

## 1. A Metabolomics Approach to Investigate Kukoamine B—A Potent Natural Product With Anti-diabetic Properties

Y.Y. Li, D.A. Stewart, X.M. Ye, L.H. Yin, W.M. Pathmasiri, S. L. McRitchie, T.R. Fennell, H.Y. Cheung, S.J. Sumner

*Frontiers in Pharmacology*, January 2019, Volume 9, Article 1575.

### **Abstract**

*Due to the surge in type 2 diabetes mellitus (T2DM), treatments for chronic metabolic dysregulations with fewer side-effects are sought. Lycii Cortex (LyC), a traditional Chinese Medicine (TCM) herb has a long history of being widely prescribed to treat T2DM as alternative medicine; however, the bioactive molecules and working mechanism remained unknown. Previous studies revealed kukoamine B (KB) as a major and featured compound for LyC with bioactivities for anti-oxidation and acute inflammation, which may be related to anti-diabetes properties. This study aims to understand the efficacy and the mode of action of KB in the diabetic (db/db) mouse model using a metabolomics approach. Parallel comparison was conducted using the first-line anti-diabetic drugs, metformin and rosiglitazone, as positive controls. The db/db mice were treated with KB (50 mg kg<sup>-1</sup> day<sup>-1</sup>) for 9 weeks. Bodyweight and fasting blood glucose were monitored every 5 and 7 days, respectively. Metabolomics and high-throughput molecular approaches, including lipidomics, targeted metabolomics (Biocrates p180), and cytokine profiling were applied to measure the alteration of serum metabolites and inflammatory biomarkers between different treatments vs. control (db/db mice treated with vehicle). After 9 weeks of treatment, KB lowered blood glucose, without the adverse effects of bodyweight gain and hepatomegaly shown after rosiglitazone treatment. Lipidomics analysis revealed that KB reduced levels of circulating triglycerides, cholesterol, phosphatidylethanolamine, and increased levels of phosphatidylcholines. KB also increased acylcarnitines, and reduced systemic inflammation (cytokine array). Pathway analysis suggested that KB may regulate nuclear transcription factors (e.g., NF-κB and/or PPAR) to reduce inflammation and facilitate a shift toward metabolic and inflammatory homeostasis. Comparison of KB with first-line drugs suggests that rosiglitazone may over-regulate lipid metabolism and anti-inflammatory responses, which may be associated with adverse side effects, while metformin had less impact on lipid and anti-inflammation profiles. Our research from holistic and systemic views supports the conclusion that KB is the bioactive compound of LyC for managing T2DM, and suggests KB as a nutraceutical or a pharmaceutical candidate for T2D treatment. In addition, our research provides insights related to metformin and rosiglitazone action, beyond lowering blood glucose.*

## **2. Chemical Profiling of *Lobelia chinensis* with High-Performance Liquid Chromatography/Quadrupole Time-of-Flight Mass Spectrometry (HPLC/Q-TOF MS) Reveals Absence of Lobeline in the Herb**

H.X. Wang, Y.Y. Li, Y.Q. Huang, C.Y. Zhao, H.Y. Cheung

*Molecules*, 2018, 23(12), 3258

### **Abstract**

*Lobelia chinensis* is a kind of herbal medicine widely distributed and used in Asia. The chemical components of this herb, however, have not been well studied until now. Lobeline, as an essential and famous bioactive compound in *Lobelia* genus, has been assumed to be present in *L. chinensis*. In order to ascertain its presence and, more importantly, proper use of this herb, chemical profiling this herb with highly sensitive and high-resolution analytical mass spectrometry was applied. In this study, high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (HPLC/Q-TOF MS) method was employed to systematically profile the chemical constituents of *L. chinensis* for the first time. Comparative chemical profiling study of *L. chinensis* and *Lobelia inflata* was also conducted to provide evidence whether lobeline is present or not. Piperidine alkaloids except for lobeline, alkaloid-lignan hybrids, flavonoids, polyacetylenes, nonanedioic acid, and some new phytochemicals were successfully identified in *L. chinensis* simultaneously. Comparing to the chemical profiles of *L. inflata*, lobeline was found to be absent in *L. chinensis*. All of the secondary metabolites in *L. chinensis* were determined with the HPLC/Q-TOFMS method. The absence of lobeline in *L. chinensis* was confirmed after this extensive study.

## **3. Asparagi Radix Dried at Ambient Air Temperature while Lycii Fructus at Freezing Temperature Favours the Production of Its Native and Non-cohesive Fine Powder by Centrifugal Impact Pulverizer**

H.Y. Cheung, F. Wang, T.C.K. Lau

*Hong Kong Pharmaceutical Journal*, 2018, 25(1), 16-21

### **Abstract**

Some raw foods and medicinal herbal substances are unsuitable for drying at conventional high temperature range whenever there is high content of water and mucilage substances. If they were air dried above 80°C, they could be discolored as well as coagulated into masses. This change in physical property affects their flowability and subsequent handling. After systematic investigations, we show that a suitable drying condition could be established for successful pulverizing them. *Asparagi Radix* could be dried at an ambient air temperature not more than 50°C, while *Lycii Fructus* could be freeze dried for sufficient

time prior to crushing in order to produce native and non-cohesive fine powder by centrifugal-impact pulverizer.

