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Oral Presentations

O-1
TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY (2D-LC) IN THE ANALYSIS OF PHARMACEUTICALS AND AUTHENTICATION OF CHINESE HERBAL MEDICINE

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One-dimensional liquid chromatography (1D-LC) is routinely applied to the analysis of all kinds of samples in different fields. With the introduction of UHPLC instruments and sub-2micron particle columns, the separation efficiency was greatly improved. To resolve all components of complex samples, however, 1D-LC does not provide enough resolving power, or peak capacity. In addition, to separate compounds co-eluting in 1D-LC, increasing the separation efficiency by increasing the number of theoretical plates might not be sufficient and employing different selectivities is the stronger tool.

Two-dimensional liquid chromatography (2D-LC) enables separating compounds sequentially employing different selectivities. In heart-cutting and multiple heart-cutting 2D-LC, one or several fractions from the effluent of a first dimension separation are transferred to a second dimension separation with different selectivity. Heart-cutting and multiple heart-cutting 2DLC can for example be used for separation of co-eluting impurities in pharmaceutical impurity analysis. In comprehensive 2D-LC (LC\texttimes{}LC), the entire sample is subjected to separation in two dimensions. If the first and second dimension separations are completely independent of each other and no loss in resolution results from the transfer of first dimension effluent to the second dimension, the total peak capacity of a comprehensive 2D-LC separation equals the product of the first and second dimension peak capacities. Because of the high resolving power, LC\texttimes{}LC is ideally suited for a comprehensive analysis of complex samples.

In Chinese herbal medicine (CHM), one aspect of traditional Chinese medicine (TCM), preparations of several herbs are used and the pharmaceutical efficacy is regarded to depend on the synergistic effects of multiple components of the herbs. The analysis and authentication of CHM is a challenging task because of the complexity of the herbs used and the natural variability of the plants. Generally, chromatographic fingerprinting is regarded as an effective method for authentication of CHM. Due to the complexity of the herbs used comprehensive two-dimensional liquid chromatography (2D-LC) is the method of choice for their analysis.

O-2
CURRENT AND FUTURE APPROACHES TO THE QUALITY OF CONTROL OF CHINESE HERBAL MEDICINES

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Quality of control of Chinese herbal medicines is currently mainly based on specifications of pharmacopoeias. Challenges are the limited specificity of some of these methods, the use of various grades and processed materials, which cannot easily be distinguished, and the increasing use of granules, which are so far not considered in most pharmacopoeias. For identity and purity testing, microscopy and TLC fingerprint analysis are currently mainly used. DNA barcoding should be applied in the future in critical cases. However, it may not work for granules and processed herbs. Therefore, metabolic profiling with multivariate data analysis should be evaluated for this purpose. Also spectrometric methods, like NMR and NMR may be used. In all cases, first, an agreement on the acceptance criteria has to be achieved. For contaminants, like heavy metals and pesticides, harmonized and globally accepted limits should be elaborated. They should refer to the final use of the products, like with limits for microbial contamination. For processed herbs, the mode of processing should be defined and endpoints should be specified. Assays should be related to therapeutically or pharmaceutically relevant compounds. However, the therapeutically relevant active constituents of medicinal plants are often not yet known. Therefore, the selection of single marker compounds for the assay is still common, but questionable. Introducing two markers of different polarity gives more information on the quality of an herbal drug. However, in the future, a metabolomics based approach using high resolution chromatographic and spectroscopic techniques (HPTLC, LC-MS, NMR, IR) with multivariate analysis will be the most appropriate way to standardize raw material and herbal extracts.

O-3
MHC-2D LC STRATEGY FOR THE HOLISTIC QUALITY CONTROL OF NOTOGINSENG–CONTAINING CHINESE PATENT MEDICINES

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Current China Pharmacopoeia (ChP) standards employ diversified and case-dependent assay methods to evaluate the quality of different Chinese patent medicines (CPM) that contain Panax notoginseng as the monarch drug. These conventional HPLC-based approaches utilizing complex sample preparation procedure can easily result in low analytical efficiency and possible component loss. We thereby propose a “monomethodheterotrait matrix” (MHM) strategy, that is, developing a universal multi heart-cutting two-dimensional liquid chromatography (MHC-2D-LC) approach that facilitates the simultaneous quantitation of five Panax notoginseng saponins (noto-R1, Re, Rg1, Rb1, and Rd)
in eight different CPMs. The MHC-2D-LC system was constructed on the dual-gradient liquid chromatography instrument equipped with a Poroshell SB C18 column and a Zorbax SB-Aq column for respective 1D and 2D separation. Methodical validation was performed in terms of specificity, linearity, intra-/inter-day precision, stability, and recovery, and the LODs and LOQs (loaded masses). The validated MHC-2D-LC approach was subsequently applied to quantify five saponins in 30-batch different CPMs, showing superiority over the ChP assay methods in respect of specificity (avoiding co-elution), resolution (Rs>1.5), sample preparation (easy-to-implement ultrasonic extraction without repeated re-extraction), and transfer rate (minimum component loss). It is the first application of an MHC-2D-LC method in quantitative assessment of CPMs. The MHM strategy represents new methodology for quality control of CPMs that involve complex chemical composition.

**O-4**

**COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY COUPLED WITH QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY FOR CHEMICAL CONSTITUENTS ANALYSIS OF TRIPTERGIGUM GLYCOSIDES TABLETS**

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Comprehensive two-dimensional liquid chromatography platform (LC × LC) coupled with quadrupole time-of-flight (QTOF) mass spectrometry (MS) is developed to separate, identify and relatively determine the chemical constituents of two types of tripterygium glycosides tablets (TGT). The types and relative contents of the constituents discovered in two kinds of TGT tablets were subsequently compared. C8 and C18 column were used for the separation of the first and second dimensional chromatography (1D and 2D) respectively, and an integrated shift and full gradient mode was used in 2D. Using this LC × LC-QTOF-MS platform, 92 and 132 constituents were detected in TGT preparations from Hubei and Hunan manufacturers respectively (HTGT and HY-TGT), most of which belonged to the diterpenoid, triterpenoid and alkaloid families. 50 and 90 compounds were unique in HB-TGT and HY-TGT, respectively, and their relative contents proportion were 52.0% and 54.2% of HB-TGT and HY-TGT, respectively. Furthermore, two TGT tablets could both lead to obvious change in biochemical parameters, oxidative stress related parameters and histopathological status to different degree. In all, the LC × LC-QTOF-MS platform offer a powerful and efficient method for characterizing, identifying and semi-quantifying chemical components in TGT preparations.

**O-5**

**THE MODERNIZATION AND INTERNATIONALIZATION OF TRADITIONAL MEDICINE, CHALLENGES AND OPPORTUNITIES**

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Traditional Chinese medicine (TCM) is the main body of natural medicine in China, and TCM prescriptions are the main application forming TCM. For thousands of years, Chinese people have learned to use multiple herbal medicines in proper proportions for therapeutic use, in order to overcome the side effect of some herbs, as well as to bring synergy effects. From individual herbal medicines to TCM prescriptions, it relates to hundreds of years of clinical practice, experience accumulation and precipitation. It gradually developed into a profound, unique TCM culture. Modern medical research shows that "multiple components act on multiple targets", "overall regulation", such characteristic features of TCM, are especially suitable for the treatment of various chronic diseases, degenerative diseases, as well as modern puzzling and incurable diseases. World Health Organization (WHO) emphasized that there is growing concern about traditional medicine for its important role in the diagnosis and treatment of human disease. However, TCM prescription, a profound and unique TCM culture, the heritage of human beings, have so far failed to go abroad. Why it is so difficult for modernization of TCM prescriptions? How to speed up the internalization of TCM and its products? The authors will share opinions on this topic.

**O-6**

**INTRODUCTION OF ASIAN HERBAL MEDICINES IN THE EUROPEAN PHARMACOPOEIA: RELEVANCE OF THE CURRENT ASSAY POLICY**

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Herbal drugs have a long tradition as medicines for the European market. They are recognized within the regulatory framework of the European Union (EU) on medicinal products like all kinds of medicines. All herbal drugs and the products made thereof have to proof their quality and safety prior to gaining access to the market. The concern about the safety of TCM herbal drugs in the EU was the basis of the decision in 2005 of the Commission of the European Pharmacopoeia (Ph Eur) to establish TCM herbal drug quality monographs, thus describing the most important Chinese Materia Medica (CMM) important for the European market. Ever since more than 50 Monographs on TCM herbal drugs have been elaborated by a highly specialized TCM Working Party of the Ph Eur Commission and are legally implemented for all the EU member states. The basis for this program is the actual English edition of the Chinese Pharmacopoeia (Ch P) 2010. However, a simple adapt/adopt procedure is not feasible due to significant differences in the legal framework when comparing Ph Eur
and Ch. P. One main difference is the consistent inclusion of an assay in all monographs in order to determine/quantify defined natural products for each herbal drug. The result of the assay of any natural product must be stated in the definition chapter of the monograph and it will consequently be described in the assay chapter in detail. In some cases, when evaluating a new monograph, concerns about the practicability of introducing new, sometimes rather expensive marker compounds are actually disputed. This concern is mainly valid in cases where there is not enough evidence that these envisaged constituents contribute to the clinical efficacy of the herbal drug in question. Consequently, the compounds to be assayed can only function as analytical markers not determining the overall efficacy of the CMM in question. When it is not obvious that the constituents, which are chosen for an assay are not responsible for the quality assessment of a TCM herbal drug, other approaches have to be considered in order to replace the classical assay. One possible way to avoid the assay methodology could be the application of analytical markers or standardized herbal drug extracts by semi-quantitative HPTLC. This will be discussed with some examples. In these cases, however, a test for 'extractable matter' should be added to complete the respective monograph. In some monographs the assay is an essential prerequisite, mainly when toxic compounds have to be determined and limited. Also when herbal drugs are intended to be utilized in any authorized herbal medicinal product, the assay of any marker substance is essential for legal purposes. The ongoing discussion about these assay problems in the framework of the Ph Eur is currently being evaluated with the respective shareholders. The results of these discussions will be published and different opinions from outside and will help to come to final conclusions.

O-7 CHROMATOGRAPHIC HERBAL FINGERPRINTS: DEVELOPMENT, VALIDATION AND DATA HANDLING

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The use of herbal medicines is gaining importance, even in Western medicine. With any herbal extract/medicine, however, there are many unknown components, which are often only present in low amounts. Identifying only one or some compounds hardly describes the complex extracts, and will thus not be reliable enough for their quality control. A possible solution is the use of ‘fingerprint technology’, which can, for example, be performed by means of High-Performance Liquid Chromatography (HPLC). This technology has been approved by the World Health Organization, the Chinese State Food and Drug Administration, the USA Food and Drug Administration, and the European Agency for the Evaluation of Medicinal Products. The idea is to develop a complete chromatographic pattern of the herbal extract, i.e. a chromatographic fingerprint, in which usually as many compounds as possible are separated. When using the fingerprint technology, three main steps are considered, i.e. the fingerprint development, its validation, and the extraction of information from the fingerprint. Fingerprint development can be further divided into a sample preparation step and an actual fingerprint development step (i.e. determining the optimal analytical conditions) [1]. To validate the entire fingerprints, a set-up can be used to determine simultaneously the repeatability and the time-different intermediate precision of e.g. the retention time and peak area/height of given peaks in the fingerprint [2]. Considering the data handling, depending on the goal of the study, (1) data pretreatment, (2) identification and quality control, (3) exploratory analysis, (4) pattern recognition, (5) curve resolution, and/or (6) multivariate calibration techniques can be used [3]. Different aspects of the data handling of chromatographic herbal fingerprints will be discussed and illustrated with some examples.

O-8 QUALITY CONTROL OF TRADITIONAL CHINESE MEDICINES: INVESTIGATION ON CHEMICAL MARKERS

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Traditional Chinese medicines (TCMs) have played an important role in the treatment of diseases for thousands of years. With the rise of the combination therapy for multi-gene diseases, TCMs attracted more attentions gradually. However, TCMs is a complex system which could contain tens, or even hundreds of compounds. Selection of chemical markers is thus a key to the quality control of TCMs. In recent years many efforts have been made on single-and multi-components quantification, or fingerprint chromatography, but they ignored the complex interaction of components in TCMs. Therefore, we proposed a bioactive equivalence oriented feedback screening strategy to discover the exact composition of effective components from a complex mixture. This strategy mainly include chemical profile of TCM, trapping and preparing candidate BECCs, bioactivity assays of candidate BECCs, and bioactive equivalence evaluation. By comparing the variations of whole effect of TCM before and after knock out the candidate BECCs, the bioactive contributions of candidate BECCs could be evaluated. This strategy was successfully applied to screen the BECCs from Cardiotonic Pill (CP). We found the combination of 10 salvianolic acid, 4 saponins and 4 diterpene quinones as the BECCs of CP on treating myocardial ischemia through assessing the gold standard of myocardial infarction area. Their total content accounted for 15.01% of CP, which could be regarded as the chemical marker of this preparation. The BECCs screening strategy could open up a new way to improve quality
control of herbal products and facilitate the discovery of combinatorial drugs.

O-9
DEVELOPMENT OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY: A KEY ENGINE OF TCM MODERNIZATION

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Traditional Chinese Medicine (TCM) has been popular for thousand years in prevention and treatment of chronic diseases synergistically with Western medicine while producing mild healing effects and lower side effects. Although many TCMs have been proven effective by modern pharmacological studies and clinical trials, their bioactive constituents and the remedial mechanisms are still not well understood. Researchers have made great efforts to explore the real theory of TCM for many years with different strategies. Development of high performance liquid chromatography (HPLC) and mass spectrometry within recent decade can provide scientists with robust technologies for disclosing the mysterious mask of TCM. We reviewed important innovations of HPLC and mass spectrometry in the application of TCM analysis from single compound identification to metabolomic strategy.

O-10
PHYTOCHEMICAL, BIOLOGICAL AND ANALYTICAL WORK ON ASIAN PLANTS

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Based on their health promoting properties several herbal drugs used in Asian Traditional Medicine have infiltrated the European market. Because of the growing evidence suggesting that phytosterogens might protect against various cancers, cardiovascular diseases, osteoporosis and menopausal symptoms, many commercial preparations containing isoflavone extracts from e.g. Glycine max. and Pueraria lobata have established a firm market position as nutritional supplements and phytotherapeutic preparations. Red Yeast Rice (RYY), traditionally popular in Asia for colouring of food, drinks and textile and for its medicinal properties, is becoming a popular product worldwide because of its positive effect on cholesterol and therefore preventing cardiovascular diseases (CVD). RYY is a traditional Asian fermentation product of Monascus purpureus, mainly produced in China. The active compounds, the monacolins including lovastatin and monacolin K are competitive inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase, an enzyme in the metabolic pathway of cholesterol production. For companies providing commercial herbal preparations it is very important to control the quality of the bulk materials and the finished products. The ‘Guideline on quality of herbal medicinal products/traditional herbal medicinal products’ covers the general quality aspects of these products. Control of herbal substances and of herbal preparations should be in accordance with the ‘Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products’. This guideline addresses specifications, i.e., those tests, procedures, and acceptance criteria used to assure the quality of these substances and products at release and during the shelf life. Furthermore, as described in the guideline on the Quality of Combination Herbal Medicinal Products/Traditional Herbal Medicinal Products, one has to individually identify and assay the different constituting herbal substances or preparation in the release and shelf life testings of a combination product. Therefore methods used in identification, tests and assay should be specific and stability indicating based on a chromatographic profile. Methods set in official monographs should be used, but when the described methods are not appropriate or when no official monograph exists, a comprehensive specification including a suitable assay should be drawn up. Creating a new method includes two steps: (1) the development and (2) the validation according to the current EMA/ICH Guidelines on validation by investigating defined characteristics, such as the calibration model, the specificity and selectivity, the precision and the accuracy. The major challenges in developing and applying such methods for the quality control of single and combination products will be discussed for isoflavone containing products (soy, pueraria, red clover) and RYY supplements with respect to national and European requirements.

O-11
QUALITY-DESIGN IN TRADITIONAL CHINESE MEDICINE: A CASE STUDY ABOUT ISATIS INDIGOTICA FORT.

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Quality is the basis for the efficacy of Traditional Chinese Medicine (TCM), affecting herbs, formulations, and even the practice of TCM itself. In our laboratory, we used Isatis indigotica, a prevalent Chinese medicinal herb, as a model to illustrate strategies and methods for TCM Quality-design study. First of all, tetraploid I. indigotica (2n=28) with better yield, higher antiviral activity and enhanced resistance was obtained from its natural diploid progenitor (2n = 14) after selection for five years. Chemical investigation demonstrated the lignans including lariciresinol and larch lignan glycosides represented important efficacious substances for the antiviral effect, which accumulated more in tetraploid I. indigotica than diploid progenitor. A further comprehensive survey of global gene expression performed by using an Arabidopsis thaliana whole genome Affymetrix gene chip revealed the variation of gene expression between autotetraploid and diploid I. indigotica, providing a pool of candidate genes for improving I. indigotica quality through
transgenic manipulation. Next, in order to facilitate the process of isolating these quality-related genes (especially gene family) such as that involved in biosynthesis of effective metabolites, *L. indigotica* transcriptome sequencing was performed. By using this gene database, a large number of quality-related genes were isolated and intensively investigated, including stress resistance-involved transcription factor family IiWRKYs, signal transduction-involved gene IiCPK1 and IiCPK2, plant development-involved gene IiSDD1, lignins biosynthetic pathway regulatory factor family IiHLHs, and a series of lariresinol biosynthetic pathway genes such as IiPAL (DQ115905), IiC4H (GU014562), Ii4CL (GU937875), IiCCR (GQ872418), IiCAD (GU937874), IiCH (JF826963), IiCoAOMT (DQ115904), as well as IiDir and IiPLRs gene families. Lastly, gene-based metabolic engineering study was performed to improve quality. For example, overexpression of IiPLR1 greatly enhanced lariresinol production in *L. indigotica* hairy root cultures with the content of 353.9 µg/g of dry weight, which was appropriately 6.3-fold more than that in the wild-type counterpart; transgenic *L. indigotica* expressing Bt Cry1Ac and Pinellia ternata agglutinin gene (pta) significantly enhanced plant tolerance to moths and aphids. In conclusion, our study not only prompted the possibility to obtain *L. indigotica* with higher quality, but also provided a reference for the study of TCM Quality-Design.

**O-12**

**SIMULTANEOUS QUANTITATION OF FIVE PANAX NOTOGINSENG SAPONINS IN EIGHT CHINESE PATENT MEDICINES BY MULTI HEART-CUTTING TWO DIMENSIONAL LIQUID CHROMATOGRAPHY**

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Current China Pharmacopoeia (ChP) standards employ diversified and case-dependent assay methods to evaluate the quality of different Chinese patent medicines (CPM) that contain Panax notoginseng as the monarch drug. This study is aimed to develop a two-dimensional liquid chromatography (2D LC) based assay approach to unify and simplify the quality assessment of Notoginseng containing CPMs. Multi heart-cutting 2D LC (MHC-2D LC) was used to separate and quantify five major saponins (noto-R1, Rg1, Re, Rb1, and Rd) in eight different CPMs. The content determination results were also compared with those obtained using the ChP standard methods. The MHC-2D-LC system was constructed on a dual-gradient liquid chromatography instrument. Method validation was performed in terms of specificity, linearity (r2 and F-test), intra-/inter-day precision (0.4%-7.9%), stability (1.2%-3.9%), and recovery (90.2%-108.7%), and the LODs and LOQs (loaded masses) of the five analytes varied between 4.0-11.0 ng and 6.0-33.0 ng, respectively. The validated MHC2D- LC approach was subsequently applied to quantify the five saponins in thirty batches of different CPMs. The method demonstrated superiority over the current ChP assay methods in respect of sensitivity (avoiding co-elution), resolution (Rs>1.5), sample preparation, and transfer rate (minimum component loss). This is the first application of an MHC-2D LC method for the quantitative assessment of the constituents of CPMs. The established approach represents a new, strategically significant methodology for the quality control of CPMs.

**O-13**

**WARM NEEDLING FOR OSTEOARTHRITIS: A SYSTEMATIC REVIEW AND METAANALYSIS**

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The purpose of this systematic review was to evaluate the effectiveness of warm needle treatment of osteoarthritis. Fourteen databases were searched from their inception through April 2015. Randomized clinical trials (RCTs) were included if a warm needle was used either as the sole treatment or as a part of a combination therapy for osteoarthritis. Risk of bias was assessed according to the Cochrane criteria. Of the articles that were identified from 14 databases, 956 had relevant titles, and of these, 55 RCTs were deemed eligible. All of the included trials reported beneficial effects of warm needle compared with conventional treatments including drug therapy and exercise. Most of the included trials showed positive effects of the warm needling for the treatment of osteoarthritis compared with conventional therapies. However, the total number of RCTs, the total sample size was small and the risk of bias were too high to draw firm conclusions. Future rigorous RCTs will be necessary to assess the clinical efficacy of warm needle for this condition.

**O-14**

**COLLECTING AND ANALYSING OUTCOMES DATA FROM ACUPUNCTURE TRAINING INSTITUTIONS: A COLLABORATIVE PROJECT**

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If clinical research is to be relevant to real-world decision making it requires interventions that reflect usual practice. Observational studies may provide the best approach to defining this. Sitting studies in the student clinics of acupuncture teaching institutions (ATIs) has potential benefits for the institutions as well as for researchers. This is the first such multi-centre study accredited ATIs were
invited to participate, using a protocol based around MYMOP (Measure Yourself Medical Outcome Profile). MYMOP questionnaires were to be completed at the first, second, third, sixth and tenth sessions. Data were to be collected from all consenting new patients during 2013-14. Overall coordination and training was provided by the British Acupuncture Council’s Research Manager. Seven ATIs participated but only five provided data. Despite the common protocol there was considerable variation in the content and format of the data provided, most critically in the MYMOP recording points used. Some data fields were entirely missing for some ATIs; other items were missing from individual patient records due to random errors. MYMOP protocol violations were common. Cleaning the data prior to SPSS analysis was a lengthy, untidy process. Of 1200 records received only about one third can be used for the main analysis. Results will be presented at the meeting. Successful multi-centre studies of practice require that both individual and institutional participants are sufficiently incentivised and committed.

