Joint meeting of the GP-TCM Research Association

The TCM Chemistry Specialty Committee

and the TCM Pharmaceutical Analysis Specialty Committee of

the World Federation of Chinese Medicine Societies

12th - 15th July 2015, Mons, Belgium

"Traditional medicines: Science meets culture". Joint meeting GP-TCM RA / WFCMS. Mons 2015

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 axinellae, 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, pro oxidant effect of the extract will also be investigated to understand the mechanism of its activity.

*US Harput has been supported by the Turkish Academy of Science, Young Scientist Award (TUBA-GEBIP/2013)

P-103

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

Yukihiro Shoyama
Faculty of Pharmaceutical Science, Nagasaki International University, Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan

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* speceis 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

Hope T. Sounouvou [1], Urbain C. Kassehin [1,2], Fernand A. Gbaguidi [1], and Jacques H. Poupaert [2]
1 Medicinal Organic Chemistry Laboratory, Benin Center of Ecological Drug Discovery, School of Pharmacy, FSS,UAC, 01 BP 188, Cotonou, Bénin
2 Medicinal Chemistry, Louvain Drug Research Institute, UCL, Av. E. Mounier, 73, 1200 Brussels, Belgium

Introduction: There is a renewed interest for thiosemicarbazones in terms of antimicrobial, antiviral, and antitumor activity. With notable exceptions (e.g. thiopental) thiocarbonyl-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.I_2 complex catalysis. The characterization of the products was performed by determining the melting points and chromatographic behavior as well as ^{1}H-NMR and ^{13}C-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

Habib Toukourou[1], Amoussatou Sakirigui [2], Georges C. Accrombessi[2], Joëlle Quetin-Leclercq[3], Jacques H. Poupaert[4] and Fernand A. Gbaguidi[1,2]
1 Medicinal Organic Chemistry Laboratory, Benin Center of Ecological Drug Discovery, School of pharmacy/ UAC, 01BP 188, Cotonou, Bénin
2 Laboratoire de Chimie Organique Physique et de Synthèse, UAC 01BP 4521, Cotonou, Bénin
3 Pharmacognosy, Louvain Drug Research Institute, UCL, Av. E. Mounier 72, 1200 Bruxelles, Belgium
4 Medicinal Chemistry, Louvain Drug Research Institute, UCL, Av. E. Mounier, 73, 1200 Brussels, Belgium

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 hemi-synthesis from *Cymbopogon citratus* essential oil. The aim of this work was to determine activity against Mycobacterium tuberculosis of citral hemi-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 many compounds including citral as major component (72.91%). The structure of citral thiosemicarbazone was determined by spectroscopic analysis (MS, IR, ^{1}H-NMR, and ^{13}C-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

E. Tsiokanos [1], N.Tsafantakis [2], C. Kanakis [2], C.Stévigny[1], A-L. Skaltsounis[2]
1 Laboratoire de Pharmacognosie, Bromatologie et Nutrition humaine, Université Libre de Bruxelles, ULB, 1050 Bruxelles.
2 Laboratoire de Pharmacognosie et Chimie des substances naturelles, Université Nationale et Capodistrienne d'Athènes, Panepistimiopolis Zografou 15771 Athènes

Flavonoids are secondary metabolites from aromatic plants and one of the most present classes in the vegetable kingdom. This is thanks to its 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 neoeriocitrin, naringin, neohesperidin, melitidin and brutieridin. The antioxidant activity of these samples was measured, and it turns out that the neoeriocitrin 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

Rui-Hong Feng[1], Jin-Jun Hou[1], Yi-Bei Zhang, Hui-Qin Pan, Peng Qi, Shuai Yao, Lu-Ying Cai, Min Yang, Baohong Jiang, Xuan Liu, Wan-Ying Wu[*], De-An Guo[*]
Shanghai Research Center for Modernization of Traditional Chinese Medicine, National Engineering Laboratory for TCM Standardization Technology, Shanghai Institute of Materia Medica, Chinese Academy of Science, Shanghai 201203, China

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-methanolwater (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 alkalization 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 isocorynoxeine, 21 mg corynoxeine, 46 mg