#### **4. Atractylodis Rhizoma - Its Phytochemistry, Biological Activities and Medicinal Uses**

K. W. To, T.C.K. Lau, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2017, 24(4), 145-148

##### Abstract

*Atractylodis Rhizoma*, also known as Cangzhu, is the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz of the Asteraceae family. The former is a plant indigenous to central China, Japan and Korea while the later to northern China. The knotty-lumpy rhizome of these two plants are used as a medicine. They are dug in spring or autumn and dried. When use the herb is sliced after soaked with water. Due to its high content of atractylodin, which is one of the major volatile oil, *atractylodes* is used for strengthen digestion, treatment of stomachache, bloating, fluid retention, diarrhea, loss of appetite and weight loss due to cancer, allergies to dust mites and swelling joint pain. When combined with other herbs, it also has anti-lung cancer effect.

#### **5. Comparison of the Constituents and Functions of White and Red Ginseng Prepared from *Panax ginseng***

J.L. Tsai, V. Wong, T.C.K. Lau, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2017, 24(4), 137-144

##### Abstract

*Ginseng* is a medicinal plant widely used for various purposes. It has been used for promoting immunity, neurological function, relief stress, and for prevention of cancer based on its antioxidant activities. The biological effects of ginseng have been observed in people with cancers, diabetes or cardiovascular diseases. *Panax ginseng* C. A. Meyer, also known as Asian ginseng, is a valuable and important source for both white and red ginseng. The major bioactive components of this herb are ginsenosides, of which about 80 ginsenosides have been identified and isolated. However, the ginseng roots, which are white in nature, could be changed into red whenever it is steam-heated. It has been claimed that they have different effects on human health. In this article, the chemical components and structures, biological effects and pharmacological properties of both white and red ginseng are reviewed. Bioactive constituents, such as polysaccharides, ginsenosides, peptides, polyacetylenic alcohols, fatty acids and mineral oils of the white ginseng and red ginseng were compared. Biological activities, including anti-aging activities, anti-diabetic activities, immunoregulatory activities, anti-cancer activities and neuroregulation activities were described and contrasted. Based on the current available data and information, it is concluded that further research

studies are necessary in order to fully unveil the differences between these two types of ginseng.

## **6. Chemical Components and Biological Activities of *Angelicae sinensis* (Danggui) and Its Closely Related Species**

H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2017, 24(3), 105-109

### Abstract

*Danggui (DG), also known as Chinese Angelica Root, is considered one of the most important remedies in traditional Chinese medicine for female ailments. It is the dried root of Angelicae sinensis. It can promote blood circulation. Recent analytical studies reveal that phthalides, aromatic acids and their esters, polysaccharides are the main chemical components determining its bioactivities. Pharmacological studies indicate that DG inhibits platelet aggregation, dilates coronary arteries, lowers blood lipid levels, promotes the formation of hemoglobin and red blood cells, bi-directionally regulates uterine smooth muscles, and has anti-thrombotic, anti-arrhythmic, anti-inflammatory and analgesic effects. Efficacy of this herbal substance, however, could be affected by which parts of the herb used and also by the processing method.*

## **7. Taxifolin synergizes Andrographolide-induced cell death by attenuation of autophagy and augmentation of caspase dependent and independent cell death in HeLa cells.**

M.Alzaharna, I. Alqouqa, H.Y. Cheung

*PLoS ONE* 12(2):e0171325. Published: 9 Feb 2017

### Abstract

*Andrographolide (Andro) has emerged recently as a potential and effective anticancer agent with induction of apoptosis in some cancer cell lines while induction of G2/M arrest with weak apoptosis in others. Few studies have proved that Andro is also effective in combination therapy. The flavonoid Taxifolin (Taxi) has showed anti-oxidant and antiproliferative effects against different cancer cells. Therefore, the present study investigated the cytotoxic effects of Andro alone or in combination with Taxi on HeLa cells. The combination of Andro with Taxi was synergistic at all tested concentrations and combination ratios. Andro alone induced caspase-dependent apoptosis which was enhanced by the combination with Taxi and attenuated partly by using Z-Vad-Fmk. Andro induced a protective reactive oxygen species (ROS)-dependent autophagy which was attenuated by Taxi. The activation of p53 was involved in Andro-induced autophagy where the use of Taxi or pifithrin- $\alpha$  (PFT- $\alpha$ ) decreased it while the activation of JNK was involved in the cell death of HeLa cells but not in the induction of autophagy. The mitochondrial outer-membrane permeabilization (MOMP) plays an important role in Andro-induced cell death in HeLa cells. Andro alone increased the MOMP which*

was further increased in the case of combination. This led to the increase in AIF and cytochrome c release from mitochondria which consequently increased caspase-dependent and independent cell death. In conclusion, Andro induced a protective autophagy in HeLa cells which was reduced by Taxi and the cell death was increased by increasing the MOMP and subsequently the caspase-dependent and independent cell death.