O-15 RESEARCH OF COMPLEX AND BIOACTIVE TERPENOIDS FROM MEDICINAL PLANTS OF MELIACEAE FAMILY

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In China, many plants of Meliaceae, distributed mainly in Southern China, were well-known Chinese folk medicines using to treat dysentery and fever. Recently, plants from Meliaceae were the research focus of natural products because of their diverse structures and interesting biological properties. In order to discover novel and bioactive natural products from Chinese folk medicinal plants, we have investigated the chemical constituents and bioactivity of several medicinal plants from Meliaceae family mainly distributed in Xishuangbanna of China. Various methods and technologies, such as ODS, Sephadex LH-20, MCI, P-HPLC, Circular-PHPLC, UPLC-Q/TOF/MS, were used in our systemic separation and purification research. 1D and 2D NMR, CD exciton chirality, X-ray, HRESIMS, and so on, such structural elucidation means and techniques, were applied to elucidate their planar structures, relative and absolute configurations. Meanwhile, the anti-inflammatory, insecticidal, antifungal, HSP90 inhibitory and cytotoxic activities of the isolated limonoids were evaluated. From 2007 to 2014, 15 species of 5 genera from Meliaceae have been researched which led to the isolation of 447 terpenoids (limonoids) including 257 new compounds and 5 types of novel carbon skeletons. Many methods, especially the application of single-crystal X-ray diffraction and CD exciton chirality method, were applied to determine their absolute configuration. The novel isolates were evaluated for a series of biological activities, such as anti-inflammatory, insecticide, antifungal, cytotoxicity, and HSP90 inhibitor, more than 100 isolates showed notable bioactivities. In our research program, many novel and complex terpenoids and limonoids were isolated and identified, which have enriched the types of natural organic compounds. Through bioactivities screening according to their traditional usage, the material foundation of these traditional medicines were elucidated basically. The findings of novel limonoids with significant bioactivity have potential academic value and scientific significance for the research and development of new drugs.

O-16 ETHNOPHARMACOLOGY AND MALARIA IN AFRICA

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According to the last World Malaria Report, there were 584 000 deaths from the recorded 198 million malaria cases worldwide in 2013. The disease caused an estimated 437 000 African children to die before their fifth birthday, still in 2013. Malaria is caused by a parasite, Plasmodium sp. and transmitted by Anopheles mosquitoes. The problem of parasite resistance towards common available medicines such as chloroquine, mefloquine, quinine, is increasing. In this context, the vegetal kingdom remains the main source of pharmacologically active compounds against this parasitic infection as attested by the famous quinine, isolated from Cinchona sp., artemisinin extracted from Artemisia annua and also atovaquone derived from lapachol found in several Bignoniaceae. All these substances are related to plants with traditional use against fever and malaria. Beside these well-known examples, various new antiplasmodial compounds are frequently discovered from Nature, particularly following an ethnopharmacological approach, as reviewed by several authors in recent years. Then, the pharmacological and phytochemical study of plants from traditional pharmacopoeias can be of first interest not only to discover new antimalarial “lead compounds”, but also to valorize local vegetal species whose efficacy and safety would have been demonstrated in laboratory and clinical investigations. As demonstrated in several works from Willcox, better knowledge of plants from traditional pharmacopoeias and local valorization of validated traditional remedies in Improved Traditional Medicine (ITM) could allow the access to effective, standardized, available and affordable therapeutics for management of malaria by local populations. After this introduction, the second part of the talk will be dedicated to the presentation of some results obtained in Liège with Dicoma tomentosa from Burkina-Faso, Strychnos icaja from Cameroun and Terminalia mollis from Rwanda.

O-17 ISOLATION, CHARACTERIZATION AND MEDICAL PROPERTIES OF ANTICOMPLEMENT AGENTS FROM TRADITIONAL CHINESE MEDICINES

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The inappropriate activation of complement system may cause some life-threatening symptoms such as rheumatoid arthritis, systemic lupus erythematosus (SLE) and acute respiratory distress syndrome (ARDS). In our efforts to obtain natural anticomplement agents from traditional Chinese medicines (TCMs) for prevention and treatment of the complement-associated diseases, bioactivity-guided fractionation and isolation was thus performed, and the anticomplement constituents were characterized chemically and pharmacologically. The anticomplement agents obtained from TCMs (Bupleuri Radix, Houttuyniae Herba, Arnebiae Radix, Moutan Cortex, Indigo Naturalis, etc) were found diverse in structure. The polysaccharides and flavonoids commonly showed good anticomplement activity by interacting with the different components in complement activation cascade. The Bupleurum polysaccharides showed anti-complementary and immunomodulating activities and exhibited beneficial effects on the ARDS and SLE in rats and mice. The Houttuynia cordata polysaccharides (HCP) exhibited anti-complementary and antipyretic activities, and attenuated the acute lung injury induced by lipopolysaccharide in rats. It’s interesting that HCP exhibited protective effect on mice infected by influenza A virus (H1N1), although it had no direct inhibition against the virus. The results demonstrated that the polysaccharides from TCMs are valuable anticomplement agents.

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O-18
THE REGISTRATION OF TCM PRODUCTS UNDER EU TRADITIONAL HERBAL MEDICINAL PRODUCT DIRECTIVE

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The Traditional Herbal Medicinal Products Directive (THMPD) 2004/24/EC is a regulatory process established to enable high quality herbal medicines to be registered as an OTC product, with medical claims (as allowed for OTC drugs) on the packaging and in the patient information leaflet.

For those products which are eligible to be registered they must firstly, be only for mild and self-limiting ailments, and for use without the supervision of a medical practitioner, secondly they have to have been in use for thirty years (of which at least fifteen years will have been in the EU), and thirdly they must meet pharmaceutical standards of quality and stability.

Since the directive went into effect in 2005, over three hundred herbal products have been registered under the THMPD, and more than one hundred different herbs are included in all granted products in the UK. Many of these products are simply different manufacturers’ brands of the similar products and there are hardly any traditional herbal medicines from outside of Europe that have been approved. After several years of working on the project and extensive discussions with MHRA, Phynova Joint and Muscle Relief Tablets was granted a Traditional Herbal Registration (THR) in February, 2015 and it has been to consumers since June, 2015. In collaboration with a Chinese Pharmaceutical company, Phynova has submitted second THR application for Isatis Colds/Flu Relief in April, 2015. The successful first registration of a TCM product under the THMPD scheme was due to Phynova’s extensive pharmaceutical expertise in the research and development of TCM derived botanical products. Phynova is a life science company that specialises in the development of botanical drugs and functional ingredients from Chinese medicinal plants. Phynova utilises stringent standardisation and quality control procedures in the production of all its herbal extracts, and complies with all of the rigorous regulatory requirements for manufacturing and distributing herbal medicinal products. Phynova works within the EU pharmaceutical GMP framework, and manufactures all of its botanical extracts in accordance with these practices. In our presentation we would like to share our experience in the registration of TCM drugs as a THR.

O-19
BRIEF INTRODUCTION TO WORK OF ISO/TC 249 TRADITIONAL CHINESE MEDICINE

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ISO (International Organization for Standardization) is the world’s largest developer of voluntary International Standards. International Standards give state of the art specifications for products, services and good practice, helping to make industry more efficient and effective. Developed through global consensus, they help to break down barriers to international trade. ISO was founded in 1947, and since then has published more than 20000 International Standards covering almost all aspects of technology and business. From food safety to computers, and agriculture to healthcare, ISO International Standards impact all our lives. Based on 3 000 years of experience, traditional Chinese medicine (TCM) is used in over 160 countries. Including a wide range of medicines, devices, massages and diets, TCM has a common basis with other traditional systems. As TCM techniques and medicines are exported globally, this makes the need to maintain quality, safety, and efficacy of traditional practices and products even more important. Thus, International Standards need to be developed. In 2009, ISO established a technical committee, named 249 Traditional Chinese medicine, to develop the international standards in the field of Traditional Chinese medicine. The secretariat of ISO/TC 249 is allocated to China (SAC, Standardization Administration of China). The Chair of ISO/ TC 249 is retired Dr. David GRAHAM, the former national manager of therapeutic goods administration (TGA) in Australia. The Secretary of ISO/TC 249 is Mr. SHEN Yuandong, professor of Shuguang hospital affiliated with Shanghai University of Traditional Chinese Medicine. Until now, thirty-five
member bodies are participating in ISO/TC 249. The technical work scope of ISO/TC249 focuses on standardization in the field of medical systems derived from ancient Chinese medicine which shall be able to share one common set of standards. Both traditional and modern aspects of these systems are covered. The committee focuses on quality and safety of raw materials, manufactured products and medical devices and of informatics, including service standards limited to involving the safe use and delivery of devices & medicines. Since 2010, six plenary meetings have been held successfully. Nowadays, ISO/TC 249 have established five working groups and one joint working group with ISO/TC 215 health informatics. It has also established liaison with some related international organizations, e.g. World Health Organization (WHO), ISO/TC 215, IEC/SC 62D, World Federation of Chinese medicine societies (WFCMS) and World Federation of Acupuncture-Moxibustion societies (WFAS). In ISO/TC 249, there are two published standards and thirty-three projects under development, covering ginseng seeds and seedlings, acupuncture needles, herbal decoction apparatus, coding system of Chinese medicines, quality and safety of natural materials and manufacturing products made with natural materials used in and as traditional Chinese medicine (TCM), etc. The aim to establish ISO/TC 249 is to strengthen TCM to be used in a more scientific, effective and safer way. At present, ISO/TC 249 is continuously exploring and improving its work and it also needs to learn the experience from related international organizations and receive great contributions from member bodies. With the increasing needs in TCM standardization globally, ISO/TC 249 intends to: explore the possibilities to cooperate with related international organizations, expand the working scope to the field of TCM services, develop other ISO deliverables, TS, TR, PAS publicize the published standards to encourage more and more countries to adopt them.

O-20
A SYSTEMS PHARMACOLOGY VIEW OF THE BIOACTIVITY OF THE EXTRACT FROM RHIZOME DIOSCOREA NIPONICA MANKINO

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Key for worldwide acceptance of Traditional Chinese Medicine (TCM) is the ability to provide scientific evidence combined with a quality control system based on the bioactive ingredients. Modern scientific technology tools are now available to accomplish standardization of TCM products in order to achieve a high level of efficacy and safety, enhancing the introduction into the international markets. Despite the complexity of ingredients and the aspect of synergistic bioactivities in TCM, so far the analysis for quality control was mainly limited to major components for each herb without evidence for a direct relationship with bioactive components. In a Systems Biology approach, the multi-dimensional chemical and pharmacological approach enables linking of the complex metabolic profile of herbs with biological effects and is therefore a key for quality control of TCM material medica, while providing simultaneous scientific evidence for the underlying efficacy and worldwide acceptance of TCM products. The root extract of Dioscorea nipponica is a well-known component in Traditional Chinese Medicine formula’s. The bioactivity of this extract has been described in Chinese medicine as (1) activating blood circulation to dissipate blood stasis (2) promoting qi circulation to relieve pain and muscle tension due to stress (3) relieving internal resistance of stagnant blood. In order to translate the explanatory system-level functionalities in Chinese medicine into a Western biochemical, functional and clinical understanding an extensive series of pharmacological experiments were used to obtain insights in various effects of prevention and treatment in the cardiovascular domain. Moreover the active component profile consisting of saponins were studied and found to be of a system synergistic nature. Integrating this information with the outcome of clinical trials which over 16,000 patients were involved together with results from over 100 million people treated yielded a systems pharmacological view on the working mechanism and insights in the effectiveness and safety profile of this product produced and controlled under GALP and GMP. This product was the first HMP herbal medicine product registered in the EU that was produced in China. It underpins a new view on evaluation of herbal medicine products in general and creates an important systems pharmacology concept applicable in a wider sense.

O-21
MOLECULAR UNDERSTANDING OF NATURAL POLYPHENOLS: FREE RADICAL SCAVENGING AND INTERACTION WITH BIOMEMBRANES

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Quantum calculations (mainly DFT) and molecular dynamics are increasingly effective tools to evaluate the physical chemical properties of natural and bio-inspired compounds. Free Radical Scavenging Capacity. Thermodynamic parameters (mainly bond dissociation enthalpies (BDE) of the O-H phenolic bond) allowed an accurate prediction of the antioxidant capacities of natural compounds. Based on the Transition State and the Marcus Theories (for atom-and electron-transfers, respectively), kinetics was also evaluated providing a better prediction of the antioxidant behavior in solution or in the organism. Pro-oxidant effects have also been studied for flavonoids and stilbenoids, mainly showing their capacity to form dimers and oligomers after oxidation. Interaction with Lipid Bilayer Membranes. Over the past years, building models to figure out drug-membrane interaction has deserved much interest. Membrane penetration / accumulation / crossing / positioning play a crucial role in drug delivery and
metabolism in the human body. Molecular dynamics is
molecular picture of the location of natural polyphenols in
lipid bilayers and possible cooperative interaction with
other antioxidants a much promising tool, complementary
to experimental measurements, to get into this knowledge.
Such theoretical simulations have been performed to
provide an accurate picture of the interaction between food
flavonoids & polyphenols and lipid bilayer membranes,
predicting their location, orientation and partitioning. We
really aim at using advanced molecular modeling methods
for an applicative purpose in food and cosmetic industries.
The predictive character of these methods allows building
molecular guidelines for a better and safer use of food
flavonoids and polyphenol.

O-22
TRADITIONAL CHINESE MEDICINE IS MARCHING
TOWARDS EVIDENCE-BASED MEDICINE

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Traditional Chinese medicine (TCM) has over 3000 years of
history treating diseases in China and has played a pivotal
role in the Chinese health system. But the experience-based
feature of traditional Chinese medicine requires for its
scientific evidence in quality, safety and efficacy to evolve
into evidence-based medicine. In the past 20 years or so,
great progress has been achieved on the modernization of
traditional Chinese medicine. Government launched a
number of “heavy-weight” programs and allocated central
budget for the development of TCM. Phytochemical
investigation of Chinese herbal medicines was the most
active research area for the clarification of its active
principles and new drug lead discovery. So far over 5000
new chemical compounds were isolated with many being
bioactive ones and some of them have been in the process of
new drug development. In this lecture, an overview of
research progress in TCM resource investigation,
comprehensive phytochemical analysis, pharmacokinetic
studies, TCM pharmacology, proteomics and TCM-based
drug discovery and exemplified through several commonly
used TCM herbs or preparations including Salvia miltiorrhiza, Panax notoginseng, Ganoderma lucidum,
Glycyrrhiza uralensis, Danqi Tablet, etc., which may help
the audience to understand where TCM stands in China.
In addition, the current research progress on the elaboration
of quality standards of traditional Chinese medicines will also
be introduced with several concrete examples. The future
perspective of TCM modernization will be finally projected.

O-23
ACCELERATED DISCOVERY AND PROFILING OF
PHYSIOLOGICALLY ACTIVE COMPONENTS IN
COMPLEX SAMPLES BY HPTLC-EDA-HRMS

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On the one hand, the separation of thousands of
compounds in a complex extract is thrilling, but may be still
be separated unsatisfactorily. Hence, the question arises
where to stop in high-sophisticated separation science?
Which technical effort is economically justifiable in routine?
On the other hand, the separation itself does not imply an
effect-directed answer to questions such as “Which
compounds of the thousands of compounds are effective?”.
In contrast to high-sophisticated, comprehensive separation
science, a streamlined methodology is presented in the
following that is able to answer these effect-directed
questions without the need for a perfect separation,
however, with clear limitations. The first part of the
streamlined methodology is an effect-directed screening of
up to 22 raw extracts in parallel. Thus, this part
(HPTLC-UV/Vis/FLD-(bio)assay) can be described as a
non-targeted, effect-directed detection of single or also
coelecting effective compounds of the sample. The second
part is a highly targeted characterization of the effective
compounds discovered via the hyphenation to structure
elicating techniques (HPTLC-HRMS or NMR or ATR
FTIR). For a direct link, the hyphenation
HPTLC-UV/Vis/FLD-(bio)assay-HPLC-HRMS or NMR or
ATR FTIR is studied. Here, the bioactive zone of interest is
selectively eluted via a short orthogonal column into the
HRMS or into a microvial for NMR or ATR-FTIR recordings.
Considerable information can be obtained when the same
sample is applied multifold on the plate. Thus, information
on effective compounds in a complex sample and their sum
formulae can be obtained from a single chromatographic
run. Depending on the bioassay or enzymatic assay selected,
for example, antibiotics, oestrogens, acetylcholinesterase
inhibitors, xanthine oxidase inhibitors or tyrosinase
inhibitors are discovered in complex samples.
HPTLC-UV/Vis/FLD-(bio)assay examples are given, taking
3 to 20 min per sample for the discovery of the bioactive
components. Every technique has its limitation. For volatile
or oxidation-prone compounds, this streamlined
methodology is limited in the information content.
Nevertheless, it may serve as a survey on effect-directed
components in complex samples. Benefits may result from
the side-by-side sample comparison, the matrix-tolerance,
the avoidance of carry over and of discrimination, the
always fresh adsorbent, the comparatively low-tech
workflow as well as the multiple evaluation of the
separated sample saved on the plate.

O-24
APPLYING NOVEL ANALYTICAL TECHNOLOGIES
TO FACE NATURAL PRODUCTS CHALLENGES

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Herbal medicines have been used since ancient times for the
treatment of many diseases and illnesses. Modern chromatographic and spectroscopic techniques have had a tremendous impact on the analysis of herbal medicine to facilitate research as well as enhancement of safety and efficacy of commercial products. Today, with the advances in technology, and a variety of analytical solutions available to tackle different analytical problems, the challenge for researchers resides on being able to keep up with the pace of the technology advancement, understand and how to use them effectively to answer the analytical questions at hand quickly and effectively.

The goal of this presentation is to share with scientists some examples of the latest updates of analytical technologies, from chromatographic separation to mass spectrometry detection to informatics platform through application examples. Example technology mentioned include: supercritical fluid chromatography (SFC), nano-fluidic chromatography, QToF MS, as well as the latest development on Ion Mobility (IMS) Mass Spectrometry.

O-25

VASORELAXANT DITERPENES: FROM TRADITIONAL MEDICINE TO NEW INHIBITORS OF VOLTAGE-DEPENDANT CALCIUM CHANNELS

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Crotalaria zambesiaca Muell. Arg. (Euphorbiaceae) (Syn. C. amabilis Muell. Arg., C. gratissimus Burch.) is a shrub or small tree reaching 10 m in height. The leaf decoction is used in Benin as anti-hypertensive, anti-microbial (urinary infections) and to treat fever associated with malaria. We analysed the in vitro and in vivo vasorelaxant activity of different extracts as well as their toxicity and identified in the dichloromethane extract diterpenes that inhibit the KCl-induced contraction of male Wistar rat aorta in a concentration-dependent manner. Their vasorelaxant effect was compared to a series of synthetic trachylobanes on KCl-induced contractions in order to evaluate the structure-activity relationships. The cytotoxicity of all these compounds was also evaluated on HeLa cells as some trachylobanes were shown to be cytotoxic and pro-apoptotic. Analysis of the mode of action showed that their activity is associated with the blockade of L-type voltage-operated calcium channels. These results indicate that C. zambesiaca leaves contain vasorelaxant diterpenes which could account, at least partially, for their use to treat hypertension. Nevertheless, as some of these compounds are cytotoxic, this plant has to be used with caution and more in vivo toxicity studies are needed before this plant could be recommended in medicine.

O-26

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM ENDOPHYES ASSOCIATED WITH MEDICINAL PLANTS

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In the field of natural products, plants have proven their interest as a source of lead compounds in drug discovery. Nevertheless, in the context of biodiversity preservation, researchers recently focused their work on renewable sources, like micromycetes, which present the ability to modulate their metabolism depending on the substrate. Fungi are one of nature’s most promising source for finding new drug candidates and antimicrobials. They have interesting potential as the new way of manufacturing biological medicines and a wide spectrum of new value added bio-based products. The use of fungal processes and products can lead to increased sustainability through more efficient use of natural resources. Particularly, plant-associated endophytic fungi, a group of poorly investigated microorganisms that colonize internal tissues of all plant species and able to produce some important pharmaceuticals as their host plants (such as the world’s first billion-dollar anticancer drug paclitaxel), represent a sustainable and reproductive source of plant-associated natural products, and have undeniable application in the development of compounds in drug discovery. In this case, this would not only reduce the need to harvest slow-growing and possibly rare plants but also preserve the world’s ever-diminishing biodiversity. The project led in our research group is aiming at a better understanding of the communities of medicinal plants-associated endophytes, especially their diversity and their interactions, as well as the search for new antiparasitic compounds. Medicinal plants from South America and North Africa were investigated for their culturable fungal endophytes diversity: 409 ascomycetous strains were isolated from 21 plants freshly collected. ITS rDNA was sequenced for 346 isolates: 71% of the isolates belong to the Sordariomycetes class, and 29% are Dothideomycetes. Secondary metabolome of all the strains is currently under investigation concurrently with a primary bioassay screening on Leishmania infantum. Our major goals are to correlate the potential bioactivity to the UHPLC-ESI-QTOF MS profiles to highlight bioactive markers, and to correlate these chemical profiles to the DNA sequences to discriminate fungal strains using both molecular dereplication techniques and chemical diversity analysis.

O-27

TURMERIC – COULD IT BE A MULTI-TARGETED HERB FOR COLORECTAL CANCER ADJUVANT THERAPY?