## **8. Quality control of Lycium chinense and Lycium barbarum cortex (Digupi) by HPLC using kukoamines as markers.**

Y.Y. Li, R.Di, W. L. Hsu, Y. Q. Huang, H.Y. Cheung

*Chinese Medicine*, 2017, 12:4, DOI 10.1186/s13020-016-0121-x

### Abstract

*Background:* Lycii Cortex (LyC), composed of Lycium chinense and Lycium barbarum cortex and having the Chinese name Digupi, is used to treat chronic diseases like cough, hypertension, and diabetes in Eastern Asia. However, chromatographic methods, such as TLC and HPLC, to determine the phytochemical composition of LyC have not been included in any official compendiums. This study aims to establish a validated HPLC method for quality control of LyC.

*Methods:* Kukoamines A and B (KA and KB, respectively) were selected as markers for the HPLC method. An acetic acid solution was adopted for sample extraction because it facilitated the release of kukoamines and effectively prevented their degradation. Optimal separation of the kukoamine isomers was achieved on hydrophilic ligand coated C18 columns with a gradient elution of acetonitrile and 0.1% (v/v) trifluoroacetic acid. The average contents and proposed contents for LyC were calculated with a t test and an uncertainty test based on 16 batches of authentic samples.

*Results:* The method was validated with linearity ( $r^2 = 0.9999$  for both KA and KB), precision (RSD = 1.29% for KA and 0.57% for KB), repeatability (RSD = 1.81% for KA and 0.92% for KB), and accuracy (recovery of 90.03–102.30% for KA, and 98.49–101.67% for KB), indicating that the method could offer reliable results for quality control analysis of LyC. At the 95% confidence level, the calculated content limits were 1.45 mg/g for KA and 4.72 mg/g for KB.

*Conclusion:* Compared with conventional morphological identification, the HPLC method involving KA and KB contents offers precise, objective, and quantitative results for quality control of LyC.

## 9. Lipidomic Profiling of Dried Seahorses by Hydrophilic Interaction Chromatography Coupled to Mass Spectrometry.

Q. Shen, Z. Dai, Y. W. Huang, H.Y. Cheung

*Food Chemistry*, 2016, 205, 89-96

### Abstract

*Dried seahorse is a precious raw food material for cooking soups. In this study, a lipidomics strategy using the techniques of solid-phase extraction (SPE) and hydrophilic interaction chromatography–tandem mass spectrometry (HILIC–QTOF/MS) was developed for extraction, visualization, and quantification of phospholipids in dried seahorses. The parameters of SPE were optimized, and 1 mL of sample and chloroform/methanol (1:2, v/v) were found to be the best loading volume and eluting solvent, respectively. Afterwards, each phospholipid class was successfully separated on a HILIC column and analyzed by mass spectrometry. A total of 50 phospholipid molecular species were identified and determined, including 15 phosphatidylcholines (PCs), 14 phosphatidylethanolamines (PEs), 12 phosphatidylinositols (PIs) and 9 phosphatidylserines (PSs). In comparison to previously methods, this strategy was robust and efficient in extraction, characterization, and determination of phospholipids. The dried seahorse was found to contain large amounts of polyunsaturated fatty acyl phospholipids which are beneficial to human health.*

## 10. Determination of Antioxidants in Smilacis Glabrae Rhizoma by High-Performance Liquid Chromatography with Ultraviolet and Mass Spectrometry Detection.

Z.F. Zhang, L.Y. Lu, Y. Liu, R. Zeng, J. Xie, Y.Q. Huang, Q.Shen, H.Y. Cheung

*Analytical Letters*, Accepted author version posted online: 18 Feb 2016

### Abstract

*Smilacis Glabrae Rhizoma is a traditional food in China. Although phytochemical and pharmacology studies have been performed, the antioxidant constituents have not been characterized with the exception of astilbin. In the present study, six antioxidants in Smilacis Glabrae Rhizoma were determined by high-performance liquid chromatography with ultraviolet and mass spectrometry detection using 1, 1-diphenyl-2-picrylhydrazyl and 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) assays. These analytes were 5-O-caffeoylshikimic acid, polydatin, neoastilbin, astilbin, neoisoastilbin, and isoastilbin. The relative Trolox equivalent antioxidant capacity of each compound was determined using the two protocols. Moreover, antioxidant fingerprints were constructed for quality evaluation of Smilacis Glabrae Rhizoma and principal component analysis was performed to provide a guide for customers and producers to*

ensure the quality of the raw materials. The methodology may be employed for the analysis of *Smilacis Glabrae Rhizoma* and potentially for other plant-based products.

## **11. Identification and Characterization of Kukoamine Metabolites by Multiple Ion Monitoring Triggered Enhanced Product Ion Scan Method with a Triple-Quadrupole Linear Ion Trap Mass Spectrometer.**

Y.Y. Li, H.X. Wang, C.Y. Zhao, Y.Q. Huang, X.W. Tang, H.Y. Cheung

*Journal of Agricultural and Food Chemistry*, 2015, 63, 10785-10790

### Abstract

*Kukoamines are a series of bioactive phytochemicals conjugated by a polyamine backbone and phenolic moieties. Understanding the structural diversity of kukoamine metabolites in plants is meaningful for drug discovery. In this study, an LC-MS/MS method was established for kukoamine profiling and characterization from lycii cortex (LyC) via a triple-quadrupole linear ion trap mass spectrometry (Q-TRAP). On the basis of the typical fragmentation of kukoamine, a diagnostic ion, which represents the features of the backbone and phenolic substitute, was chosen as the product ion for precursor ion scan, and then the screened precursor ions were applied to a successive multiple ion monitoring triggered enhanced product ion scan (MIM-EPI) to simultaneously present the profile survey and MS/MS acquisition. Because the MIM narrowed the ion scan range in Q1 and the ion trap enhanced the ion fragments passing through Q2, the qualitative capability of quadrupole MS can be greatly improved, especially for capture of the uncommon metabolites. There are 12 kukoamine metabolites identified from LyC, with either spermine or spermidine backbone and with conjugation of one to three dihydrocaffeoyls or other kinds of phenolic moieties. Except for kukoamines A and B, other metabolites were identified in LyC for the first time. This approach can be utilized for metabolite identification in other substrates.*

## **12. Differences between Tufuling and Fuling Based on Their Chemical Constituents, Biological Effects and Medicinal Use.**

H.Y. Cheung, C.H.F. Chan, V. Wong

*Hong Kong Pharmaceutical Journal*, 2015, 22, 111-115

### Abstract

*The scientific name for Tufuling and Fuling is Smilax Glabra Rhizoma and Poria Cocos Sclerotia, respectively. Due to the similarity between these herbs, whether in their Chinese and English names or in their morphological appearance when sliced thin, it can be so hard to distinguish them from each other. Our survey reveals that the latter, instead of the former, has been used as one of the main ingredients in Gui-ling-gao in a few brands on the market since 2009. It is important to*

*note, these two herbs are actually two different species and would give rise to different medicinal effects on the human body. This review article aims to differentiate the content of their bioactive components and medicinal uses based on what were reported in the literature. It is concluded that they should not be used interchangeable.*

### **13. Potential neuroprotective effects of herbal compounds**

J.T. Baibado, H.Y. Cheung

*International Journal of Pharmaceutical and Biological Science Archive, 2015, 3, 24-26*

#### Abstract

*Herbal bioactive compounds have been investigated to possess neuroprotective properties. They are involved in the modulation of different signaling pathways that may facilitate neuroprotection. In this brief review, some of the promising compounds and their potential neuroprotective effects have been reported. They can be potential sources of therapeutics for neurodegenerative disorders.*

### **14. Biological functions of the metabolites from *Euphorbia hirta* L.**

J.T. Baibado, H.Y. Cheung

*International Journal of Pharmaceutical and Biological Science Archive, 2015, 3, 27-29*

#### Abstract

*Euphorbia hirta L. contains various biologically active compounds that can be beneficial to human health. It has been known for its antibacterial, antioxidant, and anti-inflammatory effects. In addition, recent findings have shown that it has anti-venom, wound healing property, anti-cancer, anti-diabetic, and even molluscicidal effects. Although it has folkloric use against dengue fever and oral herpes, further study should be done to confirm its antiviral properties. However, it may have histopathologic adverse effects which are dose dependent.*

**15.** H.Y. Cheung, Y. Y. Li, R. Di. "Process for isolating kukoamine". US patent No: US9012687B2. 2015.07.29

**16.** H.Y. Cheung, M. Yang, S.H. Cheung. "Comprehensive Authentic System for the Quality Assurance of Edible Bird's Nest Based on Its Physical, Chemical and Biochemical Properties". PRC patent No: CN103364497B. 2015.04.21.

## 17. Chemical Constituents and Bioactivities of Plantaginis Herba

H.X. Wang, C.Y. Zhao, Y.Q. Huang, F. Wang, Fang, Y. Y. Li, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2015, 22, 29-35.

### Abstract

*Plantago*, which is derived from the dried whole plant of *Plantago* species (*Plantaginaceae*), is a herbal medicine distributed and utilized worldwide for centuries. *P. asiatica* L. and *P. depressa* Willd. are the endorsed official sources of the herb in China. The former is also an official medicinal herb in Japan while *P. lanceolata* L. is commonly used in Europe. Apart from these official species, many other species such as *P. major* L. and *P. ovolata* L. are used in folk medicines for the treatment of a variety of diseases such as hematuria, chronic inflammation and constipation. In addition to common primary metabolites, a great number of bioactive constituents have been isolated from the herb, e.g. phenylethanoid glycosides, flavonoids, iridoids, triterpenoids, phenolic acids and so on. A variety of useful biological activities such as diuretic, anti-inflammatory, antioxidative, antiviral, antibacterial and immunomodulatory effects was reported, while very few contraindications have been described. This article aims to review (1) the complexity of this herb; (2) what components of this herb promote human health and (3) what sorts of scientific evidences are available.