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Turmeric is a medicinal herb in Asia and as dietary supplement in western countries. Its major active component, curcumin, has good reputation for chemopreventive effects in different cancers. However, poor oral bioavailability of curcumin hinders the wide clinical application of this compound. Our research group made attempts to advance the utilization of the herb turmeric. The presence of lipophilic components (a/b-and aromatic-turmerones) of turmeric was previously shown to facilitate the absorption of curcumin into human intestinal Caco-2 cells. Our recent findings suggested that oral bioavailability of curcumin could be enhanced when curcumin is present in turmeric ethanolic extract. Besides, the anti-proliferative activities of a/b-turmerones in human breast and colon cancer cells, as well as in vitro and in vivo anti-angiogenic activities of aromatic-turmerone have also been demonstrated. Hence, turmeric extract was anticipated to possess anti-tumor effects in colon tumor-bearing mice. In this study, the hypothesis has been verified that turmeric extract plus bevacizumab, a monoclonal antibody approved as first line therapy with chemotherapy in colon cancer, exerted noteworthy anti-tumor activities in human colon tumor-bearing mice. The anti-tumor and life span prolonging efficacies of turmeric extract were comparable to those of the first line chemotherapeutics, 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX). Unlike FOLFOX treatment, the absence of hematologic side effects of turmeric extract treatment was another remarkable finding. In conclusion, our study demonstrated that turmeric ethanolic extract plus bevacizumab possessed potent anti-tumor effects in colon tumor-bearing mice without observable side effect, strongly suggesting the adjuvant use of turmeric in colorectal cancer.

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O-28
QUALITY CONTROL OF HERBAL MATERIAL: ALTERNATIVE AND/OR COMPLEMENTARY TOOLS BASED ON BIOSENSORS, DNA PROFILING, NEAR INFRARED SPECTROSCOPY AND NUCLEAR MAGNETIC RESONANCE

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Phar. Eur. Herbal Drug (HD) monographs state which aspects have to be considered for quality assurance through the relevant chapters “Definition”, “Characters”, “Identification”, “Tests”, and “Assay”. Identification of botanical material is achieved by macroscopic and microscopic morphology, generally examined by a trained expert. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are unknown and markers should be used which cannot be really related to the quality. The other critical points in the definition of the quality of the HD are represented by the purity tests, in particular some of them (heavy metals, aflatoxins and pesticides) are laborious and time consuming, requiring a significant investment in equipment, materials, and maintenance. Based on the recent studies, genome-based methods for identification of plants at the species level can be successfully based on. DNA fingerprinting can differentiate between individuals, species and populations and is useful for the detection of the homogeneity of the samples and presence of adulterated or misidentified raw materials. In some cases phytochemical and genetic data can be correlated. The generation of molecular “barcodes” can be obtained from fresh and dried plant parts, plant extracts, processed herbal drugs, as well as finished products such as herbal teas, tablets and capsules. NMR can provide direct NMR fingerprint determination (complete assignment of the signals by 1D and 2D experiments), quantitative NMR and chemometric analysis (the metabolite fingerprint is based on the distribution of intensity in the NMR spectrum to provide sample classification). NIR spectroscopy is a fast qualitative and quantitative analytical method, getting knowledge about plant species and/or its geographic origin. Finally, the development of chemical and biological sensors is currently one of the most active areas of analytical research. Immobilization of specific enzymes led to recognize definite class of compounds such as cysteine sulfoxides, glucosinolates, cyanogenic glycosides, and polyphenols. Other recognition elements are nucleic acids to evaluate the ability of different molecules to bind DNA. Sensors have also been developed for the detection of heavy metals in botanicals. Moreover, the analysis of micotoxins and pesticides, could represent another field of possible application.

O-29
DEREPLICATION STRATEGIES IN NATURAL PRODUCT RESEARCH: VARIOUS TOOLS AND METHODOLOGIES BEHIND THE SAME CONCEPT

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The development of new therapeutic drugs will certainly benefit from an ever improving knowledge and understanding of the living beings chemistry. However, the identification of innovative ‘drugable’ molecules within the immense biodiversity of forests, soils or oceans still requires considerable investments in technical equipment, time and human resources. An important part of this process is the quick identification of the known substances in order to concentrate the efforts on the discovery of new ones. A range of “dereplication” procedures are currently emerging to meet this challenge, as key strategies to reduce the duration and improve the performance of natural product
screening programs. Initially defined in 1990 as “a process of quickly identifying known chemotypes”, dereplication is today a not so univocal concept and has evolved over the last twenty years in different ways. An overview of the de-replication related studies in natural product chemistry research from 1990 to 2014 will be presented. It brought to light that five distinct de-replication workflows can be distinguished depending on the nature of the starting material, on the employed analytical technique, and above all on the final objective. De-replication can thus be used as an untargeted workflow for the rapid identification of the major compounds whatever their chemical class in a single natural sample or for the acceleration of bioactivity-guided fractionation procedures. In other cases de-replication is fully integrated in metabolomic studies for the untargeted chemical profiling of natural extract collections or for the targeted identification of a predetermined class of natural metabolites. Finally a quite distinct de-replication approach mainly based on gene-sequence analyses is frequently used for the taxonomic identification of microbial strains. At last, a focus will be made on a recently developed de-replication method, which combines chromatographic, spectroscopic and data mining tools for the analysis of natural metabolite mixtures without purification of each individual substance. Its principle consists in rapidly generating a series of simplified fractions from a crude natural extract by centrifugal partition extraction, with each fraction subsequently analyzed by \(^{13}\)C NMR. After automatic collection and binning of all \(^{13}\)C NMR signals across the spectra of the fraction series, the resulting data set is subjected to hierarchical clustering analysis for pattern recognition. Strong signal correlations caused by the same structure in different fractions are directly visualized as clusters and subsequently assigned to a molecular structure using a natural metabolite database.

O-30

NOVEL APPROACHES FOR THE DETERMINATION OF MULTIPLE PARTICULAR PESTICIDES IN TRADITIONAL CHINESE MEDICINES WITH COMPLEX MATRICES

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The pesticide contamination of Traditional Chinese Medicines (TCMs) takes different ways, which caused the complexity of pesticide residues. The effective control of pesticide residues and the establishment of relevant detection methods are very important for ensuring the safety of TCMs. Our group has been committed to the determination of pesticide residues in TCMs for years. Modular detection methods for hundreds of pesticide residues in TCMs were developed, some of which were included in the appendices of Chinese Pharmacopoeia 2015 edition. It is important to point out that the modular methods are not suitable for all circumstances, due to the complexity of TCMs matrices and the particular characteristics of some pesticides. Therefore, specific methods tailored to some particular pesticides and matrices were developed, including toxaphene analogues detection method and individualized pesticide residue detection method for complex TCMs containing volatile oil. Considering the complicity of pesticide residue detection in TCMs, continuous and systematic research will be needed to improve and consummate the detection technologies in the future.

O-31

DEVELOPING AND PREPARING TCM REFERENCE STANDARDS FOR PHARMACOPEIAS

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TCM reference standard (TCM RS) plays an important role in identification, characterization and quality control of herbal raw material, slices, extracts and finished products. It is widely used in: 1) authentication of TCM material; 2) quantitative determination of herbal chemicals and marker compounds; and 3) limit tests of toxic impurities. TCM RS are provided in three different categories in the form of voucher specimen, phytochemical reference standards and plant extract (mixture) reference standards. The reference standards chosen for a given TCM material should be specific and characteristic of the material. Most TCM chemical reference standards have complex molecular structure and are difficult to synthesize. Extraction from herbs followed by isolation is the major way of obtaining these chemical reference materials. The difficulties lie in the fact that an herb contains a large number of structurally similar compounds. These compounds have similar physical chemical properties and separation of them presents unique challenges. The commonly used process for preparing TCM chemical reference standards includes the following steps: target compound literature search, raw material candidate screening, extraction process development, target compound separation and purification research, extracted compound structure reconfirmation and full characterization including stability and homogeneity study of the extracted phytochemical material. Shanghai Nature Standard R&D and Biotech Co. Ltd specializes in producing high quality phytochemical extracts from botanicals and TCM herbs. The company has developed over 400 TCM RS and operates under ISO 17025 quality management system. Our highly purified phytochemicals (purity>=98%) isolated from herbs are widely used by official pharmacopoeia, industry and academic research as reference standard or research material. The company is a major supplier of Chinese Pharmacopoeia TCM RS and United States Pharmacopeia’s herbal and dietary supplement bulk reference material.

O-32

RECOMMENDATIONS FROM THE HERBAL MEDICINES AND PRACTITIONERS WORKING
GROUP IN THE UK: RAISING THE STANDARDS OF MANUFACTURED HERBAL PRODUCTS

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The UK government set up a Herbal Medicines and Practitioners Working Group (HMPWG) to assess possibilities of: 1. Setting up Statutory Regulation of Herbal Medicine Practitioners; 2. Allowing continued public access to valuable herbal medicines. On 26th March 2015 the HMPWG published the key recommendations after over a year of meetings and consultation with experts. [https://www.gov.uk/government/publications/advice-on-regulating-herbalmedicines-and-practitioners]. The HMPWG report did not support Statutory Regulation (SR) of herbalists, thus causing much disappointment to the TCM sector. Noteworthy key points in the report are: 1. The not recommending SR was based on a full review showing “insufficient evidence that without SR Herbal Medicines and Practitioners are putting the public at risk”, implying a public recognition of the general safety of TCM; 2. SR would not have allowed the supply of unlicensed medicine according to the UK government’s interpretation of the EU commission v Poland judgement indicating a clear UK official recognition of the shortcomings of the EU-THMPD for traditional medicine practice. [http://www.ema.europa.eu/htms/human/hmpc/hmpcli st.htm] The report recommends the UK Chinese Medical Council (CMC) working with the authorities to raise standards of manufactured herbal medicines in order to protect Chinese Medicine practice and industry with a unified approach.

O-34 HOW TO CONDUCT A GOOD CLINICAL TRIAL: AN EXAMPLE OF CHINESE HERBS FOR PSORIASIS

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It is important for high-quality trials to be conducted in compliance with GCP principles and the CONSORT statement. We showed an example of such trial. A double-blind, randomized, placebo-controlled trial was conducted, aimed to evaluate the effectiveness and safety of formula PSORI-CM01 for psoriasis. A pilot study was performed to assess the feasibility of the trial. For quality control of the formula, UHPLC-ESI-MS method was established for determination of marker compounds of the PSORI-CM01, and reliability validation was performed. To ensure high-quality implementation of the trial, researchers finished training before the trial. The monitoring of the trial was entrusted to a CRO company in China. The Data Monitoring Committee will assess the safety data and the efficacy outcomes. The primary outcome, relapse rate, is based on PASI assessed blindly during the trial. The inter-rater agreements in the PASI assessment were evaluated in all sites. The pilot study showed the PSORI-CM01 group (n=15) had a positive trend to get the 50% improvement of PASI compared with the placebo (n=17). The reliability validation including spike recoveries, precision, and repeatability, showed that quality control of PSORI-CM01 met the requirements of the CONSORT statement. Regular monitoring and auditing were employed during the process of the trial. The inter-rater ICC was from 0.84 to 0.98 in different sites. Excellent inter-researcher agreement for PASI measurements was obtained. The results suggest that with careful training and implementation of quality control procedures, high reliability of outcome measurements provide good quality assurance for the trial.

Cardiotoxic Pills® (CP) is a compound preparation of Chinese medicine consisting of Salvia miltiorrhiza, Panax notoginseng and Borneol, and it has been approved in 1994 by the China State Food and Drug Administration for treating ischemic angina pectoris. It has passed the Phase II clinical trials by the US Food and Drug Administration in 2009, December, demonstrating its potential to prolong the tolerant time in treadmill test for induction of angina, reduce the incidence of angina, and decrease the dosage of nitraten taking, while without finding any side effect. A recently published study in China showed the CP ability to decrease the occurrence of chest pain and arrhythmia in patients with heart attack receiving intervention. Our studies have demonstrated that pre-treatment with CP significantly inhibits the cardiac microcirculatory disturbance and myocardial apoptosis induced by I/R. Furthermore, administration of CP once a day starting from 3 h after reperfusion until 6 days was found to prevent post-infarction myocardial fibrosis induced by I/R, possibly due to the pivotal role of the microcirculatory disturbance and myocardial injury in the pathogenesis of myocardial remodelling after I/R and maybe attributing to the antioxidant ability of CP. These results substantiate the potential of the clinical use of CP as a management for the patients after interventional therapies.
O-35
MARINOBUFAGENIN AS A POTENTIAL BIOMARKER FOR PRE- ECLAMPSIA RISK ASSESSMENT: PURIFICATION FROM BUFO MARINUS TOAD VENOM AS SUPPORT FOR A HUMAN PLASMA LC-MS/MS ASSAY

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Marinobufagenin (MBG) is a bufadienolide compound belonging to the cardiac glycosides class. The bufadienolides are present in humans as well as in some plants and animals. But the major source for these compounds is located in the parotid and skin gland secretions of some toad species. MBG is acting as a human endogenous cardiac inotrope and is demonstrating growing interest in the early diagnosis of volume expansion-mediated hypertensive states such as preeclampsia (PE) and end-stage renal disease hypertension. Mammalian MBG acts as an inhibitor of the a1-isofrom of Na+, K+-ATPase with vasoconstrictive and cardiotonic properties, resulting in hypertension and natriuresis. Elevated endogenous MBG levels have been described in pregnant mammals and especially in preeclamptic patients. The rise of endogenous MBG appears prior the development of the main symptoms of PE, leading us to consider MBG as one of the potential target in the biomarker panel for PE. A sensitive and accurate analytical method is needed to assess MBG in as lower level as possible in plasma. Currently, only marinobufagenin-like material has been found in humans using two published quantification methods based each on immunoassays. These techniques suffer from a lack of specificity due to cross-reactivity and tend to exhibit high variability at low concentrations. Our aim is to develop a MBG assay using a more specific and easy to access technique, such as LC-MS/MS. An algorithm dealing with the MBG plasma levels might be established by clinicians in the future, in order to predict, in combination with other clinical and biological markers, the risk for preeclampsia in pregnant women. The MBG standard compound is currently not commercially available nor is it synthesizable. It forced us to develop an effective extraction and purification method of MBG from freshly collected and crystallized toad Bufo Marinus venom. A LC-MS based assay designed to determine MBG in human plasma is being optimized, giving us the opportunity to investigate MBG in non-pregnant healthy volunteers plasma as well as in early pregnant women plasma. A pre-extraction procedure was elaborated to concentrate and clean the plasma sample prior to its analysis. Pure MBG has been successfully extracted from the Bufo Marinus toad venom and the identity of the compound has been confirmed by different spectral techniques. The purification procedure is being optimized for standardization. The designed assay for plasma MBG determination is based on a solid phase extraction procedure preceding a LC-MS/MS measurement using 2 mass transitions. Preliminary results allowed us to authenticate the presence of MBG in non-pregnant women as well as in early pregnancy. Further optimization and validation of the LC-MS assay are needed to quantify MBG plasma levels. However, these pioneering observations, are giving the clinicians a promising perspective for early preeclampsia risk assessment in pregnant women.

O-36
CHINESE HERBAL FORMULA FOR PSORIASIS VULGARIS: A REPORT OF SERIES STUDIES

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We conducted series studies to assess the effectiveness and safety of Chinese herbal formula PSORI-CM01 for psoriasis vulgaris (PV), and indicate mechanisms and ingredients of the formula. An observational study, experimental study and a pilot randomized controlled trial was performed. To define the chemical profiles and control the quality of PSORICM01, UHPLC-ESI-LTQ/Orbitrap-MS method was utilized for simultaneous identification and quantification of multiple ingredients. A metabolomics approach was also used to investigate the global biological characterization of the patients and the therapeutic metabolomics mechanism of the formula. Finally, a pilot study was conducted to assess the effect of PSORI-CM01. In the observational study, 22 PV patients were observed before and after treatment of PSORI-CM01. There was a reduction of 8.7 at PASI score after 2 months compared to baseline. A total of 108 compounds were identified by the UHPLC-ESI-LTQ/Orbitrap-MS method. In metabolomics study, a total of 41 psoriasis patients and 19 healthy volunteers were enrolled. The metabolic variations visualized not only between the healthy group and psoriasis group, but also between the psoriasis group before and after 12 weeks of treatment with the formula. The pilot study enrolled 32 PV patients and randomly allocated to an experimental group (n=15) and a placebo group (n=17). The experimental group showed a positive trend to get the 50% improvement of PASI than control group and had a longer maintenance efficacy. Two adverse events were reported. The formula PSORI-CM01 was effective for patients with PV. The results indicated metabolomics mechanism and basis of ingredients of the formula.

O-37
BEIJING UNIVERSITY OF CHINESE MEDICINE: A MAIN FORCE OF TCM PRESERVATION, INNOVATION AND PROMOTION
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Beijing University of Chinese Medicine (BUCM) was founded in 1956, and is one of the earliest established traditional Chinese medical institutions for higher education in the People’s Republic of China. Today, BUCM has 10 schools with 2,462 staff and 27,833 students, 3 affiliated hospitals with 1960 medical staff and equipped with 2200 beds average daily out-patient capacity is over 7000 each. Scientific research funding is over $16.5 million per annum to promote the TCM leading areas in disease prevention and treatment (cardio-cerebral vascular disease, encephalopathy, tumor, diabetes, pain, etc.), the TCM herbs/formulae fundamental research and development of new herbal medicine. BUCM has established itself as a leader in the field of TCM culture promotion worldwide and international cooperation. The university collaborates with 103 international institutions from 29 countries and regions in areas of TCM education, scientific research and medical treatment. It’s also the earliest Chinese Medicine Institution to accept international students in China, and set up the first overseas Chinese medicine hospital in 1991 at the south-east part of Germany. Multiple overseas TCM centers are set up recently as well: St. Petersburg TCM Center, partner with St. Petersburg Water Group and Pavlov State Medical University of St. Petersburg and officially certified by the Traditional and Ethnical Medicine Committee of Russia; Sydney TCM Center, partner with University of Western Sydney (UWS), Australia – the collaboration agreement was signed in Canberra on Nov. 17, 2014, in the presence of President Xi Jinping, People’s Republic of China and Prime Minister Tony Abbott, Australia; Washington TCM Center, partner with Medical School of Georgetown University, United States. Additionally, a Journal of Traditional Chinese Medicine Science (in English) is published to facilitate international academic communication. All of these efforts make BUCM ranking first among Chinese Medicine universities for 8 consecutive years in aspect of TCM international cooperation. In her nearly 60 years journey, BUCM preserves Chinese medicine as a vital part of traditional Chinese culture and continuously strives for further development of Chinese medicine in both prevention and treatment of diseases. Along with China’s “The Belt and Road Initiatives” now, BUCM is expanding its international cooperation to maximum TCM’s contribution to the health of human beings.

O-39
TRADITIONAL HERBAL MEDICINES IN LITHUANIA

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Lithuania is an Eastern European country with 60 miles of sandy coastline to the open Baltic Sea. The country can be divided into 4 ethnographic regions with specific nature scenery, soil, traditions and linguistic dialects. Lithuania experienced many political changes through the centuries leading finally to regained independence and EU membership in 2004. Geographical, climatic conditions, cultural and political changes strongly influenced the traditional use of medicinal plants growing wildly or being cultivated locally. Collection, growing, storing and preparation of medicinal plants is included in the officially approved list of Lithuanian law of Traditional Crafts. The most reliable written sources about traditionally used medicinal plants date back to the second part of XIX century. The analyses of more than 300 bibliographical sources revealed the number of medicinal plants used since 1873. The number of medicinal plants decreased from 380 in 1873 to 141 in 2013. While analysing the use of medicinal plants during different historical periods, the biggest decline was observed since 2004 when Lithuania joined the EU. This

O-38
AN ORIGINAL DIAGNOSTIC SYSTEM FOR MALARIA TO SCREEN THE ANTIPLASMODIAL ACTIVITY OF AFRICAN HERBAL MEDICINES

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Malaria, caused by protozoa of the genus Plasmodium, causes some 198 million of confirmed cases and about 584,000 deaths per year. Its management needs both accurate diagnostic and treatments availability. The current diagnostic techniques (rapid diagnostic tests and microscopy) appear either too expensive, slowly sensitive or difficult to implement. The hemozoin (“malaria pigment”) is currently considered as a possible marker to diagnose the disease. A fluidic device is developed in our laboratory to exploit the paramagnetic susceptibility of hemozoin for its isolation from biological matrixes; detection is ensured by a photometric system based on a 405 nm monochromatic led and photosensor. Optimization and validation of the device is on-going; the current prototype, can reliably and consistently detect 0.05 g/mL of hemozoin, corresponding to 80 parasites/μL, lower than the 200 parasites/μL limit recommended by the World Health Organization. Since the biosynthesis of hemozoin is a way of heme detoxification, overcoming this process leads to enhance toxicity against Plasmodium species. Thus we have developed a TLC bioautographic assay to detect antimalarial compounds acting by the inhibition of hemozoin formation for the screening of natural sources.

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can be explained by a more stringent legislative framework applicable to traditional herbal medicines and higher quality and safety standards. At the same time the number of herbal food supplements increased dramatically. The most frequently used traditional medicinal plants in Lithuania are Mentha × piperita L. and Valeriana officinalis L. They are followed by 12 other plants: Althaea officinalis L., Arctostaphylos uva-ursi (L.) Spreng., Artemisia absinthium L., Atropa belladonna L., Glycyrrhiza glabra L., Juniperus communis L., Matricaria recuita L., Mentha infausta L., Quercus spp., Rheum palmatum L. /Rh. officinale Baillon, Salvia officinalis L. and Taraxacum officinale Web. Most of those plants are grown locally and are present in herbal medicines available at the moment on the Lithuanian market.

O-40
RECOGNITION OF THE HERBAL PHARMACOPOEIAS OF THE FRENCH OVERSEAS REGIONS AND COLLECTIVITIES

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French overseas departments and territories are known for their rich and diverse tropical florae, associated with traditional health knowledge, skills, and practices. Many people find necessary to further highlight this still very much alive cultural heritage, because transmission to future generations is not assured. Official recognition of the medicinal character of major local medicinal plants, and in particular inclusion of these plants on the "List of Traditionally Used Medicinal Plants", a list which is part of the French official Pharmacopoeia, is one of the measures recommended by several overseas organizations of scientists and health professionals. Until recently, very few medicinal plants of the French overseas regions were included on this list, but a special focus of the experts of the French Pharmacopoeia Committee for Medicinal Plants & Essential Oils on the medicinal floras of Martinique, Guadeloupe, La Réunion and French Guiana allowed the inclusion in recent years of over 60 medicinal plants on this list. The presentation will first describe a few characteristic features of the traditional practices and health resources of the communities of the French overseas regions (Martinique, Guadeloupe, French Guiana, La Réunion, New Caledonia and French Polynesia). The integration process of the medicinal plants of these regions in the French Pharmacopoeia will be the focus of the second part of the presentation. In addition to the importance of the cultural recognition of these traditions, it is also important to note that the integration process involves economic issues that may contribute to the development and marketing of herbal health products by local entrepreneurs.

O-41
RESEARCH OF ANTIMICROBIAL AND RESISTANCE MODIFYING AGENTS FROM BENINESE PLANTS USED IN TRADITIONAL MEDICINE

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In a world of increasing resistance to current antibiotics, search of novel therapeutic options is urgently needed. The aim of this work was to screen plant crude extracts for direct or indirect (inhibition of resistance) antimicrobial activity. Four crude extracts from 12 plants traditionally used in Benin for the treatment of infections were obtained by successive extraction with hexane, dichloromethane, ethyl acetate, and methanol. Staphylococcus aureus MRSA ATCC33591 (resistant to ß-lactams by production of ß-lactamase and of an additional ß-PBP2a) with low affinity for most ß-lactams) and SA1 (resistant to quinolones by overexpression of the efflux pump NorA) were used as test organisms. Direct antimicrobial activity was tested by determination of Minimal Inhibitory Concentrations (MIC). Indirect activity was screened by looking for interaction between antibiotics and extracts using checkerboard titration and calculation of Fractional Inhibitory Concentration Index (FICI). Combined antibiotics were ampicillin (resistance due to ß-lactamases and ß-PBP2a), oxacillin (resistance due to ß-PBP2a only), norfloxacin (substrate for efflux by NorA) and moxifloxacin (poor substrate of NorA). Synergy was defined by a FICI ≤ 0.5 and additivity, by a FICI = 1. The methanol extracts of Vitellaria paradoxa and Cola gigantea leaves and stem barks were additive or synergistic with oxacillin and ampicillin against MRSA ATCC33591 (FICI: 0.28 ≤ 1), suggesting a possible inhibition of ß-PBP2a. The methanol extract of Topinanthus bangwensis aerial parts improved only the activity of ampicillin (FICI 0.3751), suggesting ß-lactamase inhibition. The methanol extract of Holarrhena floribunda aerial parts and the dichloromethane extract of Spirosernum penduliflorum aerial parts increased the activity of norfloxacin against strain SA1 (FICI 0.375-1) without modifying that of moxifloxacin, which suggests the presence of compounds inhibiting the efflux pump NorA. Ongoing work aims at identifying the active compounds in these extracts.

O-42
Study on Bioactive Compounds with Novel Structures from Toxic Plants in China

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Natural products play an important role in the discovery of leads for the development of drugs for the treatment of human diseases. In China, much of nature sources remain to be explored, particularly, the toxic plants, which leave no doubt that a host of novel, bioactive chemotypes await discovery. There are more than 900 species of toxic plants in China. Recently, the bioactivities of extracts of over 200...
important toxic plants were investigated in our group. It was found that more than 30 toxic plants showed cytotoxic activities, anti-inflammatory activities and vasodilator activities, of which 20 toxic plants were further studied by bioassay-guided technique. From these plants, more than 1200 compounds were isolated, including 15 new skeleton compounds and more than 350 novel compounds, of which more than 80 compounds exhibited significant bioactivities (IC50: 10-6-10-8) to different targets. Moreover, relationships between structure and function of some new natural products with potent activities were investigated. It lays a foundation for study on innovative drugs and elucidation of the bioactive substances of toxic plants.

O-43
TRADITIONAL CHINESE MEDICINE AND MODERNIZATION “NOBLESSE OBLIGÉ”

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Comprehensive overviews of the EU regulatory system become more and more well-known, also outside Europe and regulatory knowledge and experience is increasing among Chinese companies. Nevertheless, possible implications for TCM in the light of “modernization”, should remain in the center of discussion. In this presentation some scenarios from a regulatory point of view and for the European situation will be explored, taking advantage of the academic platform at the University of Mons. At one side, some encouraging developments and achievements are identified regarding modernization of TCM, as summarized above. On the other hand, some open pathways are still badly used, or only slowly progress. This lecture will focus on possibilities to increase the flow of experience, to increase the number of real applications and which factors in the European system could endorse this. With strictly personal reflections on the role of regulators, scientists, Herbal committees, pharmaceutical companies, and European decisions made in London, in Strasbourg, in Brussels, and not at least, in China.

O-44
ARISTOLOCHIC ACID NEPHROPATHY: AN UPDATE

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Aristolochic acid nephropathy (AAN) is a rapidly progressive interstitial nephritis leading to end-stage renal disease and urothelial malignancy. It was originally reported in Belgium in a group of more than 100 patients who had ingested slimming pills containing powdered root extracts of a Chinese herb, Aristolochia fangchi. Although botanicals known or suspected to contain aristolochic acid (AA) are no longer permitted in many countries, several AAN cases are regularly observed all around the world. The incidence of AAN is probably much higher than initially thought, especially in Asia and in the Balkans. In Asian countries, the complexity of the pharmacopoeia represents a high risk for AAN because of the frequent substitution of the botanical products by AA-containing herbs. In the Balkan regions, the exposure to AA found in flour obtained from wheat contaminated with seeds of Aristolochia clematitis could be responsible for the so-called Balkan-endemic nephropathy. Finally, despite the Food and Drug Administration’s warnings concerning the safety of botanical remedies containing AA, these herbs are still sold via the Internet. Clinically, except some reported cases of acute renal failure secondary to tubular necrosis, the initial presentation of AAN is usually silent and renal failure is discovered by routine blood testing. However, a markedly increased urinary excretion rate of low molecular weight proteins is early observed, reflecting the fact that the proximal tubular cells are the main target of AA. In vitro studies confirmed this point. Microscopically, an extensive interstitial fibrosis with atrophy and loss of proximal tubules is the predominant lesion. An inflammatory cell infiltrate may also be seen in the interstitium. Urothelial abnormalities are very often present (atypias, hyperplasia) in relation with the high carcinogenicity of AA. In end-stage AAN patients currently treated by dialysis or renal transplantation, we found that 40-45% of them displayed multifocal high-grade transitional cell carcinomas, mainly in the upper urinary tract. In nephropturectomy pieces, the detection of DNA adducts specific to AA metabolites (aristolactams) is still possible even 20 years after exposure to AA. An overexpression of p53 protein has been regularly observed in tumor samples as well as a specific AAG-to-TAG mutation in codon 139 (Lys →Stop) of exon 5 in p53 gene, probably triggering the tumorigenesis process. Moreover, kidney recipients for end-stage AAN are at high risk of bladder cancer and require a regular follow-up by cystoscopy. In such patients suffering from non-muscle invasive bladder carcinoma after renal transplantation, Bacille Calmette-Guerin (BCG) therapy represents an effective option under specific conditions. Experimentally, rat and mouse models of AAN have been developed in the lab. Up to now, attempts to counteract AA induced renal toxicity (such as angiotensin-converting enzyme inhibitors or human bone morphogenetic protein-7) have been unsuccessful. A better understanding of the pathophysiological AA-induced mechanisms should help to develop strategies susceptible to stop or -at least-to slow down the progression of renal lesions.

O-45
UPLC-QTOF-MS FOR METABOLITES IDENTIFICATION IN CACO-2 CELLS OF BENZYLISOQUINOLINE ALKALOIDS IN NELUMBINIS PLUMULA

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The aim of the study was to identify main metabolites of benzylisoquinoline alkaloids from Nelumbois Plumula after biotransformation by Caco-2 cells. Caco-2 cells were seeded to a 6-well plate and cultured for a period of time until 80% of each well was filled with cells. Then, cell medium was replaced and the norcoclaurine, liensinine, isoliensinine and neferine were respectively added to the wells with final concentration of 100 μM. After incubation of 3, 6 and 12h respectively, Caco-2 cells were harvested and washed twice with ice-cold PBS. Through repeated freeze-thaw and ultrasonication, samples were homogenized with 200μL methanol and then centrifuged at 15000 rpm for 20 minutes. The supernatant was then collected and injected for UPLC-QTOF-MS analysis. The structures of the metabolites were elucidated by molecular masses, retention times, MS and MS/MS spectra comparing with those of the parent drugs. Results: The procedure identified that the major metabolites of norcoclaurine are methyl-norcoclaurine and norcoclaurine-glucuronide, the major metabolite of liensinine is dimethyl-liensinine, the major metabolite of isoliensinine is demethyl-isoliensinine, the major metabolites of neferine are liensinine, isoliensinine and their further demethylation products. After biotransformation by Caco-2 cells, metabolites of norcoclaurine, liensinine, isoliensinine and neferine were identified. Methylolation, demethylolation and glucuronidation were the main metabolic pathways. UPLC-QTOF-MS provided a simple, rapid and sensitive way for identification of metabolites of benzylisoquinoline alkaloids from Nelumbois Plumula by Caco-2 cells.

O-47
CHEMISTRY AND BIOACTIVITY OF POLYSACCHARIDES FROM TRADITIONAL CHINESE MEDICINE MAHUANG

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The long-term clinical practice of traditional Chinese medicine (TCM) confirms its importance and essential role in the health care system in China, especially in the prevention and treatment of chronic diseases. TCM is the holistic medicine under the guidance of system theory, emphasizing harmony between human and nature, focusing on equilibrium and balance, and focusing on state of functional system and normal function of the human organism viewing it as the integral entity. The theory of TCM property is one of the basic theories of the science of TCM. It is the connection between the TCM theory and the clinic. The interpreting of the scientific meaning of TCM property is one of the critical problems for the modernization process of TCM. Nowadays, though a large number of researchers are engaged in the study of TCM, some are deviating from unique characteristics of Chinese medicine. With the developing of their research work, people come to realize the importance of TCM characteristics, and begin to lay more emphasis on its special clinical effect, rational theory system and rich practice experience. Otherwise, the essential character of TCM cannot be fully and correctly explained. It is essential to clarify the role of TCM theory, principles of TCM and material basis. In a manner of speaking, this is an effective way to develop TCM industry in the direction of TCM theory with the idea of modern scientific technology. This leads us to conclude that Chinese medicine is the subject area with the most potentiality and the possibility for original innovation. Next, I will introduce our research practices, “Mahuang” and “Yangjinhua”. Ephedrae Herba (Mahuang) is a Chinese herbal medicine with a long history of application in China. Amphetamine alkaloids in Mahuang were recognized as major active components due...
to various medicinal effects for a long time. However, there is still more to confuse us about its nature and flavour as well as efficacies and others. In combination with ancient Chinese literatures, it indicated the existence of “cold” and “bitter” on nature and flavor as well. Our studies have showed that the main source of “bitter and cold” material basis on nature and flavour of Mahuang was the polysaccharide component rather than the alkaloid component. The immunologically pharmacological experiments in vivo and in vitro have proved that Mahuang polysaccharides had the obvious immunosuppressive function. The finding of immunosuppressive activity of Mahuang polysaccharides further deepens the understanding of Mahuang efficacy. Mahuang polysaccharides could be a therapeutic candidate for some autoimmune diseases.

O-48
NEW STRATEGIES FOR THE CONTROL OF BACTERIAL INFECTIONS: MODULATORS OF QUORUM SENSING AND BIOFILM FORMATION FROM MALAGASY DALBERGIA SPECIES

Tsiry Rasamiravaka, Pierre Duez and Mondher El Jaziri

Considering the WHO warning about the emergence of a ‘post-antibiotic’ era during the 21st century in which common infections and minor injuries will have a dramatic impact on human death toll, search for new potential antibacterial drug targets became a necessary need. Targets that are extensively explored concern the modulation of bacterial quorum sensing (QS) mechanisms which are key regulators of virulence factor expression and biofilm formation with the putative advantage to generate less selective pressure as compared to antibiotics. Indeed, this approach does not kill bacteria but could render them less virulent and unprotected enough to be easily eradicated by immune defence. Madagascar is a huge pool of endemic plant with known and unknown medicinal properties. Four Malagasy endemic Dalbergia species have been screened for their anti-virulence activity and eight extracts were found to inhibit P. aeruginosa PAO1 QS mechanisms. Interestingly, extracts of Dalbergia trichocarpa bark have been shown to disrupt specifically QS and biofilm formation. One of the active compounds has been isolated and identified as oleanolic aldehyde coumarate (OALC) which inhibits the production of QS-dependent virulence factors and biofilm formation in P. aeruginosa. Regarding the mechanism of action, this novel bioactive compound has been shown to inhibit the expression of the las and rhl systems and the global activator gacA which consequently inhibit the production of QS-controlled virulence factors including, pyocyanin, elastase and rhamnolipids as well as the production of extracellular polysaccharides. Furthermore, OALC disorganizes established biofilm structure and improves the bacterial activity of tobramycin against biofilm-encapsulated PAO1 cells. Finally, a significant reduction of Caenorhabditis elegans paralysis was recorded when infected with OALC-pre-treated P. aeruginosa, as compared to non-treated control groups. Taken together, these results evidenced triterpenoid coumarate esters as suitable chemical backbone to target bacterial virulence mechanisms. Besides, this finding demonstrated the potent source for novel and unrevealed anti-virulence compounds of Malagasy flora and the potentiality of this approach to strengthen our antimicrobial therapeutic arsenal with the ongoing struggle against bacterial infection.

O-49
INNOVATIVE BIOLOGICAL AND CHEMICAL STUDIES OF HERBAL MEDICINES APPLICATION OF MASSLYNX AND UNIFI PLATFORM SOLUTION TO ACCELERATE THE IDENTIFICATION OF INGREDIENTS AND THEIR METABOLITES OF TCM

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Traditional Chinese Medicine (TCM) has been used in prevention and treatment of disease in clinical practice for thousands of years, with an indispensable role of multiple ingredients. Thus, a rapid and effective chemical ingredients analysis was of necessary to be established for the evaluation of the holistic quality of TCM. As could afford the data with high resolution and high sensitivity, UPLC/Qtof MS has been widely used for the analyses of complex samples, including herbal medicines and even TCM formula. However, no significant breakthrough has been made in terms of the overall workflow of chemical recognition. Currently, typical LC-MS based workflow still includes manually observing the chromatographic peaks individually, searching possible structural information from various internet libraries, and checking literature to rationalize MS fragmentation pattern of the target component. The author will introduce some case studies on the identification of ingredients and their metabolites of TCM with some new chemical ingredients screening and identification strategy based on UPLC/Qtof-MS coupled with the application of Masslynx and UNIFI informatics platform.

O-50
SPHINGOLIPIDOMIC AND CYCLOPEPTIDOMIC STUDIES ON CORDYCEPS

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Cordyceps is a precious Chinese medicine with various pharmacological activities. In 1995, a potent immunosuppressive active sphingolipid named myriocin was isolated from the culture broth of *Isaria sinclairii*. FTY720, a sphingolipid analogue modified from myriocin, has been developed into an important oral drug for the treatment of multiple sclerosis and organ transplantation. In 2005, cordycepinpeptide A, a cyclopeptide with strong cytotoxicity, was isolated from the culture solution of Cordyceps. These studies collectively demonstrated that sphingolipids and cyclopeptides represent active components of Cordyceps. Objective: To provide chemical evidence for pharmacological effects, quality control and rational exploration of Cordyceps and mycelium preparations. Methods: Deep profiling of sphingolipids and cyclopeptides in Cordyceps was carried out by using UHPLC-Q-TOF MS. Quantitative profiling of sphingolipids was achieved by using UHPLC-QQQ MS. Results: Using the improved sphingolipidomic approach, 464 sphingolipids were identified in Cordyceps based on accurate mass and high-resolution MS/MS. Additionally, 185 sphingolipids in Cordyceps and mycelium preparations were quantitatively analysed. Multivariate analysis was performed to deal with quantitative results, revealing significant difference between sphingolipidome of wild Cordyceps and mycelium preparations. For the profiling of cyclopeptides, 11 cyclopeptides were identified in Cordyceps and 44 cyclopeptides were found in mycelium preparations. Conclusion: Our study represents the most comprehensive profiling of sphingolipids and cyclopeptides in Cordyceps so far. The results revealed remarkably diversified sphingolipids in this precious herbal medicine. Given the increasingly appreciated biological activity, sphingolipids and cyclopeptides can be regarded as the most promising active components responsible for the beneficial effects of Cordyceps and its related products.

O-51
CHALLENGES AND OPPORTUNITIES OF CLINICAL STUDY IN CHINESE MEDICINE

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Clinical trials are experimental studies done in clinical setting. Such prospective biomedical or behavioral studies on human beings are designed to answer specific questions about interventions, including new treatments and known interventions that warrant further study and comparison. Starting from 1980s, more and more of Chinese herbal medicines have been put into the frames of clinical trials to test its efficacy and safety, and currently, those of results attracts many attentions from main stream of medicine. Based on analysis of trials with Chinese herbal medicine published in PubMed, we found that many of the trials tested the efficacy and safety of a kind of Chinese herbalmedicine, which is very exciting. On the one hand, it is crucial to assess its efficacy and safety in a strict approach, thus to demonstrate whether this herbal medicine is effective or not. From this point of view, we believe it is necessary to increase the alert about the following points of the trials with Chinese herbal medicine, protocol design, quality control of herbal medicine, implementation and good publication practice with Chinese herbal medicine. On the other hand, some of points may deserve further consideration in the future. Firstly, Chinese medicine is a broad range of medicine practices adapting the holistic approaches which may include various forms of herbal medicine, acupuncture, massage, exercise (qigong), and dietary therapy, etc. The treatment that practitioners provide to patients, may not only a formula, but a systematic remedy, which may include a formula of herb, a course of acupuncture treatment, a recommendation about exercise, food, and emotion modulation, etc. The combination of these therapeutic elements takes effects based on the synergistic effects among each other. Secondly, the trial always focuses on the intervention itself during the fixed experimental periods. The interesting thing is that although researches try very hard to minimize the difference among groups of participants, those factors still have the effects on the trial results, especially including the activities inside and outside the participants. From this point of view, the trial, especially focusing on a single intervention, should be reviewed critically. Thirdly, the experts in trials always want to isolate participates from their real life, to put them in a “Vacuum” condition, which does not match with their real life. From these points of view, it is necessary to assess the efficacy of Chinese medicine in a real holistic approach. A new approach to assess the efficacy and safety of Chinese medicine may need to be developed, which reflects the nature of Chinese herbal medicine.

O-52
FROM DANGGUI LONGHUI WAN TO BIOACTIVE INDIRUBIN ANALOGUES

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Indirubin, indigo and isoidigo are the core representatives of a rather small category of bisindole alkaloids referred to as indigoids. These compounds are the coloured constituents of the natural dyes Indigo and the famous molluscian Tyrian purple, used throughout the centuries for textile dying. In contrast with Indigo dyes, the major constituents of molluscum purple dyes are brominated indigoids. Nowadays, the use of natural dyes is very limited due to their replacement with cheaper synthetic dyes. Nevertheless, indigoids and especially indirubins have come to the timeliness due to the vast range of biological activities, which in many cases have their origin in traditional medicine. Chronic myelotic leukemia has been treated from the traditional Chinese medicine with the recipe Danggui Longhui Wan, a mixture of 11 herbal medicines. Eventually, the antileukemic activity was attributed to indirubin, which was detected in the mixture
as a minor constituent. During the following years, indirubin and its halogenated analogues were found to possess a vast range of biological effects in stem cells, cardiac, renal, and pancreatic cells, even in parasitic organisms. In addition, brominated indirubins have been utilized as tools for the exploration of neurodegeneration and cancer. In most of the cases, all of the above effects can be associated with the interaction of indirubins with important molecular targets such as members of the family of protein kinases, placing them among the most promising nature-derived drug candidates.