## 18. Preventing H<sub>2</sub>O<sub>2</sub>-induced toxicity in primary cerebellar granule neurons via activating the PI3-K/Akt/GSK3 $\beta$ pathway by kukoamine from Lycii Cortex

Y. Y. Li , S.Q. Hu, Y.Q. Huang, Y.F. Han, H.Y. Cheung

*Journal of Functional Foods*, 2015,17, 709-721.

### Abstract

*Lycii Cortex* (LyC) is widely used as a traditional anti-ageing food ingredient in soup, congee and tea for preventing degenerative disease. However, its bioactive components and modes of action have never been characterized scientifically. The aims of this study are to: 1) examine the neuroprotective effects of LyC against oxidative stress; 2) identify the bioactive components; 3) elucidate the possible molecular mechanisms of the key components. Phytochemical profiling using LC-MS/MS revealed that 7-(4-amino-butoxy)-6-methoxycoumarin, kukoamines A and B, and lyciumins A and B are the major characteristic compounds in LyC. LyC crude extract and kukoamines were effective in preventing cell damage in the H<sub>2</sub>O<sub>2</sub>-induced primary cerebellar granule neurons (CGNs) model, but kukoamines were more efficacious and potent than the extract. Western blot analysis revealed that KB, the most abundant kukoamine, was capable of inhibiting the neuronal apoptosis via activating the PI3-K/Akt/GSK3 $\beta$  pathway. Thus, our explorative results indicated that kukoamine, the major compounds in LyC, acts as a promising neuroprotectant

against the H<sub>2</sub>O<sub>2</sub> induced toxicity in CGNs model via activating the PI3-K/Akt/GSK3 $\beta$  pathway. Furthermore, molecular insight to substantiate the application of LyC or kukoamines for prevention and treatment of neurodegenerative diseases in which oxidative stress is involved is demonstrated.

## **19. Sensitivity improvement of kukoamine determination by complexation with dihydrogen phosphate anions in capillary zone electrophoresis**

Y.Y. Li, R. Di, H.W. Leung, Y.Q. Huang, H.Y. Sun, H.Y. Cheung

*Electrophoresis*, 2015, 36, 1801-1807

### Abstract

A novel complexation between kukoamines and dihydrogen phosphate ions (DPI) during capillary zone electrophoresis (CZE) was discovered to improve the UV signal of kukoamine by around 30-fold. This complexation formed by electric current was attributed to the hydrogen bonding of hydroxyl and amino (or amide) groups between the analyte and electrolyte anions. The established CZE method is low-cost, easy to operate, and eco-friendly, and it was shown to be superior to HPLC in terms of separation capability, efficiency, specificity, and sensitivity. We believe that our CZE method can be applied as an alternative to HPLC for kukoamine assay. The approach described here can be also extended for analyzing other compounds with similar functional groups.

## **20. Improvement of reproducibility and sensitivity by reducing matrix effect in micellar electrokinetic chromatography for determination of amino acids in turtle jelly.**

L.Q. Li, Y. Cai, M. Yang, Q. Shen, K. M. Yu, H. Y. Cheung

*Electrophoresis*, 2015, 36, 1186-1195

### Abstract

Matrix effect (ME) is commonly seen in electrophoretic separation, but this phenomenon lacks any systematic study. Our work aimed to find out the relationship between separation efficiency and current, and then figure out an effective, simple, and economic solution to overcome the negative impact of ME. This present study showed that small amount of NaCl ( $\leq 0.005$  mg/mL) in the sample had no impact on the separation but enhanced the sensitivity. However, when concentration of NaCl increased above 0.005 mg/mL, it alleviated the separation efficiency, sensitivity, and migration time. Besides, increasing NaCl concentration resulted in increasing turning point (TP). The study of relationship of current and NaCl concentration indicated that when the TP of a sample is higher than 62.36  $\mu$ A, desalination is necessary. Since the reported desalination methods are either expensive or complicated, we developed a simple and economic method by simply adding

12 times (volume) of chloroform/methanol (2:1, v/v) into the sample. When applied this method to turtle jelly, the number of theoretical plate (N) of 20 amino acids got up to 3-fold enhancement. This article is protected by copyright. All rights reserved. This article is protected by copyright. All rights reserved.