O-53
THE USE OF TRADITIONAL CHINESE MEDICINE FOR IN VITRO FERTILIZATION IN CHINA: CLINICAL EVIDENCE

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In vitro fertilization (IVF), as an important assisted reproductive technology (ART), is chosen by over 1,000,000 infertile couples each year, which is linked to over 3,000,000 babies born worldwide. However, an IVF cycle may be unsuccessful. The success of IVF is not guaranteed, and patients often have to undergo more than one cycle of treatment before they are successful. IVF treatment may lead to anxiety and ectopic pregnancy. 3-5 unsuccessful IVF cycles might negatively affect the reproductive endocrinology of the IVF women. In recent years, many infertile couples have chosen of Traditional Chinese Medicine (TCM) as an adjunct when they undergo IVF. However, the current evidence makes it difficult for clinical practitioners and patients to make a decision on whether to choose TCM as an adjunct when undergoing IVF treatment. Transcutaneous electrical acupoint stimulation (TEAS) is a new development in traditional electro-acupuncture. In the TEAS treatment, the skin electrodes are placed on the acupoints of the patients, instead of piercing the skin with the traditional acupuncture needles, which can also increases the reproducibility of the acupuncture-like stimulation. In a prospective, randomized and controlled study (the trial was registered at Chinese Clinical Trial Register (ChiCTR) with the identifier ChiCTR-TRC-14004493, http://www.chictr.org.cn/proj/show.aspx?proj=7934), we found that TEAS using a frequency of 2/100Hz could help to reduce the anxiety levels associated with IVF and to improve the IVF outcomes, which provides an alternative treatment to alleviate the anxiety levels of the infertile women undergoing IVF. Auricular acupressure (AA), a micro-acupuncture technique similar to reflexology, is effective in reducing state anxiety in both healthy volunteers and the adult patients awaiting their surgery. It was also effective in improving functions of the endocrine system. It can be easily accepted by people for its effectiveness and non-invasion. In the prospective, randomized and controlled study (the trial was registered at Australian New Zealand Clinical Trials Registry (ANZCTR) with the identifier ACTRN 1261100899943, http://www.ANZCTR.org.au/ACTRN126-11008999943.aspx), AA was performed by using acupressure seeds named wáng bù liú xìng (Vaccariae Semen). The seed was kept in place by a piece of patch and fixed onto the acupoint selected. The subjects were asked to press the acupoints four times a day (08:00, 12:00, 16:00 and 20:00 h respectively) with 15 min each time by themselves. The AA was conducted on the two ears simultaneously, which consisted of six days (from 12:00 h of one day before TVOR to 20:00 h of the next day of ET). We found that AA could help to reduce anxiety levels associated with IVF and improves the outcomes of IVF partly through increasing the levels of NPY in the follicular fluids. More TCM theory based, large-size, randomized and multicenter trials should be conducted before a final decision is made on whether to choose TCM to improve IVF outcomes in clinical practice.

O-54
DESIGNING A FEASIBILITY STUDY ON THE USE OF ACUPUNCTURE FOR PHANTOM LIMP PAIN

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People with diabetes are 30 times more likely to undergo amputation than the general population. A total of 3.2 million people in the UK have been diagnosed with diabetes and by 2025 this number is estimated to be 5 million. One potential complication post amputation is phantom limb pain (PLP), a prevalent and often chronic condition which is difficult to treat. To inform a feasibility study on acupuncture for PLP, a mixed methods approach was adopted using the MRC framework for developing and evaluating complex interventions. A systematic review of randomised controlled trials and a narrative synthesis of case series / reports were carried out using Chinese, Korean and English databases. Online Delphi technique, designed using 2 case studies, was carried out to develop consensus with registered acupuncture practitioners on PLP treatment guidelines. Qualitative semi-structured interviews with in-patient amputees at a rehabilitation unit assessed acceptability of acupuncture intervention and identified outcome measures which could be used in a feasibility study. The systematic review identified only two poor quality non-randomised controlled trials. The narrative synthesis identified 28 acupuncture case series / reports using a variety of different treatment approaches. The Delphi study obtained consensus on a basic protocol which could be used in a subsequent trial. Interviews captured amputees lived experience of PLP, their need to address symptoms and identified that acupuncture and participating in a feasibility study would be acceptable. Seven appropriate outcome measures were identified. The study phases described above helped to design a feasibility embedded mixed methods research study (randomised controlled trial and semi structured interviews. The trial has been registered (ClinicalTrials.gov: NCT02126436) and the protocol published. The trial commenced in October 2014,
has currently recruited 11 participants and is anticipated to complete by October 2015.

O-55
DENSE CRANIAL ELECTROACUPUNCTURE STIMULATION FOR MDD: A PET/CT RANDOMIZED CONTROLLED STUDY

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The purpose of this study was to determine the effectiveness of Dense Cranial Electroacupuncture Stimulation (DCEAS) as additional therapy and neuroimaging correlates in patients with major depressive disorder (MDD) using PET-CT. A single-blind, randomized, controlled trial was conducted in 25 MDD patients treated with antidepressants combined with sham or active DCEAS with 3 sessions per week for 6 weeks. Clinical outcomes were measured using the Hamilton Depression Rating Scale (HAM-D-17), Zung Self-Rating Depression Scale (SDS), Clinical Global Impression-Severity Scale (CGI-S) and Insomnia Severity Index (ISI). 18F-FDG PET/CT and fMRI brain scans were conducted at baseline and endpoint. A total of 23 subjects (12 in sham and 11 in DCEAS) were included in PET study with linear mix model analysis. There was a significant difference in favor of DCEAS on the slope in SDS (P < 0.035). While glucose hyperactivity in the cerebellum was normalized in both treatment groups, the reversion of the reduced glucose activity metabolism in the left prefrontal cortex was only observed in DCEAS-treated patients (P < 0.001 uncorrected). Additional treatment with DCEAS could more vigorously improve brain glucose metabolism in MDD (the study was supported by GRF: 786611).

Posters

P-56
STUDY ON THE TISSUE DISTRIBUTION OF COMPOUNDS AND METABOLITES OF THE MONGOLIAN MEDICINE DIGEDA-4 DECOCTION IN ACUTE LIVER INJURY INDUCED BY D-GaIN

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The objective of this study was to study the distribution characteristics of Mongolian drug Digeda-4 decoction in rats with acute liver injury. The Mongolian drug Digeda-4 decoction was administered intragastric in rats with acute liver injury induced by D-GalN. The removal of the Liver, spleen, lung, kidney and heart, 10% tissue homogenate was prepared with physiological saline, remove the protein, the moving elements were analyzed by LC-MS and NMR of Liver, spleen, lung, kidney and heart, identified it structure.

Three compounds were detected in liver, including 2 prototype components of swertiamarin and androsin, and a new metabolite; 7 compounds were detected in spleen tissue, including 4 prototype components and 3 new metabolites; 6 compounds were detected in kidney tissue, including 3 prototype components and 3 new metabolites; 2 compounds were detected in lung tissue, all prototype components; no compounds were detected in heart tissue. Detected prototype components include swertiamarin, androsin, β-rhamno-epinapside, picroside, and hexadecanoic acid.

The compounds from the Mongolian drug Digeda-4 decoction are widely distributed in the liver, spleen and kidney, to a lesser extent in the lung but not in heart. The results indicate that the drug not only has curative effect on liver disease but may also have therapeutic effects on spleen and kidney diseases, with potential interest for further development.

P-57
CONTENT DETERMINATION OF HYPERIN IN RHODODENDRON BY HPLC

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As one of the cultural heritages of the nation, Mongolian Medicine was formed and developed in the long-term medical practice of the Mongolian people, and has become a major constituent part in the Traditional Chinese Medicine. In the past, Mongolian medicine made great contributions to the living and breeding of generations of the Mongolian people and has a positive impact on the formation of the civilization in the northern China. The Mongolian medicine, Rhododendron, is the dried flower of Rhododendron micranthum. It has functions of eliminating phlegm and stopping cough, revitalizing and nourishing. In order to control the inherent quality of RSP and to ensure its clinical efficacy, we establish a HPLC method for content determination of hyperin in Rhododendron. Using Diamonsil C18 (250mm×4.6mm, 5um), the mobile phase was acetonitrile-0.5% phosphoric acid solution (15:85), with the flow rate of 1.0 ml/min, column temperature of 30°C and the detection wave at 360 nm, the sample size was 10 μL. A good linear relationship was obtained between the peak areas and the concentrations of hyperin in the range from 0.0575 to 0.408 μg (r=0.9997), the mean recovery rate was 98.41% (n=6), RSD=1.54%. This method is convenient, rapid, accurate, and can bring about good recovery; it can be used for content determination of hyperin in Rhododendron.

P-58
IN VITRO AND IN VIVO EVALUATION OF THE SAFETY OF SOME BENINESE PLANTS USED IN TRADITIONAL MEDICINE

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About 80% of population in developing countries use traditional remedies in their usual health care and plants used in traditional medicine are an interesting alternative to expensive and hardly available modern medicines, mainly in rural areas. Moreover, they are a promising source of new drugs structurally innovative. Therefore it is important to investigate their biological properties and we focused on 5 beninese plants: *Byrsocarpus coccineus* Schumach. & Thonn. (Connaraceae), *Carpolobia lutea* G. Don (Polygalaceae), *Holarrhena floribunda* T. Durand & Schinz (Apocynaceae), *Keetia leucantha* (K. Krause) Bridson and *Keetia venosa* (Oliv.) Bridson (Rubiaceae).

In order to validate their safety, we evaluated toxicity of dichloromethane extracts and also aqueous decocations being the major formulation traditionally used, in vitro on two cellular strains, WI38 and J774, and in vivo on female NMRI mice according to the highest tolerated dose model. All lipophilic extracts and aqueous decocations showed a low cytotoxicity on both strains (IC50 >35 and 100 µg/ml respectively) and no toxic signs with a total cumulative dose of 800 mg/kg (i.p. and p.o. respectively). These results provide evidence of their safety in acute model and the different extracts will be investigated in vitro/ in vivo to validate their traditional uses, especially anti parasitic ones for most of them.

**P-59**

**PHENOLIC PROFILE, ANTIBACTERIAL, NEUROPROTECTIVE AND CYTOTOXIC PROPERTIES OF THREE ALGERIAN MENTHA SPECIES**

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This study was designed to evaluate the antibacterial (direct effect and reversal of antibiotics-resistance), neuroprotective (acetylcholinesterase and carboxylesterase inhibition) and cytotoxic (MTT assay on 2 human cancer cell lines) properties of three Mentha species [M. pulegium L. (MP), *M. rotundifolia* (L.) Huds (MR), and *M. spicata* L. (MS)] harvested in Bejaia (Algeria). The phenolic compounds were characterized by HPTLC and HPLC and quantified using colorimetric assays. Results and conclusions: The extracts of MP and MS showed inhibitory activity against *Staphylococcus aureus* C 100459 with MICs values of 0.5 and 1.0 mg/mL, respectively. An additive effect was found between MS extract and streptomycin, whereas indifference effects were observed for the other tested combinations. The MP extract showed high neuroprotective and cytotoxicity effects compared with those of MS and MR despite the fact that (i) the MS extract showed the highest total phenolics content (26.4 ± 1.2 mg GAE/g DM; n = 3); and (ii) the MR extract harbored the highest flavonoids content (3.8 ± 0.1 mg QE/g of DM; n = 3). Phenolic acids identified in analyzed species were rosmarinic, caffeic, ferulic, and chlorogenic acids; while detected flavonoids were rutin, apigenin, naringenin, kaempferol, and quercetin. The overall results indicate that the studied plant extracts might be prospective sources for biological active compounds.

**P-60**

**THE ROLE OF DIPYRIDAMOLE IN PREVENTING ANGIOGENESIS IMPAIRMENT BY HOMOCYSTINE AND ADENOSINE: A MODEL FOR SCREENING HERBAL BIOACTIVES.**

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Herbal bioactives have been shown to influence the pathogenesis of homocysteine associated vascular complications. However, there are no simple cellular models to study their role in preventing angiogenesis impairment by homocysteine and adenosine. Using dipyridamole, an inhibitor for nucleoside transport, we examined its mechanism of action on impaired angiogenic processes caused by homocysteine and adenosine in EAhy926 endothelial cells. Our results showed that dipyridamole restored the extracellular adenosine and intracellular S-adenosylhomocysteine concentrations disrupted by the combination of homocysteine and adenosine. Dipyridamole restored the impaired proliferation, migration and formation of capillary-like tubes of EAhy926 cells caused by the combination of homocysteine and adenosine. Mechanism analysis revealed that dipyridamole induced the phosphorylation of MEK and ERK, and its effect on cell growth was attenuated by the MEK inhibitor, U0126. Dipyridamole protected against impaired angiogenesis caused by homocysteine and adenosine, at least in part, by activating the MEK/ERK signalling pathway, and this could be associated with its effects in suppressing intracellular S-adenosylhomocysteine accumulation. This present study suggests that this cellular model could be used to investigate the angiogenetic properties and other CVS synergic actions of combinational extracts and isolated components of herbal medicines for homocysteine-associated vascular complications.
P-61
SESQUITERPENES AND MONOTERPENOID WITH ACETYLCOLINESTERASE (ACHE) INHIBITORY ACTIVITY FROM VALERIANA OFFICINALIS VAR. LATIOFOLIA

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Four new sesquiterpenoids, volvalerenal H-J (1-3) and Volvaleranic Acid K (4), along with a new monoterpenoid named Densispicnins C (5), were isolated from the ethanol extract of the root of Valeriana officinalis var. latiofolia. The structures of new compounds were determined on the basis of 1D and 2D NMR, and HRESIMS. And the isolations were evaluated by measuring acetylcholinesterase (AChE) inhibitory activity. Compounds 2 and 4 showed AChE inhibitory activities of 22.4±0.8% and 58.9±1.3% at a concentration of 0.30 μM, respectively.

P-62
SESQUITERPENES FROM CURCUMA PHAECAULIS AND THEIR INHIBITORY ACTIVITIES ON NITRIC OXIDE PRODUCTION IN RAW 264.7 CELLS

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Curcuma phaeacaulis Valeton is used in traditional Chinese medicines for the treatment of blood-related disorders such as blood stasis and inflammation. Our previous studies have found that sesquiterpenes were mainly isolated from C. wenyujin and C. phaeacaulis, while diarylheptanoids were the major compounds from C. kwangsiensis. And most of the isolated compounds exhibited remarkable inhibitory activities against nitric oxide production. As part of our ongoing research for biologically active sesquiterpenoids from the genus Curcuma, materials in the remaining fractions were further fractionated using silica gel chromatography as well as HPLC to afford four new cadinane-type sesquiterpenes named phacadinanes A-D (1-4), four known eudesmane-type sesquiterpenes, and ten known guaiane-type sesquiterpenes. Furthermore, inhibitory effects of the isolated compounds on nitric oxide production in LPS-activated macrophages were evaluated. Hydrocortisone (43.80 μM) was used as a positive control. Phacadinanes B, curculide, isozedoarondiol and phaeocaulisin C showed strong inhibitory activities on NO production with IC50 values of 2.25, 0.80, 1.38 and 1.61 μM, respectively, which indicates their potential as promising compounds for the further research and development of anti-inflammatory agents.
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In order to identify plants and recipes used in the treatment of malaria in Bagira, an ethnobotanical study was conducted from December 2013 to February 2014, by interviewing 85 traditional healers (46.9 ± 12.0 average age; range: 19-79 years). The direct interview using a questionnaire was used to collect ethnobotanical information. A specimen of each plant was collected with traditional healers and deposited in the Lwiro and Kipopo herbaria where plants have been identified. The GraphPad Prism Version 5 software was used for statistical data processing. Fifty-three plants belonging to 24 families, dominated by Fabaceae (23 %) and Asteraceae (22 %), and distributed in 49 genera were identified. These antimalarial plants are also used to treat signs corresponding to about 150 pathologies. A total of 66 monoherbal and 16 polyherbal recipes were reported to be prepared from these plants. In both cases, leaves are the most used organ with 56 % and 86 % citations, respectively; recipes are prepared by decoction (59 % and 63 %), mainly administered orally (96.9 % and 100 %). Cinchona calisaya Wedd. (citation frequency, 54.1 %) Ekebergia benguellensis Welw ex CDC (54.1 %), Ochna schweffurthiana F Hoffm (50.6 %), Julbernardia paniculata (Benth.) Troupin (48.2 %) Psorospermum corymbiferum Spach (47.1 %) and Rothmannia engleriana (K. Shum) Keay (47.1 %) are the 6 plants most cited. Antimalarial plants and recipes are known in Bagira. Phytochemical study, pharmacology and toxicology are in progress in our research unit to capitalize on this knowledge.

P-65
PATTERN ORIENTED APPROACH OF HERBAL MEDICINES, USING CAPILLARY ELECTROPHORESIS

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Capillary Electrophoresis (CE) is a powerful analytical tool to analyse water soluble substances. Many herbal medicines are prepared as infusion or similar preparations. The aim of the technique is to trace origin and crop of TCM by using fingerprint. Comparred to other analytical techniques, CE method is simple, robust and also orthogonal to for example HPLC. Several herbal medicines from China and from Europe were analysed using different CE techniques. In order to show different fingerprints, green tea has been chosen, as it is easily available in Belgium. Following methods were applied: Free Zone Electrophoresis (CZE) for charged UV absorbing analytes, Micellar Electro Kinetic CE (MEKC) for charged and non-charged UV absorbing analytes Direct UV for negative charged analytes, such as anions and organic acids Carbohydrate CE for mono-and oligosaccharides. After optimisation of the sample preparation, several herbal medicines could be analysed using CE. When comparing different origin of green tea, Principal Component Analysis (PCA) allows assessing differences in origin for those green teas.

P-66
THE EFFECTS OF THE ETHANOLIC FRACTION OF CORIOLUS VERSICOLOR ON THE A549 NON-SMALL-CELL LUNG CANCER CELL LINE

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Coriolus versicolor has demonstrated anti-cancer effects via polysaccharide-peptides (PSP) and polysaccharide Krestin (PSK). However, many other bioactive compounds within Coriolus versicolor (CV) may not have been identified. Our primary focus was to determine whether the ethanolic extract of Coriolus versicolor demonstrated any anti-cancer effects. The crude ethanolic extract was utilized, as was the S6 subtraction. In this study, the experiments were performed upon the A549 human nonsmall-cell lung carcinoma cell line. A 24h exposure to 50 ug/mL of the crude extract resulted in a suppression of cell viability (91.21±4.146 %), whilst a 50 ug/mL of the S6 subtraction resulted in 73.44±8.76 %. LDH assays and Annexin V/prodipom iodide staining did not demonstrate any significant increase compared to the control, indicating that the extracts do not result in apoptosis. Our secondary focus was upon metastasis, and TGFβ, as a known stimulator of epithelial-mesenchymal transition, was used. A 24h pretreatment with the crude extract and S6 subtraction decreased the TGFβ induction of vimentin. However, pre-treatment also exacerbated the inhibitive properties of TGFβ on E-cadherin protein expression. The inhibitory effects of the ethanolic extract was determined to occur upstream in the cell signaling pathway, where TGFβ exposure results in the phosphorylation of Smad 2/3 and Akt. The crude extract and S6 subtraction demonstrated significant inhibitive properties on A549 cell proliferation, as well as the ability to reduce the effects of TGFβ on vimentin expression by reducing the phosphorylation of Smad 2/3 and Akt.

P-67
A NEW ALKALOID FROM THE ROOTS OF REHMANNIA GLUTINOSA

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The present phytochemical study was undertaken to investigate the chemical constituents of the 95 % EtOH extract of the dried roots of *Rehmannia glutinosa*. The compounds were isolated and purified by Diaion HP-20, Toyopearl HW-40, silica gel column chromatography and preparative HPLC, and the structures were identified on the basis of spectral data and physicochemical properties. Five compounds were isolated and identified from the 95 % EtOH extract as methyl-3-(6-oxo-1,2,3,6-tetrahydropyridin-4-yl) propanoate (1), daphneresinol (2), phenyl-O-ß-xylopyranosyl(1.6)-O-ß-glucopyranoside (3), syringin (4), darendoside A (5). Compound 1 was a new compound, compounds 2-5 were isolated from this plant for the first time.

**P-68 POLYPHENOLS, CYTOTOXICITY AND ENZYMATIC ACTIVITIES OF THREE ERIAECEAE SPECIES FROM ALGERIA**

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In Algerian traditional medicine, *Erica arborea* (EA), *Erica multiflora* (EM) and *Arbutus unedo* (AU) are reported as antiseptic, diuretic, astringent, depurative, and to treat scalds and wounds. The methanolic extracts of their leaves and aerial parts were screened for enzyme inhibitory and cytotoxic activities. TLC and HPLC chromatographic profiles based on flavonoids allowed to easily distinguish between the three investigated species. Inhibitory effects were evaluated on both acetylcholinesterase and carboxylesterase and cytotoxicity was assessed using the MTT assay on U373 astroglial human cancer cells. The amounts of different phenolic compounds were determined using colorimetric assays. AU presented the highest amounts of total phenolic compounds and flavonoids. AU and EA weakly inhibited the enzyme carboxylesterase with IC50 of 14.3 ± 0.8 and 8.8 ± 3.4 mg/mL, respectively. The leaves of AU (23.2 ± 2.4%) and the flowered aerial parts of EA (19.1 ± 7.4%) and EM (13.7 ± 0.2%) exhibited a weak anti-acetylcholinesterase activity. All the extracts presented an appreciable cytotoxicity on U373 astroglial human cancer cells with an IC50 of 15.2 ± 1.5 µg/mL for AU, 11.3 ± 7 µg/mL for EM and 15.5 ± 4.5 µg/mL for EA. There are no correlations between the polyphenolics/flavonoids content and the observed enzyme inhibitory and cytotoxic activities. Acknowledgements: The authors acknowledge Mrs Manuela De Lorenzi, Mrs Marie Faes and Mr Olivier Vaillant for their invaluable technical help.

**P-69 A PROSPECTIVE STUDY TO EVALUATE THE SAFETY AND EFFICACY USING REMOVING BLOOD STASIS HERBALS FOR PATIENTS WITH INTRACRANIAL HEMORRHAGE OF HYPERACUTE STAGE AND RELATIVE FACTORS OF HEMATOMA ENLARGEMENT (A MULTICENTER PROSPECTIVE RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED CLINICAL STUDY)**

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The aim was to investigate whether using a removing blood stasis method in hyperacute intracranial hemorrhage stage can lead to hematoma enlargement and its clinical efficacy. A multicenter retrospective randomized double-blind placebo-controlled clinical study. We recruited patients aged 18 years or older and presenting at less than 6 h from symptom onset in 8 research centers. All the patients were randomized divided into group A, group B and group C. A total 80 participants were enrolled at the 8 hospitals. 12 patients (15.8%) occurred hematoma enlargement within 24 hours. 3 (11.5%) in Group A, 4 (16.0%) in Group B, and 3 (20%) in Group C whose hematoma were enlarged. Unvaried analysis find the history of ICH (OR:12.6; 95%CI: 1.0-152.2; P=0.046), the time from symptom onset to hospital (OR: 0.48; 95%CI: 0.28-0.84; P=0.010, and HDL (OR: 0.025; 95%CI: 0.0014-0.42; P=0.011) are associated with hematoma enlargement. Multivariate analysis indicates that the time from symptom onset to hospital (OR: 0.469; 95%CI: 0.231-0.952; P=0.036) and HDL (OR: 0.030; 95%CI: 0.001-0.867; P=0.041) is independent with hematoma enlargement, and small hematoma volume may increase the risk of hematoma enlargement (OR: 0.850; 95%CI: 0.710-1.018; P=0.078). It is safe using removing blood stasis method in hyper acute intracranial hemorrhage stage and can not increase the risk of hematoma enlargement. The shorter the time from symptom onset to hospital, the higher morbidity from hematoma enlargement. Low level of HDL can increase the risk of hematoma enlargement.