## **21. Phytochemical profiles, antioxidant activities of functional herb *Abrus cantoniensis* and *Abrus mollis***

M. Yang, Q. Shen, L.Q. Li, Y.Q. Huang, H.Y. Cheung

*Food Chemistry*, 2015, 177, 304-312

### Abstract

*It has been claimed that consumptions of *Abrus cantoniensis* (AC) and *Abrus mollis* (AM) as folk beverages and soups are good to cleanse liver toxicants and prevent liver diseases. There is scant information on the phytochemical profiles and antioxidant activities of these two varieties. Five major phytochemicals in these two cultivars were qualitatively and quantitatively compared using UPLC-PDA. A high level of total phenolic content (TPC) and total flavonoid content (TFC) was found in AC and AM. AC, in general, showed some antioxidant activities comparable to that of BHT, and stronger radical scavenging activities and higher reducing power than that of AM ( $p < 0.05$ ). When principal component analysis (PCA) was applied, high correlation between TPC, TFC and their antioxidant activities was found. Hence, this study proved that, both AC and AM could serve as antioxidant-rich component in foods or beverages to promote health function.*

## **22. Antioxidants purified from *Hedyotis Diffusa* Herba enhanced the apoptosis of ATRA-induced HL-60 cell**

C.K. Pun, C.Y. Zhao, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2014, 21, 25-32

### Abstract

*Antioxidants, such as p-coumaric acid and caffeic acid purified from *Hedyotis*, were found to enhance the anti-cancer activity of all-trans retinoic acid (ATRA) on human promyelocytic leukaemia cells (HL-60). The medium Lethal Dose (LD50) of the p-coumaric acid and caffeic acid was 0.205 and 0.017 mg/mL, respectively, while the LD50 of ATRA, which was the lowest amongst the three compounds, was 0.002 mg/mL. When each of these antioxidants was mixed with ATRA in different ratio, different effects were noted. Combining p-coumaric acid antagonism at low ratio ranges but became synergistic above 0.4 affected fractions. This situation was the same in different combination of caffeic acid and ATRA. The combination index indicated that synergism took place in higher mixing ratios. Confocal microscopic photos of the cell revealed that some HL-60 cells differentiated into polymorphonuclear leukocytes, which is a typical programmed cell death after*

treatment with the ATRA. The formation of polymorphonuclear features was more severe whenever these antioxidants were added to the ATRA-induced apoptosis of HL-60 cells. The actual mechanism of synergetic effect, however, was not known.

## **23. Isolation and purification of cyclicpeptides from the root bark of Lycii Cortex**

H.S.C.W. Leung, Y.Y. Li, R. Di, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2014, 21, 59-64

### Abstract

*Lycii Cortex (LyC) is Chinese medicine used extensively for the treatment of diabetes, lung disease, hematemesis, hypertension and inflammation. Today, more than 50 compounds, some are octapeptides, which exhibit significant renin and ACE inhibitory activities. Since ACE inhibitors are correlated to the hypoglycemic effect, it is reasonable to speculate that these octapeptides might be responsible for the anti-diabetic function of LyC. In order to obtain sufficient quantity and high quality of the peptides for both in vitro and in vivo studies of their anti-diabetic effects, it is necessary to extract and purify these substances. However, there is no suitable method available or reported so far. The goals of this study were (1) to extract and isolate the peptides from the bark, (2) to separate and purify them to a level that meets the criteria of the Hong Kong Materia Media Standards, (3) to confirm the structure of purified substances by the LC-MS and NMR. In this proposed study, lyciumins A and B were isolated from the extract solution. After preliminary separation through a Sephadex LH-20, eluent was passed through a C18 column to enrich the content of octapeptides. Purified compounds were obtained through a final purification step on a semi prep-HPLC. Under optimized condition in the LH-20 column, two cyclicpeptides were prepared from the bark of Lycii Cortex in large quantity. After passing through the dispersive solid phase matrix followed by prep-HPLC method, Lyciumins A and its isomer, Lyciumins B, were isolated and obtained from the crude extract. UV-adsorption spectrum of these two fractions showed a characteristic peak of peptide bond; thus, it was assumed the presence of some peptide compounds. After further characterization using the LC-MS methodology, it revealed that these two compounds have the same side chain and ring structure identical to what have been described in literatures for Lyciumins. Through these series of treatment, both Lyciumins A and B were obtained with a purity over 98% was achieved.*

## 24. Chemical Constituents and Biological Activities of *Lobelia chinensis*

H.X. Wang, C.Y. Zhao, Y. Y. Li, Y.Q. Huang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2014, 21, 108-211

### Abstract

*Lobelia chinensis* Lour is a species of commonly used herb in traditional Chinese medicine. It is listed in current Chinese Pharmacopoeia and conventionally utilized for cleansing body heat and toxins. Although this herb is belonged to the genus *Lobelia*, its chemical constituents and biological activities are different from *L. inflata*, which is an indigenous species in North America. The major bioactive constituents of *L. chinensis* are secondary metabolites belonged to piperidine alkaloids and flavonoids. These chemical components offer various health benefits, including anti-virus, anti-cancer and choloretic effects which have been confirmed and validated using modern pharmacological methods. In this article, some recent researches of the chemical constituents, biological activities and the medicinal applications of *L. chinensis* are reviewed.

## 25. Biochemical and Biological-Based Studies of Asian Ginseng

Y.X. Wang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2013, 20, 33-45

### Abstract

*Panax ginseng* C.A.Mey (*P. ginseng*), one of the most popular and best-selling herbs worldwide, has been used as a valuable folk medicine for thousands of years. It contains triterpenoid saponins, polysaccharides, volatile oils, polypeptides, polysacetylenes, amino acids and other bioactive compounds. The major bioactive components of this herb are triterpenoid saponins, including ginsenosides Rb1, Rb2, Rc, Rg, Re and Rf. Of which ginsenoside Rf is the most characteristic component. Biological vice, the herb has CNS-regulating, substance metabolism improving, age-delaying, immune system boosting, hematopoiesis promoting, anti-myocardial-ischemia, endocrine-enhancing and anti-tumor effects. In this review, an emphasis has been placed upon the biological activity and clinical applications associated with ginsenosides, particularly with respect to some new discoveries based on recent studies from chemoprofing studies and system biology.

## 26. “Chinese Medicines are Toxic” is a Misconception

W.M. Lo, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2013, 20, 120-123

### Abstract

*Recent widespread rumors that “Chinese Medicines are Toxic” are misunderstood. As experts commented whether a substance is toxic or not, very often depends on its physicochemical form, part, method of processing, and period of consumption. Depending on how and when it is used, the effect of Chinese Medicines (CM) could also be significantly different. Various results have been described and it is misleading to be uniformly saying that they are toxic. In Europe and the United States, most CM are regulated under food standards and safety. This reflects that the majority of CM are safe as long as they are properly prescribed and used. Since the philosophical approach between Western and Chinese medical practice is different, consumers have frequently been misled by the media on the effects of CM. The worse aspect of CM is their improper use by people as far as the safety issue of CM is concerned.*

## 27. Distinction of the Bulbs of *Fritillariae thunbergii* Miq., *Fritillariae ussuriensis* Maxim and *Fritillariae huperhensis* Hsiao et K.C. Hsia by concert techniques

Z.F. Zhang, T.J. Baibado, Y.J. Xu, Y.S. Cheung, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2013, 20, 208-211

### Abstract

*Fritillariae bulbus (FB), Known as Beimu in Chinese, is an herb commonly used as antitussive and expectorant in Traditional Chinese Medicine (TCM). There is difficulty in distinguishing the authentic species in herbal markets due to the complexity of its botanical origin and its similar morphology with other species. Therefore, a comparative analysis was undertaken through macroscopic and microscopic characterization and thin layer chromatography (TLC) analysis. The three FB species that were investigated include *Fritillariae thunbergii* Miq., *Fritillariae ussuriensis* Maxim and *Fritillariae huperhensis* Hsiao et K.C. Hsia. The fixed, sectioned, and stained plant materials, as well as its crude powder were studied using bright field microscopy according to the usual microscopic techniques. The results of the microscopic features were systematically and comparatively described and illustrated. The three species have distinct microscopic characteristic differences. The TLC profile was developed to show their different chemical characteristics. The methods employed distinguish clearly the three species; thus, providing specific criteria by which samples of these bulbs can be identified.*

## **28. Concert Approach to the Authentication, Qualitative Evaluation and Bioactivity Assessment of Beimu**

Y.X. Wang, Q. Shen, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2013, 20, 212-219

### Abstract

*Beimu*, which was first recorded more than two thousand years ago as a medium-grade medicinal material in *Shen Nong Ben Cao Jing*, is the dried bulb of *Fritillaria cirrhosa* D. Don (*chuan-beimu*), *F. thunbergii* Miq. (*zhe-beimu*), *F. ussuriensis* Maxim (*ping-beimu*) or other officially endorsed species of *Fritillaria* genus. These bulbs are botanical source for various pharmaceutical active components. The bulbs of genus *Fritillaria* all contain cervine-type steroidal alkaloid, which possess anti-tussive, anti-coughs, sedative and antibacterial activities. Their contents, biological effects and prices, however, vary significantly. Hence, deliberate or unintentional adulteration of *beimu* is common in the market. This article reviews some information and methods with aims to differentiate each species. Based on the data of their morphological traits and analysis of chemical profile, all these species could be authenticated and their quality could be determined. Hence, their benefits to health could be maximized.