**P-70 A SERIES OF STRATEGIES FOR SOLVING THE SHORTAGE OF REFERENCE STANDARDS FOR MULTI-COMPONENTS DETERMINATION OF TRADITIONAL CHINESE MEDICINE**

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The shortage of reference standards has become the bottleneck of quality control of TCMs. In this study, a series of strategies, including one single reference standard to determine multi-compounds (SSDMC), quantitative analysis by standardized reference extract (QASRE), and quantitative nuclear magnetic resonance spectroscopy (qNMR) were proposed to solve this problem. SSDMC, QASRE, and qNMR were developed and validated to determine four alkaloids in Mahoniae Caulis. Comprehensive comparisons among them and with the conventional external standard method (ESM) were carried out. The relative expanded uncertainty of measurement was used to compare their credibility. All new proposed methods accurately accomplished the quantification of four alkaloids in Mahoniae Caulis. These newly established strategies have their unique advantages in saving reference standards in contrast with ESM. But each method also has its drawbacks and should be applied in specific practices.

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P-71
PARTIAL RECONSTITUTION OF BOTANICAL EXTRACT PG102 BY COMBINING CHEMICAL COMPOUNDS KNOWN TO BE PRESENT IN ACTINIDIA ARGUTA: IMPLICATIONS FOR QC OF PHYTOMEDICINES

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One of the most important factors limiting the success of plant-derived therapeutics is that these products are generally a mixture containing many different molecules. The compounds responsible for bioactivities and/or clinical effects are also not yet identified. To overcome this hurdle, we investigated whether we can reconstitute bioactivities of claimed biological and therapeutic effects when chemical compounds present in a particular plant source are combined, using a previously well-characterized phytotherapeutic as a model system. PG102 is a water-soluble extract from an edible fruit, Actinidia arguta, and has previously been shown to control various factors involved in allergy pathogenesis. Six compounds present in PG102 were, individually or in the form of mixtures, tested for their effects on the expression of various Th2 cytokines and inflammatory mediators in the cell-based assay. Each chemical inhibited IL-4 expression to varying degrees. The chemical compounds were combined at a ratio present in PG102 resulting in two formulations, CQMI1H and CQM, consisting of all or the first three of the following chemicals respectively: citric acid, quinic acid, malic acid, myo-inositol, isoquercitrin, and 5-hydroxymethyl-2-furaldehyde. The mixtures reconstituted original activities of PG102 to a significant level. In the murine asthma model, CQM ameliorated asthmatic symptoms and decreased IgE and IL-5 levels. Our data indicated that a substantial portion of PG102’s anti-allergic activities can be reconstituted by mixing six chemical compounds. This suggests the possibility of developing a new type of anti-allergic agent. This approach may be useful for developing chemically defined functional products from complex botanical extracts.

P-72
USE OF CELL-BASED BIOASSAY FOR THE QUALITY CONTROL OF BOTANICAL THERAPEUTICS

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The single most important factor in modernization efforts of traditional oriental medicines is the limited understanding of active compounds responsible for claimed therapeutic effects. Indeed, the unknown identity of biologically active molecules generates major difficulties associated with botanical therapeutics in general. This includes management of raw materials, poor understanding of action mechanisms, and quality control of intermediate and final products among many others. Although many methodologies have been developed, only few have been successful, due in particular to the poor relevance of employed chemical markers in actual biological effects. We have been trying to develop more biologically relevant assays for the quality control of botanical products. In this method, primary cells or cell lines are used to test effects of particular botanical therapeutics on biologically relevant markers in a quantitative manner, allowing for the calculation of specific activities or IC50/EC50 of botanical preparations. For example, IgE or IL-4 plays an important role in all allergic diseases, and thus can be used as a marker by measuring effects of particular botanical preparations on these proteins. In arthritis, IL-18 or NO can be employed as biological markers in such assays to quantitate the effects of particular traditional medicines. In this presentation, we will show the actual examples of cell-based bioassays for the quality control of bioactivities of two botanical products, PG102, an anti-allergic agent prepared from hardy kiwi fruits, and PG201 prepared from 12 herbs, a traditional Korean medicine for arthritis. Cell-based bioassays, especially when combined with other conventional analytical tools, may provide a powerful tool for controlling the quality of botanical mixtures as well as raw materials, which may contribute to the modernization of traditional botanical medicines.

P-73
CHEMICAL VARIATION OF ESSENTIAL OIL

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CONSTITUENTS OF *OCIMUM GRATISSIMUM* LINN FROM BENIN AND INFLUENCE ON ANTIMICROBIAL AND ANTIPARASITIC PROPERTIES

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Previous work has shown great variations in the chemical composition of *Ocimum gratissimum* Linn essential oils (EOs). Our aim was to study the influence of this variation on their antimicrobial, anti-parasitic effects and toxicity. Method: EOs from aerial part collected in two vegetative stages and three moments of harvest were isolated by hydrodistillation and analysed by GC/MS and GC/FID. Oils were tested in vitro against *T. brucei brucei*, *P. falciparum*; *C. albicans*, *S. aureus* and *E. coli*. Cytotoxicity was evaluated against CHO cells and WI38 and toxicity against Artemia salina Leach. Our results confirmed the variation in the yields and chemical compositions. The most active on *C. albicans* came from the plant in full flowering stage collected at 7AM (CM=0.06 mg/mL), on *S. aureus* from the plant in pre-flowering stage at 7AM (CM=0.24 mg/mL). E. coli was insensitive to the variation of the chemical composition (CM=0.48 mg/mL). Antiplasmodial and anti-trypanosomal activities also varied depending on the vegetative stage and the harvest time; being more anti-trypanosomal than anti-plasmodial. They were not toxic against Artemia salina Leach and had a low cytotoxicity against CHO and WI38 cells. Our study shows that chemical variations of *O. gratissimum* EOs influence their antimicrobial and anti-parasitic properties.

P-75
NEW ANTIPLORIFERATIVE WITHANOLIDES FROM THE SEEDS OF DATURA METEL

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Three new with anolides, daturaselines A-C (1-3), and three new with anolides glycosides, daturasides A-C (4-6), were isolated and identified from ethyl acetate-soluble fraction of ethanol extract of Datura metel seeds. The structures of new compounds were established according to the 1D and 2D NMR spectra, combined with high resolution mass spectrometry. All isolated compounds were evaluated for their antiproliferative activities against human gastric adenocarcinoma cells (BGC-823), human hepatoma (Hepg2) and human breast cancer (MCF-7). The experiments indicated compounds 1-6 showed medium antiproliferative effects. These results made the significant contributions to illuminating the basic pharmacodynamic material for discovering new medicinal parts and expanding drug source. Simultaneously, it could provide new potential compounds as agents for drug development.

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P-76
DEVELOPMENT OF A NOVEL HPLC METHOD FOR QUALITY CONTROL OF SAFFRON (CROCUS SATIVUS L.)

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Saffron, the dried stigma of *Crocus sativus* L., finds
numerous applications in TCM. Here, a novel HPLC protocol was established and applied for the analysis of saffron samples, not only from different places of origin but also from several harvest seasons. One of the main active constituents of saffron, crocin, is also contained in jasmine (Jasminum officinale L) which is therefore used by counterfeiters in order to enable fake saffron preparations to pass pharmacopoeial tests. HPLC was performed with a Zorbax-XDB C18 column and with a linear gradient (45 min; 254 nm; 1.0 mL/min) of methanol (0.5% acetic acid) and water (0.5% acetic acid). Genuine saffron samples from different harvesting regions and years, despite their effective constituent contents being slightly different, all showed relatively similar basic chromatograms with 8 characteristic well separated peaks, three of which could be identified as crocin-I, crocin-II, and gardenoside, respectively. Consequently, the crocin-I peak was selected as the chromatogram specific reference peak in the presented HPLC method. Beside the determination of the content of the active constituents, this method was also very adapted to the determination of potential adulterations with jasmine. Whereas in the case of pure saffron the peak ratio (gardenoside / crocin-I) always exhibited values significantly below 0.5, the ratio was significantly above this value for all adulterated samples, rising with the degree of adulteration. Thus, this new HPLC method is simple, fast, accurate, and exhibits high reproducibility so that it can be used for quality control and identification of saffron.

P-77
PROSTATE CANCER BONE-METASTASIS IS INHIBITED BY CELASTROL, AN ACTIVE CONSTITUENT OF TRIPTERYGium WILFORDII HOOK.F. (雷公藤)

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Prostate cancer (PCa) is one of the most common malignant tumours and a leading cause of cancer deaths. Treatment failure of PCa is often due to bone-metastasis. Celastrol, an active constituent of the roots of Tripterygium wilfordii, has shown antitumour effects in previous studies in accordance with its use in TCM. Here we report for the first time an in-depth study of the effects of celastrol on PCa bone-metastasis and its mechanism of action. Using a PC-3 cell model, in vitro assays were performed to evaluate the effects of celastrol on proliferation, migration (wound healing assay), invasion of healthy tissues (penetration assay), and secretion of Vascular-Endothelial-Growth-Factor (VEGF) (ELISA assay). An intra-tibia injection mouse model was used to assess the effect of celastrol treatment on PCa bone-metastasis in vivo. Pre-treatment with celastrol significantly reduced proliferation of PC-3 cells in a dose-dependent manner and cell migration was much slower than in untreated controls. In the penetration assay, significantly fewer cells penetrated through the gel-membrane after celastrol administration and their skeletal invasive ability was also significantly reduced in a dose-dependent manner. Correspondingly, a significant, dose dependent decrease in VEGF secretion was observed. In the in vivo mouse model, pre-treatment with celastrol (8 µmol/L) inhibited the tumourigenicity of PC-3 cells so that almost no bone invasion occurred as compared to control injections. Histological examinations using H&E-staining showed that tibiae injected with celastrol pre-treated PC-3 cells retained their natural bone structure. Our results suggest that celastrol may have major preventive potential against PCa bone-metastasis.

P-78
INHIBITION OF THE MYD88 PATHWAY BY CURCUMIN, THE ACTIVE CONSTITUENT OF TURMERIC (CURCUMA LONGA L.), CAUSES APOPTOSIS IN HEPATIC STELLATE CELLS (HSCS)

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HSCs were divided into four groups, namely control group (A), MyD88 siRNA group (B), curcumin group (C), and curcumin + MyD88 siRNA group (D). Groups B and D were subjected to transient transfection with siRNA for 48h. Groups C and D were incubated with curcumin (25 µmol/L) for 24h. MyD88 protein expression was observed by Western Blot, apoptosis was detected by flow cytometry, mRNA expression was detected by RT-PCR. Treatment with curcumin or MyD88 siRNA significantly reduced the expression of MyD88 in HSCs (both P<0.05 vs control), even more so if cells were treated with both agents simultaneously (P<0.01 vs control). Both curcumin and MyD88 siRNA inhibited the expression the cytokines TLR2, TLR4, NF-κB, TNF-1a, and IL-1B on the mRNA level. For curcumin these effects were significant for all five (all P<0.05 vs control). For MyD88 siRNA only the effects on NF-κB, TNF-1a, and IL-1B were significant (all P<0.05 vs control). For cells receiving both treatments, a significant inhibition of the expression of all five cytokines was observed (all P<0.01 vs control). Correspondingly, a significant induction of apoptosis was observed for both agents with apoptosis rate of 20 % for the controls, 40 % (P<0.05 vs. control) for cell incubated with curcumin, 41 % (P<0.05 vs. control) for cells treated with MyD88 siRNA, and 47% (P<0.01 vs. control) for cells receiving both treatments. This study shows that curcumin promotes apoptosis of HSCs by inhibiting the expression of Myd88.
pathway related cytokines on the mRNA level.

P-79
COMBINED EFFECTS OF METRONOMIC ZOLEDRONATE WITH CORIOLUS VERSICOLOR IN A METASTATIC BREAST TUMOR MOUSE MODEL

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Incidence of breast cancer is high in women worldwide and mortality usually results from tumor metastasis. Adjuvant chemotherapeutic, such as zoledronate (ZOL), has been used in metastatic breast cancer mainly for skeletal protection. Our previous studies demonstrated that Coriolus versicolor (CV) aqueous extract exhibited anti-tumor and anti-metastatic effects in breast tumor mouse model, and together with repeated low dose (metronomic) of ZOL exerted anti-osteolysis effects in intratibial breast cancer-induced osteolysis model. In this study, the combined action of metronomic ZOL and CV was further investigated on breast tumor metastasis and immunomodulation in metastatic breast tumor mice. Female Balb/c mice, inoculated with mouse breast tumor 4T1 cells, were administered with metronomic ZOL (0.0125 mg/kg twice a week for 4 weeks), or CV (1 g/kg daily for 4 weeks), or combined ZOL and CV treatments. Results showed that the final tumor weights of combined ZOL and CV treatment were the lowest among all treated groups. The combined ZOL and CV treatment significantly attenuated ZOL-induced lung metastasis. The numbers of Ki-67-positive tumor cells and CD34-positive endothelial cells in tumor sections of the combined treatment group were also lower than the control. Besides, the CD4/CD8 T-lymphocytes ratio in tumor-bearing mice was increased in CV-treated (alone or with ZOL) groups. In conclusion, we reported for the first time the anti-tumor, anti-angiogenic and immunomodulatory effects of the combined ZOL and CV treatment in a metastatic breast tumor model.

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P-80
A BIFLAVONOID AMENTOFLAVONE FROM GARCINIA XANTHOCHYMUS FRUIT EXHIBITED

ANTI-ANGIOGENIC ACTIVITIES IN ZEBRAFISH EMBRYOS

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Edible fruits of Garcinia xanthochymus were traditionally used to treat diarrhoea and dysentery. Our previous work showed that some components of G. xanthochymus fruits induced apoptosis in colon cancer cells. In this study, the aril (edible pulp of the fruit) and seed of G. xanthochymus fruits were further investigated for their potential antiangiogenesis effects, with an aim to evaluate the anti-angiogenic effect of the crude methanolic extract and isolated compounds using transgenic zebrafish Tg(fli1a:EGFP)y1 with fluorescent blood vessels. The growth of sub intestinal vessels (SIV) and gene expression in angiogenesis signaling pathways of extract/compound-treated zebrafish embryos were examined. Our initial results showed that the methanolic extract from the aril (15 mg/mL), but not the seed, showed promising inhibitory effect on the growth of SIV in zebrafish embryos. LC-MS analysis suggested that certain Garcinia polyphenols correlated with the activity. Three representative compounds (allothyriol, xanthochymol and amentoflavone) from 3 classes of constituents (xanthone, catechol and flavonoid) were further examined for their activities in zebrafish embryos. Results showed that amentoflavone (10-20 mM), but not allothyriol and xanthochymol, could significantly inhibit the growth of SIV. It also down-regulated the expressions of Angt2 and Tie2 genes of zebrafish embryos. A detectable amount of amentoflavone in aril was demonstrated using LC-MS-TQD which may account for the anti-angiogenic effects. In conclusion, this is the first report of the anti-angiogenic activities of amentoflavone in zebrafish embryos. These findings suggested the anti-angiogenic potential of this plant species and may lead to further exploration of active constituents in different plant parts.

P-81
PROTECTIVE EFFECTS OF VERBASCOSIDE ON STATIN-INDUCED MYOTOXICITY IN ZEBRAFISH EMBRYOS

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Myotoxicity is a well-known side effect of statins, the common lipid-lowering drug. Herba Cistanches (HC, *Cistanche deserticola*) is a Chinese herb traditionally used for muscle problems. Recent studies demonstrated that HC could reduce muscle damage in post-exercised rats. Verbascoside (Vb) was found as the major constituent in the active fraction of HC and is known to be muscle protective, though its effect on statin-induced muscle toxicity has never been investigated. The aim of this study was to investigate the protective effect of Vb on simvastatin-induced myotoxicity using zebrafish embryos model. Embryos treated with simvastatin (0 -0.5 µM) at 24 hpf, with or without co- treatment of Vb (0.32 mM, maximum non-cytotoxic dose) were fixed at 48 hpf, and whole muscles were stained using the anti-slow twitch myosin F59 antibody. For each treatment group, the diameters of at least 300 muscle fibers were measured and represented as ratio of mean fiber size against control. Results showed that muscle damage at simvastatin concentrations (0.4 and 0.5 µM) were evidenced by bowing, thin and irregular fibers with diffuse appearance, while these damages were significantly improved by the co-treatment of Vb. Simvastatin caused significant dose-dependent reduction on myotube diameter as compared to control (61.5 -73.8% vs 100%). Vb (0.32 mM) significantly (p<0.001) prevented the simvastatin-induced reduction in myotube diameter at 0.4 µM (104.7 vs 73.8%) and 0.5 µM (84.6 vs 61.5%) of simvastatin concentrations. In conclusion, our study demonstrated for the first time that verbascoside could protect against simvastatin-induced myotoxicity in vivo.

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P-82
NOVEL ANTI-ANGIOGENIC ACTIVITIES OF A TRITERPENOID DEOXYACETEIN FROM CIMICIFUGA FEOITDA

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Deoxyacetain (DA) is a triterpenoid isolated from the root of Cimicifuga foetida which is a source of traditional Chinese herb “shengma” and is traditionally used as an antipterecy and analgesic agent. Previous studies showed that DA exhibited anti-proliferative effect in human breast cancer cells. Hence, the objectives of this study were to investigate the in vitro and in vivo effects of DA on angiogenesis using human microvascular endothelial cells (HMEC-1) and matrigel plug mouse model, respectively. The proliferation, motility and invasion of endothelial cells were assessed by thymidine incorporation, scratch wound and transwell migration assays, respectively. The mRNA expressions of angiogenic receptors in DA-treated endothelial cells were also examined. Our results showed that DA (3.125 -100 mM) significantly inhibited the proliferation of HMEC-1 without cytotoxicity. The motility and invasion of endothelial cells were also inhibited by 10 - 40 mM DA. Besides, this compound could down-regulate the mRNA expressions of VEGFR1 and VEGFR3 in HMEC-1 cells. Results on matrigel plug mouse model showed that oral administration of DA at 20 mg/kg for 5 days inhibited the blood vessel growth in the growth factor-containing matrigel plugs. The hemoglobin content in the plugs was significantly reduced in DA-treated mice. This is the first report of the antiangiogenic activities of DA in human endothelial cells and in mouse model. Our findings indicate that DA may act as another bioactive component in Cimicifuga species.

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P-83
SCREENING OF MEDICINAL PLANTS FROM REUNION ISLAND FOR ANTIMALARIAL ACTIVITY

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According to the last World Malaria Report, there were an estimated 584 000 malaria deaths worldwide in 2013. The problem of parasite resistance towards available medicines is increasing. Natural products could play an important role to discover new antimalarial drug. The aim of the study was to highlight the anti-plasmodial properties of plants used in traditional medicine on the Mascarene Islands. 85 extracts obtained from vegetal samples collected in La Réunion were evaluated on Plasmodium falciparum 3D7 chloroquine-sensitive strain using a colorimetric method. 21 extracts were found to have a weak anti-plasmodial activity (IC50<50 µg/mL), 18 demonstrated a moderate activity

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(15μg/mL<IC50<50μg/mL). Best results were obtained with extract from Zanthoxylum heterophyllum, Psidia retusa and Moninia rontudfolia (IC50 <15 μg/mL) that appeared to be good candidates for further investigations.

P-84
INHIBITORY EFFECT OF THE CONSTITUENTS FROM
THE RHIZOMES OF CYPERUS ROTUNDUS ON TRPV1
ION CHANNEL

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Cyperus rotundus (Cyperaceae) is used as an analgesic and sedative in oriental medicine, and has been reported to exhibit anti-nociceptive and anti-inflammatory effects. On the other hand, transient receptor potential vanilloid channel 1 (TRPV1), the so-called capsaicin receptor, is a nonselective cation channel that senses various noxious chemical and thermal stimuli. However, it has recently been reported that epidermally expressed TRPV1 is involved in heat-and UV-induced skin aging. The aim of this study was to evaluate whether C. rotundus extract and its constituents inhibit TRPV1. Using whole-cell patch-clamp recordings, we found that capsaicin-induced hTRPV1 were significantly and dose-dependently inhibited by the ethylacetate fraction of C. rotundus rhizomes and by oleanolic acid (a constituent of the ethylacetate fraction). At a concentration of 90 μM, oleanolic acid, which was one of three constituents isolated from the ethylacetate fraction, inhibited TRPV1 activity by 61.4 ± 8.0 %. In the present study, we report the first electrophysiological study undertaken to explore the inhibitory effects of C. rotundus rhizomes and of some of its constituents on hTRPV1 channel. Our findings may explain the mechanisms of the analgesic effect of C. rotundus. Moreover, although it needs further research, the possible role of C. rotundus rhizomes extract or its constituents is tremendous, because it opens up the possibility to mitigate TRPV1-mediated UV-induced photoaging or to relieve skin inflammation.

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P-85
PRELIMINARY RESULTS OF VALIDATION OF A NEW
PATIENT-REPORTED OUTCOME INSTRUMENT FOR
ADVANCED CHRONIC KIDNEY DISEASE

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The uremic symptoms of advanced chronic kidney disease (CKD) can severely impair physical function, psychological health and social life. Some patients, especially for the older, chose conservative kidney management (CKM) and Traditional Chinese medicine (TCM) but not dialysis in this stage. To measure the patients-reported outcome (PRO) from these people, Advanced Chronic Kidney Disease Quality of Life Instrument – Nondialysis (ACKDQoL-ND) was developed and validated. According to the FDA guidance for developing a PRO Instrument (2009), the development of the new questionnaire involved 10 steps: outline of the hypothesized concepts, quantitative interviews with patients, literature review, framework development, item pool construction, formulation of the first draft, refinement, initial validation, further refinement and finally revalidation. The final version of ACKDQoL-ND included 54 kidney-disease targeted items as well as an overall health rating item. A cohort of 165 participants completed it. The ACKDQoL-ND demonstrated high split-half reliability (r=0.893) and strong internal consistency (Cronbach’s α= 0.879). The content validity was assessed to test the hypothesized framework. Moderate to strong correlation (Pearson product-moment correlation coefficient between 0.389–0.826) was seen between different domain scores and total score. Exploratory factor analyses showed the ACKDQoL-ND had one single dimension (KMO=0.735, P=0.000). The ACKDQoL-ND is a simple and practical measure to measure the PRO from advanced CKD patients who receive CKM and/or TCM but not dialysis.

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P-86
ADVANCE NON SMALL CELL LUNG CANCER
ACCEPTING CHEMOTHERAPY COMBINED WITH
SHENMAI INJECTION

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The aim of the study was to observe the changes of vascular endothelial growth factor (VEGF) and the efficacy of patients with advanced non-small-cell lung cancer (NSCLC) after the treatment of chemotherapy with Shenmai Injection. This study is a randomized controlled, prospective, single blind trial. 63 eligible patients with NSCLC were divided into two groups. 32 patients belonged to the experiment group (A) and 31 patients belonged to the control group (B). The patients in A accepted chemotherapy with Shenmai Injection, and the patients in B accepted chemotherapy only. One year survival rate, Progression-Free-Survival (PFS), the value of VEGF, Performance Status, the score of Chinese clinical symptoms and adverse effect rates were measured. In group A, the change of VEGF before and after the treatment was statistically different (P<0.05). However, the
change of the VEGF was not statistically different (P> 0.05) in group B. The PFS of patients in group A was longer than in the group B (5.0 months VS. 3.9 months, P<0.05). One year survival rates in group A was better than in group B (46.9% VS 41.9%, P>0.05). In both groups, the decrease of the score of Chinese clinical symptoms before and after the treatment was significant (P<0.05). However, the difference in group A is more significant than in group B (P>0.05). The ORR (Objective-Response-Rate) was 32.25% in group A and 22.58% in group B, and the DCR(Disease-Control-Rate) was 75% in group A and 64.51% in group B (P>0.05). The changes of Performance Status were not significantly different after the treatment (P> 0.05). The adverse effects showed no significant difference. The Shenmai Injection with chemotherapy can prolong PFS and influence the VEGF of patients with NSCLC, and it is a safety and effective treatment for NSCLC.

P-87
A NOVEL DIRECT ACTIVATOR OF TFEB PROMOTES AUTOPHAGY AND LYSOSONE BIOGENESIS AND PROTECTS NEURONS IN THE BRAIN

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Autophagy dysfunction is a common feature in neurodegenerative disorders caused by the accumulation of toxic, aggregate-prone proteins. Increasing evidence have demonstrated that genetic or pharmacological activation of transcription factor EB (TFEB), a master regulator of autophagy and lysosomal biogenesis, ameliorates neurotoxicity and rescues neurodegenerative phenotypes in several animal models of neurodegenerative diseases. Current known TFEB activators are mainly inhibitors of the mammalian target of rapamycin (mTOR). By screening a series of curcumin derivatives, a potent TFEB activator has been identified in this study. The small molecule directly binds to TFEB to inhibit mTOR-TFEB-YWHA interaction, thus promoting TFEB nuclear translocation without inhibiting mTOR activity. By activating TFEB, the compound enhances autophagy and lysosome biogenesis to degrade SNCA/alpha-synuclein, both in vitro and in vivo. This novel TFEB activator deserves further studies in animal models of neurodegenerative diseases.

P-88
STILBENOIDS AND PHENANTRENES FORM ARUNINDA GRAMINIFOLIA (DAI MONORITY MEDICINE) AND ANTITUMOR BIOACTIVITY

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The objective was to isolate, elucidate and analyze the bioactive chemicals from the tuber of Arunindia graminifolia (Dai Monority Medicine). The compounds were extracted by 95% alcohol and isolated by column chromatography on silica gel, Sephadex LH-20 and ODS. The structures were determined by UV, IR, NMR and MS spectra. HPLC/PAD was applied to analyze stilbenoids and phenanthrenes in tuber, stem and alcohol extract of A. graminifolia. Anti-tumor activities against human hepatocellular carcinoma cell Bel-7402 and human gastric cancer cells BGC-823 were assessed using MTT method. Eleven chemicals with stilbenoids or phenanthrenes were discovered, two of them were new stilbenoids, named as arundininan [2-(p-hydroxybenzyl)-3-hydroxy-5methoxybibenzyl] and arundinanol [1-(p-hydroxybenzyl)-7-hydroxy-2,4-dimethoxy-9,10dihydrophenan-threne] respectively. Compounds with bibenzyl skeleton were characteristic components of A. graminifolia. HPLC/PAD was successfully applied to the simultaneous quantification of four stilbenoids namely coelonin, arundinan, shancdin, orchinol in A. graminifolia. Compound 3,3’-dihydroxy-5-methoxybibenzyl with bibenzyl ring opening exhibits stronger anti-tumor activity than those compounds with bibenzyl ring closing.

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P-89
THE POSITIVE EFFECTS OF VOLATILE COMPONENTS IN JIAWEI WENDAN PRESCRIPTION ON THE IMPROVEMENT OF THE AUTISTIC BEHAVIOR IN RATS

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The objective was to investigate the effects of volatile components in Jiawei Wendan prescription on autistic behavior in rats, to analyze the volatile constituents and then to explore its effective components based on the treatment of autism. The pregnant mice were divided into two groups according to the method that Schneider used. To establish the animal model of autism, rats in this group were given sodium valproate (VPA) 600mg/kg by intraperitoneal injection after they were been pregnant for 12.5 days, and the control group were given the same amount of saline 0.85% (NS). The offspring of rats were in developmental biology, behavioral, morphological identification. The sample concludes 60 autism rats with 35 days from the model group and 20 rats from the control group. Autism rats were randomized into distilled water (DW) group with 20 rats, Jiawei Wendan Decoction (VPA-1)
P-90 METABONOMICS CHARACTERISTICS OF MIDDLE-AGED AND ELDERLY CHINESE PEOPLE WITH QI-INSUFFICIENCY CONSTITUTION

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The aim of the work was to investigate the metabonomic characteristics of qi-insufficiency constitution among middle-aged and elderly healthy Chinese people. All healthy Chinese participants were screened from 2012 annual health check-up for middle-aged and elderly community residents (≥50 years old) at Chajiao community, Guangzhou. 40 participants were enrolled include 20 with qi-insufficiency constitution while others with mild constitution. The two groups of participants were matched with gender and age. Serum were collected for metabonomics analysis at their fasting state in the morning. HPLC/MS were used for metabolomics survey. Compounds, which got a VIP (Variable Importance in the Projection) value=1 from PLS-DA analysis, were brought into t test to detect the intergroup difference and the compounds with significant difference between groups (p<0.05) were selected out. The potential biomarkers were identified through searching the formula searchable database. (http://www.genome.jp/kegg/ligand.html andhttp://redpoll.pharmacy.ualberta.ca/~aguo/www_hmdb_ca/HMDB/). Compared to the mild constitution group, 15 potential biomarkers involved metabolic pathway of lipid, carbohydrate, energy, protein and steroid were screened out from the group with qi-insufficiency constitution. Disorders of multiple substance metabolism as lipometabolism, glycometabolism, proteometabolism and energy metabolism, etc. may be the subsancial basis of qi-insufficiency constitution among middle-aged and elderly Chinese people.

P-91 SURVEY OF PLANTS USED IN TRADITIONAL MEDICINE AGAINST MALARIA IN BUKAVU AND UVIRA/DR CONGO.

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Malaria, for its high prevalence and morbidity, is one of the major tropical parasitic diseases, particularly in DR Congo. Its therapy not only uses modern antimalarials, for which many Resistance problems are encountered, but also medicinal plants which are promising sources for new and effective antimalarial drugs. This study was conducted between May 2013 and June 2014 from southern Bukavu to Uvira to identify reputable antimalarial plants. Direct interview with a field questionnaire allowed collecting ethnobotanical data; for each plant, a specimen was harvested in the presence of the interviewed traditional healers. The names and parts of plants, methods of preparation and administration of remedies were recorded. The listed plants were identified in the Kipopo herbarium, at the National Institute of Agricultural Research of Lubumbashi, where the herbarium specimens were deposited. Thirty-two resource persons, most popular of their city, among which men are the majority (62.5 %, with a sex ratio 1.7), cited 45 plant species grouped into 40 genera and 16 families in which Asteraceae (26.7 % of plants) were predominant. The leaves (57.3 %) are the organ mostly used for the preparation of drug recipes. The decoctions (48.9 %) and beverages (72.6 %) represent the major preparation and administration methods. The population of Bukavu and Uvira uses plants in the treatment of malaria. Studies should be conducted to determine the effectiveness of these plants to isolate antimalarial molecules.

P-92 EFFECT-DIRECTED ANALYSIS OF BOTANICAL EXTRACTS BY HPTLC-UV/VIS/FLDDEA-MS

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In recent years, the consumers’ interest in a healthy lifestyle has increased, and this is often related to oxidative-stress, which is the process of damage to DNA and cellular components caused by oxidative reactions. Food products rich in antioxidants such as fruits, vegetables, and herbs may prevent oxidative damage. However, it is important to determine the bioactivity of these compounds. Many of these compounds show bioactivity (antimicrobial, anti-inflammatory etc.) and act as antioxidants in the body. Therefore, the study of their bioactivity is important for human health.

The aim of this study was to investigate the antioxidant and antimicrobial activities of 68 dried botanical samples. The antioxidant activity was measured using different assays, including the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The antimicrobial activity was evaluated against a panel of bacteria, including methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible Staphylococcus aureus (MSSA).

The study showed that many of the samples had antioxidant and antimicrobial activities. The results also indicated that the bioactivity of these compounds can be analyzed using different methods, such as high performance liquid chromatography (HPLC) and electrophoresis (PAGE).

The findings of this study have important implications for the food industry and public health. The results can help in the development of new food products with improved health benefits. Additionally, the study provides insights into the potential use of these compounds as natural antioxidants and antimicrobials.
ROLE OF MEDICINAL AND AROMATIC PLANTS IN MODERN URBAN COMMUNITIES OF BRUSSELS

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Medicinal and aromatic plants (MAPs) have been used by humans for centuries. A big part of the knowledge and interest in MAPs was lost during periods of intensive industrialisation and urbanisation. According to the estimations of the United Nations, the world urban population is expected to increase by 84 % by 2050. Therefore it is extremely important to protect the biodiversity of MAPs and regain ethnobotanical knowledge in urban areas. Belgian practices, based on the traditions of medieval gardens, can be presented as a good example on how to bring MAPs closer to the citizens and how to adapt the use of MAPs to modern life styles and urban needs. The case study on the presence and role of MAPs in the City of Brussels has been carried out using available literature, visits to the relevant sites, meetings with representatives from the competent authorities, following activities of nature related organisations and taking part in events on cultivation and protection of MAPs. Almost 50 % of the City of Brussels territory contain non-built spaces. MAPs are often found in the forests, public parks, playgrounds and collective gardens which all together contain 36 % of Brussels green areas. Wild and cultivated MAPs are present in specifically designed private, school, university, museum and library gardens as well as city farms. The research revealed that MAPs are largely used in multidisciplinary urban greening projects. Besides well-known phyto-therapeutic use, private gardens of MAPs often serve as source of edible plants, spaces for mental relaxation, physical activity or socialising. School gardens are used as outdoor spaces for the pupils to learn and play as well as tools for an integrated education. Gardens of MAPs in museums, universities and libraries bring together history, folkloric traditions and modern knowledge about MAPs. Local nature and culture centres serve as life-long learning places to explore ethnobotanical knowledge of MAPs, to create therapeutic landscapes and to improve urban well-being.

PRELIMINARY ASSESSMENT OF THE CYTOTOXICITY AND GENOTOXICITY OF AN ASSOCIATION ARISTOLOCHIA - MAGNOLIA

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Herbal medicines are widely used around the world, either for primary health care or as complementary medicines. These herbal medicines are generally considered as safe and reliable. However, herbs can sometimes cause serious health troubles. For example, the aristolochic acid nephropathy (AAN), a progressive renal interstitial fibrosis frequently associated with urothelial malignancies, was reported in a Belgian cohort after the intake of slimming pills inadvertently containing an Aristolochia species, rich in aristolochic acids (AA), known to be genotoxic through the generation of aristolactams which yield highly persistent and non-or poorly-repaired DNA adducts. Interestingly, the Belgian cases are remarkable by the rapid onset of the pathology. Available records indicate that the supposedly slimming pills prescription included roots of Stephania tetrandra, that were in fact adulterated by the roots of an Aristolochia species (probably A. fangchi), and also bark of Magnolia officinalis. The goal of the present study is to evaluate on a human intestinal epithelial cell line the cytotoxicity and genotoxicity of Aristolochia baetica and Magnolia officinalis aqueous and methanolic extracts, alone or in combination, by a MTT cell viability assay and by the detection and quantification of the phosphorylated histone g-H2AX (whole cell ELISA and Immunocytochemistry). The genotoxicity of the association of Aristolochia baetica and Magnolia officinalis extracts is significantly higher compared to the individual plant extracts. The high potentiation of AAs genotoxicity by Magnolia officinalis could then be an explanatory factor for the “Chinese herbs nephropathy” cases, observed in Belgium in the 1990s. Although this would be a very interesting example of indirect genotoxicity, this observation warrants further studies to establish the precise role of the Magnolia officinalis extracts and to identify the compounds responsible for the observed co-genotoxicity.

GLOBAL ANALYSIS OF THE METABOLITES OF CURCUMIN IN RATS BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH QUADUPOLE TIME-OF-FLIGHT TANDEM MASS SPECTROMETRY

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Curcumin, a safe natural yellow pigment with a wide range of pharmacological activities, is used both in herbal drugs and as a food coloring agents. Studies have shown that curcumin would suffer from extensive metabolism in vivo
and the predominant metabolic pathways are reduction and conjugation. In order to comprehensively study the metabolism and enrich the metabolic profile of curcumin in vivo, we carried out this research. A systematic method with highly sensitive UPLC-Q/TOF-MS was established to analyze different biological samples of rats after oral administration of curcumin. The Waters MetabolonXTM data processing software based on mass defect filter technique was used to trace all of the probable metabolites. Based on the chromatographic behavior and fragmentation patterns of 6 reference standards isolated from the urine and feces of rats, 45 metabolites (including 12 phase .and phase metabolites) of curcumin were elucidated in the biological matrices including plasma, urine, feces, bile and tissues. Among these metabolites, 32 metabolites of curcumin were discovered for the first time. Furthermore, this study firstly reported other metabolic pathways of curcumin in rats, including demethylation, dehydroxylation and cyclization. Curcumin underwent extensive metabolism and mainly existed in the form of metabolites in rats. Conjugation with sulfate and glucuronide were the main metabolic pathways of curcumin. This project was partly supported by National Natural Science Foundation of China (No. 81430095) and also by Special National Program on Key Basic Research Project (No. 2014CB560706).

P-98
SESQUITERPENES FROM THE RHIZOMES OF CURCUMA WENYUJIN

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The rhizomes of Curcuma wenyujin Y. H. Chen et C. Ling are used as a traditional Chinese medicine for the treatment of Oketsu syndrome. Recently, our research group has been examining the constituents of C. kwangsiensis, C. phaeocaulis and C. wenyujin. As part of our ongoing research for biologically active sesquiterpenoids from the genus Curcuma and to provide a potential explanation for the usage of this species as Chinese herbal medicines for the treatment of inflammatory diseases, the essential oil of the rhizomes of C. wenyujin were fractionated using silica gel chromatography as well as HPLC to yield 16 sesquiterpenoids, 56H-elem-1,3,7,8-tetraen-8,12-olide (1), isogermafenol (2), hydroxysogermafenolide (3), 4,8-dioxo-6a-methoxy-7a,11-epoxy carabane (4), 8,11-epidioxy-8-hydroxy-4-oxo-6-carabren (5), curcumene (6), curdione (7), neocurdione (8), curdionolide B (9), isocurcumol (10), isocurcumenol (11), curcumol (12), 4-epi-curcumol (13), zedoarondiol (14), procurcumenol (15) and zedoalactone A (16). All of the isolated compounds were examined for their inhibitory effects on nitric oxide production induced by lipopolysaccharides (LPS) in RAW 264.7 cells. Hydrocortisone (64.34 µM) was used as a positive control. Most of the isolated sesquiterpenes showed potent inhibitory activities with IC50 < 30 µM. This finding provides some promising compounds which could be developed into anti-inflammatory agents for further study.

P-99
ANTI-TRYPANOSOMA CRUZI ACTIVITY OF 20 MEDICINAL PLANTS USED IN NORTH EAST MEXICO

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Chagas disease is caused by the protozoan parasite Trypanosoma cruzi. This disease is also known as American trypanosomiasis and approximately 7–8 million people are currently infected (WHO, 2014). Nifurtimox has been used for over 40 years to treat Chagas disease, however, this drug is only effective during the acute phase of infection, and certain protozoan strains have developed resistance to treatment with it (Rojas et al., 2010). The aim of this study was to screen the trypanocidal activity of plants used in traditional Mexican medicine for the treatment of various diseases related to parasitic infections. Cultured Trypanosoma cruzi epimastigotes were incubated for 96 h with different concentrations of aqueous extracts obtained from Aloe vera, Cepacria obtusifolia, Coriandrum sativum, Curcuma longa, Gymnosperma glutinosum, Haematoxylon brasiletto, Iberovilla sonorae, Larrea tridentata, Nopalea cochenillifera, Olea europea, Opuntia ficus-indica, Pachycreus marginatus, Smilax aristolochiacea, Smilax hispida, Solanum marginatum, Taraxacum officinale, Tecomä stans, Trigonella foenum-graecum, Turnera diffusa and Urtica dioica. The inhibitory concentration (IC50) was determined for each sample via a colorimetric method (Molina-Garza et al., 2014). Results: Among the evaluated species, the extracts of Iberovilla sonorae, Larrea tridentata, Olea europea and Tecomä stans exhibited the highest trypanocidal activity, showing percentages of growth inhibition between 67 and 87 %, at a concentration of 300 µg/mL, and IC50 between 100-150 µg/mL. Conclusions: The IC50 values of the most active extracts are far less effective than Nifurtimox (IC50 10 µg/mL) but these medicinal plants may represent a valuable source of new bioactive compounds for the therapeutic treatment of trypanosomiasis.

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P-100
MEDICINAL PLANTS OF THE GENUS LEONURUS
AND SEVENTEEN OF THEIR ISOLATED
CONSTITUENTS SCREENED FOR EFFECTS ON PPARα,
β/δ, and γ IN AN IN VITRO LUCIFERASE REPORTER
GENE ASSAY

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Leonurus japonicus Houtt. is used in TCM to treat the
metabolic syndrome. However, up to now, no active
constituents could be identified. Here we describe the
isolation of 17 dominant constituents of L. japonicus and the
related European herb Leonurus cardiaca L. – namely
7R-chloro-6-desoxy-harpagide, ajugol, campeoxide II,
chicoric acid, ferulic acid, harpagide, isoacetoside,
isoscampeoxide II, isoleosibirin, lavandulifolioside,
leonoside A, rutin, and verbascoside, as well as four new
phenylethanoids described here for the first time with the
preliminary names LC139C, LC138C, LC141B, and LC140A1
and their screening for activity on the metabolic syndrome
related targets PPARα, β/δ, and γ in a newly developed
luciferase reporter gene assay. All 17 isolated constituents
(at 6.25, 25, 50, and 100 μg/ml each), and GW0742 (positive
control, 0.1 nM) were dissolved in DMSO and added to the
medium of the transfected COS-1 cells. For PPARα and γ,
the positive controls WY16463 (50 μM) and troglitazone (10
μM), respectively, were used. In this assay, only
7R-chloro-6-desoxy-harpagide, which was recently isolated
by our group for the first time, displayed significant activity
in the PPARβ/δ assay at 50 μg/ml while the result for 100
μg/ml was even higher than for the GW0742 positive
control. Furthermore, rutin at 100 μg/ml showed weak
PPARγ agonistic activity. For PPAR γ no significant effects
were observed. This activity of extracts of medicinal plants
of the genus Leonurus and especially of their active
constituent 7R-chloro-6-desoxy-harpagide on the PPARβ/δ
d subtype of the PPAR system strongly indicates their
potential for anti-obesity therapy.

P-101
ANTIBACTERIAL ACTIVITY OF FIFTEEN PLANTS
FROM THE NATIONAL PARK OF KAHUZI-BIEGA
(RDC) USED IN TRADITIONAL MEDICINE.

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According to the WHO, the actual antibiotic resistance
problematic constitutes a real threat for the public health.
There is therefore an urgent need for new active
compounds resulting in an increasing interest for plants
which are an important source of secondary metabolites.
The fifteen analysed plants were selected on the basis of an
ethnobotanical survey that classified them as medicinal
plants. Interestingly, these plants also appear to be eaten by
lowland gorillas (Gorilla berengei graueri), eventually for
automedication. The plants were harvested in the National
Park of Kahuzi-Biega in DR Congo and for each plant a
voucher specimen has been deposited in the National
Herbarium of Belgium. The minimum inhibitory
concentration (MIC) of the plant extracts (methanol and
aqueous) were determined by microdilution methods
against S. aureus (methicillin susceptible and –resistant
strains) and E. coli. The initial tests showed that the
methanol extract of Pleiocarpa pyranchtha Stapf. is active
against all tested strains (from 125 μg/ml to 32 μg/ml ) and
its synergy activity in association with an antibiotic has
been also tested. Whereas the others extracts showed no
effect. Therefore we decided to test the indirect effect but
only for the plants possessing the highest probability of
exhibiting active antibacterial activity according to the
ethnobotanical survey. It appears that only Carapa
grandiflora Sprague increases the activity since we observed
a slight diminution of the MIC for both extracts with
penicillin G. The following work will consist in a bioguided
fractionation of methanol extract of Pleiocarpa pyranchtha
Stapf. and purification and identification of the active
compounds.

P-102
SUPEROXIDE RADICAL SCAVENGING AND
CYTOTOXIC EFFECTS OF DIFFERENT MARINE
SPECIES FROM TURKEY’S COASTS

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In this study, acetone extracts of thirteen different species
distributed in Turkey Coasts including tunicates, sea
anemones, sponges and corals were investigated for their
superoxide (SO) radical scavenging and cytotoxic activities.
While SO radical scavenging activity was tested using alkaline DMSO method, cytotoxic activity was tested by MTT assay against Hep-2 cancer cell line. As a result of bioactivity studies, Paramuricea clavata extract showed the strongest scavenging activity (IC50=296.81 µg/mL) and extracts of Parazoanthus clavata, Halocynthia papillosa, Eunicella cavolini and Dictyonella incisa showed moderate activity comparing to that of ascorbic acid and quercetin. However, other extracts did not show any SO radical scavenging activity in tested concentrations. In the case of cytotoxic activity, only Paramuricea clavata extract showed dose dependent cytotoxic activity (IC50=228.71 µg/mL). Paramuricea clavata extract showed both SO radical scavenging and cytotoxic activity, phytochemical studies are going to perform on this species. In addition, prooxidant effect of the extract will also be investigated to understand the mechanism of its activity.

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**P-103**

QUALITY CONTROL OF HERB MEDICINE AND TCM BY FINGERPRINTING USING EASTERN BLOTTING

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We prepared many kinds of monoclonal antibodies (Mabs) against natural products and developed a new staining method using MAb named as Eastern blotting. Natural products containing glycoside were developed by TLC and the TLC plate was covered by PVDF or PES membrane and blotted. The membrane was treated with NaIO4, and then with carrier protein like BSA resulting in glycoside-carrier protein conjugate (schiff base) on membrane. Peroxidase labeled secondary MAb and then substrate were added successively. Several ginsengs were analyzed to find out unknown ginsenosides in American ginseng, and elucidated these structures. In Panax japonicus we found two unknown ginsenosides. Therefore, we prepared an affinity column combined with antiginsenoside Rb1 MAb and purified the above crude extract. Two ginsenosides can be separated by the affinity column to isolate, individually. These ginsenosides were determined compared to the data of authentic sample of ginsenosides. Although Karopanax spp. was believed to be contained no ginsenoside, we succeeded to isolate ginsenoside Rb1 by Eastern blotting monitoring using anti-ginsenoside Rb1 MAb though very low concentration. As another application of eastern blotting method the double eastern blotting was developed. The crude extract of several Panax species were developed by TLC and blotted to PVDF membrane, and then stained by anti-ginsenoside Rb1 and Rg1 MAbS using two substrates. Pinkish and blush spots appeared, individually. From this staining we characterized two type of ginsenosides which possessed protopanaxadiol or protopanaxatriol as an aglycone in a molecule. Furthermore, this staining roughly can distinguish ginsenosides having pharmacological activity against the CNS.

**P-104**

SYNTHESIS AND TOXICITY ASSESSMENT OF DRUGGABLE THIOSEMICARBAZONES

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**Introduction:** There is a renewed interest for thiosemicarbazones in terms of antimicrobial, antiviral, and antitumor activity. With notable exceptions (e.g. thiopental) thio carbonyl-containing chemical entities are commonly considered as non-druggable. Screening of a library of thiosemicarbazones showed, however, exceptional potential. The purpose of this work was both to develop an environment-friendly method for the green synthesis of thiosemicarbazones and assess their toxicity. **Methods:** Synthesis was performed under DMF I2 complex catalysis. The characterization of the products was performed by determining the melting points and chromatographic behavior as well as 1H-NMR and 13C-NMR. Acute toxicity assay on Wistar rats was performed according to the recommendations of OECD test Nr. 423 to which were added biochemical dosages. In vitro larval toxicity test was performed according to Michael et al. **Results:** Synthesis led to 12 original thiosemicarbazones in fair to good yields. Among the prominent molecules, the best one was 9-fluorenone-4-phenylthiosemicarbazone which obtained a 99% yield. The toxicity assay on Wistar rats showed that the LD50 values of 4 druggable thiosemicarbazones were all higher than 2000 mg / Kg body weight. Larval toxicity test did not evidence any particular toxicity. **Conclusion:** Contrary to their bad reputation, synthesized thiosemicarbazones were validated as druggable leads.

**P-105**

ANTIMICROBIAL ACTIVITY OF CYMBOPOGON CITRATUS ESSENTIAL OIL AND CITRAL THIOSEMICARBAZONE AGAINST MYCOBACTERIUM TUBERCULOSIS

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Introduction: Tuberculosis, caused by Mycobacterium tuberculosis became more prevalent due to drug resistance. *Cymbopogon citratus*, a crop from the African flora contains an essential oil rich in carbonyl compounds. Condensation of carbonyl compounds with thiosemicarbazides gave thiosemicarbazones which exhibit rather interesting antimicrobial properties. We produced these compounds by an in situ semi-synthesis from *Cymbopogon citratus* essential oil. The aim of this work was to determine activity against Mycobacterium tuberculosis of citral semi-synthetic thiosemicarbazone and to compare its activity with the parent *Cymbopogon citratus* essential oil. Methods: Essential oil was extracted from the plant by hydrodistillation analysed by GC. Citral thiosemicarbazone was obtained by condensing citral and thiosemicarbazide. Antimicrobial activity was achieved using Resazurin Microtiter Assay. Results: GC analysis of the essential oil showed the presence of citral as major component (72.91%). The structure of citral thiosemicarbazone was determined by spectroscopic analysis (MS, IR, 1H-NMR, and 13C-NMR). Essential oil of *Cymbopogon citratus* inhibited in vitro growth of three Mycobacterium strains at concentrations of 17.63 µg/mL, 35.26 µg/mL, and 35.26 µg/mL while citral thiosemicarbazone inhibited all the strains at 8.81 µg/mL. Conclusion: Determination of selectivity index shows that citral thiosemicarbazone is more selective and more efficient than the essential oil of *Cymbopogon citratus*.

P-106 PHYTOCHEMICAL STUDY OF CITRUS AURANTIUM BERGAMIA: ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN JUICE, TISSUES AND ESSENTIAL OIL OF THE FRUIT

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Flavonoids are secondary metabolites from aromatic plants and one of the most present classes in the vegetable kingdom. This is thanks to their antioxidant properties but also through its benefits on the human body that these molecules have become the center of interest of many researchers in recent years. They are found in large quantities in citrus species. The aim of this study is to analyze the composition of flavonoids of two varieties of Citrus aurantium bergamia, one from Italy, from Reggio Calabria and the other original from Greece, from the island of Kefalonia, and to measure their antioxidant capacity according to the method of Folin-Ciocalteu reagent. The methanolic dried extracts of juice and various tissues of the fruit (albedo and flavedo) were analyzed by three methods, HPLC-UV, LC-MS and HPTLC. Many molecules have been detected, but only five major. Two of them are present in both varieties but in varying amounts. There are neoequoritrin, naringin, neohesperidin, melittidin and brutidin. The antioxidant activity of these samples was measured, and it turns out that the neoequoritrin present in greater amounts in the juice and tissues of bergamots of Kefalonia, trap importantly free radicals from the diphenylpicrylhydrazyl. Two additional tests were performed on each sample, TFE (total flavonoids estimation) and TPC (total phenolics compounds), in order to determine the amount of metabolites present in each sample compared to a control sample of a known concentration. The Greek variety has a higher amount of phenols and flavonoids in the juice as well as in its tissues. Moreover, the essential oil of these two fruits has been extracted by hydrodistillation and then analyzed by GC-MS. The main terpenes are linalool, d-limonene, linalyl acetate and γ-terpinene present in various amounts in each variety. However the richest metabolites amount in the oil is found in the Kefalonia’s variety.

P-107 AN EFFICIENT AND TARGET-Oriented SAMPLE ENRICHMENT METHOD FOR PREPARATIVE SEPARATION OF MINOR ALKALOIDS BY pH-ZONE-REFINING COUNTER-CURRENT CHROMATOGRAPHY

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A sample enrichment method focusing on the minor targeted components was established to help them to be successfully separated by pH-zone refining CCC. Seven minor indole alkaloids in Uncaria rhynchophylla (Miq.) Miq. ex Havil (UR) were chosen to show the advantage of this method. The sample enrichment and separation were both performed with an optimized two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (3:7:1:9, v/v), where triethylamine (TEA) as the retainer and hydrochloric acid (HCl) as the eluter were added at the equimolar of 10mm. Crude alkaloids of UR dissolved in the corresponding upper phase (containing 10 mm TEA) were extracted twice with lower phase (containing 10mm TEA) and lower phase (containing 10 mm HCl), respectively, the second lower phase extract was subjected to pH-zone-refining CCC separation after alkalinization and desalination. The total content and purities of the seven minor indole alkaloids were tested by HPLC and their chemical structures were elucidated by ESI-HRMS and 1H NMR. Results: Finally, from 10 g of crude alkaloids, 4 g of refined alkaloids was obtained and the total content of seven target indole alkaloids was increased from 4.64% to 15.78%. Seven indole alkaloids, including 54 mg isocorynoxine, 21 mg corynoxine, 46 mg...
isorhynchophylline, 35 mg rhynchophylline, 65 mg hirsutine, 51 mg hirsuteine and 27 mg geissoschizine methylether were all separated from 2.5 g of refined alkaloids, with the purity of 86.4%, 97.5%, 90.3%, 92.1%, 98.5%, 92.3%, and 92.8%, respectively. The target-oriented enrichment method has a good potential for sample preparation especially for the minor ones used for pH-zone-refining CCC separation.

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P-108
QUALITY CONTROL MARKERS OF DU-HUO-JI-SHENG-TANG IDENTIFIED ACCORDING TO ANTI-INFLAMMATORY EFFECTS
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The Du-Huo-Ji-Sheng-Tang (DHJST) is the top commercial concentrated aqueous extract used in clinics for treating osteoarthritis. However, choosing the quality control markers for DHJST is an important problem for herbal medicine industry. In this study, DHJST was divided into three groups according to the medical philosophy of traditional Chinese medicine (TCM), such as Si-Wu-Tang (SWT), wind-dampness herbs (WDH) and tonifying herbs (TH). The anti-inflammatory effects of the four kinds of extracts (DHJST, SWT, WDH and TH) were evaluated in lipopolysaccharide (LPS) and IL-1β induced primary culture of rat chondrocytes (PRC) for investigating the inflammatory cytokines. The nitric oxide (NO) inhibition effects of DHJST and the combination of three kind extracts group (SWT+WDH+TH) was found to have no difference. Each group combined with TH group showed stronger NO inhibition than without TH group. The ankle inflammatory mechanisms of TH group showed more inhibitory effects than DHJST on NO, PGE2 and MMP-13 expression and MIA-induced osteoarthritis in rat model. We suggested TH group is the major active components of DHJST. Therefore, quality control markers from TH group are important. In according to HPLC analysis, there were four major peaks from Glycyrrhizae Radix et Rhizoma (Gancao) and Cinnamomi Cortex (Rougui) in TH extract which was isolated by the chromatographic method. Therefore, we suggested the anti-inflammatory effects of DHJST were majorly caused by Gancao and Rougui, and the four compounds from above two herbs will be evaluated the anti-inflammatory activities in the future.

P-109
NEURO PROTECTIVE EFFECT OF SCHISANDRA B ON AD-RELATED CELLULAR MODEL INVOLVES THE ATTENUATION OF APOPTOSIS
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Schisandrin B (Sch B), the major lignans isolated from Schisandra chinensis, exerts high antioxidant activities. However, it is unknown whether SchB protects neural cell against Aβ induced cellular apoptosis. This study aims to investigate the neuroprotective action of SchB on cellular model of AD, and revealed the underlying mechanisms. The protective effect of SchB was studied in SH-SY5Y cells treated with Aβ1–42. The viability of the cells, the apoptosis of the cells and the level of caspase-3, caspase-9, tau hyperphosphorylation at Ser396 expression were detected by MTT, FACS and Western blotting respectively. SchB significantly increased the cell viability of Aβ treated SH-SY5Y cells. SchB also prevented Aβ induced apoptosis, reduced the number of early and late apoptotic cells and decreased expression of caspase-3, caspase-9. In addition, pretreatment of SH-SY5Y cell with SchB significantly attenuated tau hyperphosphorylation at Ser396 induced by Aβ. SchB prevented cell injuries in SH-SY5Y cells exposed to the Aβ, and this effect may depend on the antiapoptotic mechanism. Moreover, neuroprotective effect of SchB depended on decreasing tau phosphorylation. Our study provided an important evidence for the potential application of SchB in the therapy of Alzheimer's disease.

P-110
EVALUATION OF THE ESTROGENIC EFFECTS OF PLANTAGINIS SEMEN AQUEOUS EXTRACTS
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The objective was to investigate the estrogenic effects of Plantaginis Semen aqueous extracts and study the mechanism of action. Mouse uterine weight test and MCF-7 cell proliferation assay were used to evaluate the estrogenic effects of Plantaginis Semen aqueous extracts. Reporter gene assay were adopted to explore the mechanism of action of Plantaginis Semen aqueous extracts. In reporter gene assay, HEK293 cells were co-transfected with pERE-TAL-luc, pβgal-Control, pCXN2-hERa or
pON2-hER8, and the expression of reporter gene luc was controlled by ERE. Results: Compared with the control group, mouse uterine weight test showed that the aqueous extracts of Plantaginis Semen could increase the uterus index of premature female mice (p<0.01 or p<0.05). And could also promote the proliferation of MCF-7 cells (p<0.01 or p<0.05). The reporter gene controlled by ERE technology showed that when mediated by ERα or ERβ respectively, the normalized luciferase activity of aqueous extracts of Plantaginis Semen was significantly higher than the activity of the control group(p<0.01). We found the estrogenic effects of Plantaginis Semen for the first time and the estrogenic effects of Plantaginis Semen were mediated by ERα and ERβ.

P-111
ANTICANCER AGENTS FROM TRADITIONAL CHINESE MEDICINE AND NATURAL PRODUCTS

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Chemotherapy is still the effective strategy for treating cancer. It is important to explore anticancer agents from Traditional Chinese Medicine and Natural products. Different cancer cell lines were included in our research, such as HL60, K562, K562/ADR, KB, KBv200 cells. Cell growth inhibition assay, Annexin V-FITC/PI double-staining assay, measurement of reactive oxygen species (ROS) generation, determination of mitochondrial membrane potential, whole-cell lysates and western blot analysis, subcellular fractionation and western blot analysis of cytosolic cytochrome c, DOX and Rh 123 accumulation and efflux were applied to investigate anticancer activities or reversing multidrug resistance. Brucine D showed potent anti-proliferative activity against human chronic myeloid leukemia K562 cells and induced apoptosis in K562 cells via the mitochondrial pathway. Brucine D also inhibits phosphorylation of AKT and ERK which involved in MAPK and PI3K pathways respectively. Quercetin induced apoptosis in KB and KBv200 cells via the mitochondrial pathway with involvement of cytochrome c, Caspase-9 and -3. Euphorbia factor L1 could reverse multidrug resistance mediated by ABCB1 through increasing intracellular accumulation of anticancer drugs in KBv200 and K562/ADR cells, involving in inhibition of ABCB1 effluxing activities. Traditional Chinese Medicine and natural products are great treasure for developing novel anticancer drugs. Brucine D and quercetin might be candidates of anticancer agents. Euphorbia factor L1 might be applied to reverse multidrug resistance.

P-112
CHEMICAL CONSTITUTES FROM THE RHIZOMES OF MATEUCCIA ORIENTALIS AND THEIR ANTIOXIDANT ACTIVITY

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The rhizomes of Matteuccia orientalis (HOO.) TREV (Dong Fang Jia Guo Jue) have been used as traditional folk remedies for rheumatalgia and traumatic hemorrhage in China. Our earlier work on the 50% EtOH eluate from the Diaion HP 20 macroporous adsorption resin column chromatography (CC) of the 60% EtOH extract of the rhizomes of M. orientalis led to the isolation of some isocoumarin derivatives and phthalide derivatives. In our further investigation on the bioactive constituents from the rhizomes of M. orientalis, one new chromosome (1), two new bibenzyl derivatives (2-3), and one lignan (4), together with ten known compounds, were isolated from the 30% EtOH eluate from the Diaion HP 20 macroporous adsorption resin CC, which exhibited significant antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (IC50 17.3 ± 0.2 μg/mL). All the structures were determined on the basis of spectroscopic analyses. Compounds 8 and 9 (IC50 25.2 ± 0.6 and 18.0 ± 1.1 μM, respectively) exhibited significant scavenging activity equivalent to vitamin C (IC50 20.0 ± 0.4 μM). Although less active than vitamin C, compounds 10 and 11 (IC50 48.8 ± 1.4 and 45.1 ± 1.8 μM, respectively) showed higher activity than BHT (IC50 57.3 ± 4.8 μM).

P-113
FLAVONOIDS AND ITS DERIVATIVES FROM CALLISTEPHUS CHINENSIS FLOWERS AND SIMULTANEOUS DERTERMINATION BY HPLC

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The aim of the study was to study the chemical constituents of Callistephus chinensis flowers (CCF) (Chinese name is Cai Ju) and establish a method for determination of seven flavonoids in CCF by using HPLC simultaneously. The compounds were isolated by repeated column chromatography with silica gel, and HPLC. The chromatographic separation was performed on a XUion C18 column with a gradient of acetonitrile and water, at a flow rate of 1mL min-1, detected at 203 nm. Seven compounds were isolated from the EtOAc extract in the CCF. Their structures were identified as apigenin-7-O-β-D-glucoside, kaempferol, hyperin, naringenin, quercetin, luteolin, and kaempferol-7-O-β-D-glucoside. The calibration curve was
linear within 2.5-200mg L-1 for isolated compounds, respectively with the correlation r>0.999. The extraction recoveries varied from 95% to 105%. Compounds 1-7 are isolated from this plant for the first time. The method of quantitative determination is accurate and selective, and can be used for the quality control of CCF.

P-114
THE EVALUATION METHODS OF VELVET ANTLER AND THE DETERMINATION OF NUCLEOSIDES AND STEROID HORMONES IN DIFFERENT PARTS FROM SIKA DEER AND RED DEER
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Velvet antler is an important and precious traditional Chinese animal medicine, and was used for strengthening the kidney, anti-fatigue, improving sexual function and so on. But the evaluation methods of velvet antler and the scientific evidence for the use of antler is insufficient. Materials and Vacuum freeze drier was used for dehydration of the fresh antler at -60℃, 6 pa for 24 h. Chromatographic separation was performed on a Agilent HC-C18 column (250×4.6 mm i.d., 5μm) with a gradient elution program using a mixture of methanol and water solution as mobile phase. Detection wavelength was set at 254 nm for nucleosides and 205 nm for steroid hormones. Results: Cryogenic vacuum freeze processing method used in this study not only protected the thermal active ingredient but also improved the efficacy. It was better than the traditional heating and cooking in the boiling water processing. The RP-HPLC method was found to be an accurate method for nucleosides (uracil, cytidine, hypoxanthine, xanthine, thymine, inosine, guanosine, adenosine) and steroid hormones (stilbestrol, ethinylestradiol, estradiol, levonorgestrel, methyltestosterone, testosterone) from the antler. The nucleosides in the top were higher than the middle and bottom, which were the same in the sika deer and red deer. But for the steroid hormones, the middle of the velvet antler was higher than the top and the bottom. The method with good linearity, precision, repeatability, stability, recovery of the eight components and six steroid hormones was applied effectively to analyze the nucleosides in the different parts of antler. It was an effective method to evaluate the quality of velvet and also provided a scientific evidence for the use of antler.

A simple and efficient HPLC method was developed for quality analysis of flavonoids from Impatiens balsamina L. (IBL) flowers, which were collected from Xingjiang, Anhui, Henan, and Hubei provinces in China. The flavonoids substances in IBL were determined by HPLC through methyl alcohol ultrasonic extraction. A mixture of acetic and water (3:1000) as mobile phase A and acetonitril as mobile phase B; The tests showed IBL from 4 different provinces all contained compounds 1-4, and of which compounds 2 and 4 were the major flavonols. The analysis indicated that the compounds 1 and 4 showed strong inhibitory activities. The proposed method is convenient, accurate and applicable for quality analysis of flavonoids in IBL. The IBL from Hubei province with high content of 4 is a good source of anti-diabetic agents. HPLC chromatograms and quantities of compounds 1-4 in IBL from different habitats.

P-115
GLUCOSIDASE INHIBITORY ACTIVITY AND HPLC ANALYSIS OF THE FLAVONOIDS OBTAINED FROM IMPATIENS BALSAMINA L. OF DIFFERENT REGIONS
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