## **29. Identification of kukoamines as the novel markers for quality assessment of Lycii Cortex**

Y.Y. Li, D. Rui, J.T. Baibado, Y.S. Cheng, Y.Q. Huang, H.Y. Sun, H.Y. Cheung

*Food Research International*, 2014, 55, 373-380

### Abstract

The aim of the study is to find suitable representative marker(s) for the quality assessment of *Lycii Cortex* (LyC). A high performance liquid chromatography coupled with electronic-spray ionization tandem mass spectrometry method with high specific multiple reaction monitoring mode was developed for simultaneous determination of sixteen secondary constituents in this herb and other cortex herbs. This method was validated with linearity ( $R^2 > 0.995$ ), precision (with RSD < 6.72% for intra-day and < 6.90% for inter-day), reproducibility (with RSD < 7.71%) and accuracy (with recovery between 90% to 116% and RSD < 8.70%). Kukoamines, the highly bioactive compounds, have been found to be the predominant and characteristic constituents in LyC. On the contrary, phenolic acids, flavonoids and coumarins, which were previously regarded as the representative components for LyC, are minor constituents and less specific in LyC. Therefore, kukoamines are recommended for use as the representative markers for the quality control of LyC.

### **30. Establishment of a holistic and scientific protocol for the authentication and quality assurance of edible bird's nest.**

M. Yang, S.H. Cheung, S.C. Li, H.Y. Cheung

*Food Chemistry*, 2014, 151, 271-278.

#### Abstract

*Edible bird's nest (EBN) is a prestigious and superior functional food. It is expensive due to its limited supply and enormous demand. Consequently, many fake products are available in the market. This report aims to design a holistic and scientific testing method for the authentication and quality assurance of EBN. The analytical system involves a concerted approach by applying the gas chromatography – mass spectrometry (GC – MS) fingerprint of oligosaccharides, the environmental scanning electron microscopy (ESEM) of microstructure, and the immunoblotting of epidermal growth factor (EGF) in EBN. The results confirmed that genuine EBN had the presence of five monoses and EGF, whereas the counterfeit EBN did not. Moreover, the content of N-Acetylneuraminic acid (NANA) and EGF are established as unique indicators for the grades of EBN. A unique three-dimensional, crater-like microstructure was also observed in authentic EBN, but not in fake products. It is concluded that the holistic approach, including chemical, physical and biochemical studies of EBN, is a reliable and scientific method for the verification of EBN.*

### **31. Pipette tip solid-phase extraction and ultra-performance liquid chromatography/mass spectrometry based rapid analysis of picrosides from *Picrorhiza scrophulariiflora***

Q. Shen, D. Wei, Y.X. Wang, L.K. Gong, Z.Y. Dai, H.Y. Cheung

*Journal of Pharmaceutical and Biomedical Analysis*, 2013, 80, 136-140

#### Abstract

*Pipette tip solid-phase extraction (PT-SPE) is a technique popular in sample preparation of biological fluids and protein hydrolysates. In this study, we developed a microtechnic using a pipette tip packed with C18 as sorbent for extraction and purification of bioactive compounds, picroside-I, II and III, in crude herbal extracts from *Picrorhiza scrophulariiflora* (*P. scrophulariiflora*). Compared to conventional SPE, PT-SPE is fast, easy to operate, and the tools are very accessible (pipette tip and tube, without expensive SPE set-up). Moreover, it is also cost-effective because significant amount of sorbent and solvents can be saved. The eluate was analyzed by ultra-performance liquid chromatography and tandem mass spectrometry (UPLC–MS/MS). Afterwards, the method was fully validated and the results demonstrated that the PT-SPE–UPLC–MS/MS method is an excellent technique for analysis of the herbal medicine. Finally, this PT-SPE–UPLC–MS/MS strategy was successfully applied to analyze the crude extracts from *P. scrophulariiflora* samples within 10 min (2 min for PT-SPE and 8 min for UPLC), 3.5*

mL solvents (including water, 0.3 mL for PT-SPE and 3.2 mL for UPLC), and 2 mg C18 sorbent for each sample. We believe this method to be very practical and, in particular, to be suitable for widespread herbal medicine analysis.

### **32. Simultaneous determination of flavonoid analogs in *Scutellariae Barbatae Herba* by $\beta$ -cyclodextrin and acetonitrile modified capillary zone electrophoresis**

Y.Y. Li, Q.F. Zhang, N.K. Cheung, H.Y. Cheung

*Talanta*, 2013, 105, 393-402

#### Abstract

A capillary zone electrophoresis (CZE) method modified by  $\beta$ -cyclodextrin ( $\beta$ -CD) and acetonitrile (ACN) was developed for simultaneous determination of seven structurally similar flavonoids in *Scutellariae Barbatae Herba* (SBH) and its preparations. Molecular selectivity of the analytes by  $\beta$ -CD was in the following order: apigenin, luteolin, quercetin, scutellarin, baicalein, rutin and wogonin, based mainly on the "molecular fit" interaction between some ligands in the C ring of the flavonoid and the cavity of  $\beta$ -CD. Flavonoids with hydroxyl substituent(s) at the C-ring, especially the 4'-monohydroxyl, were highly selected by  $\beta$ -CD although hydrophobicity of the guest molecule is the primary factor affecting the complexation. The function of acetonitrile in this study was to improve the separation of the analytes in the real SBH. The developed method was validated and applied to real samples. The principle of separation based on this CZE condition is also explained.

### **33. Taxifolin Enhances Andrographolide-Induced Mitotic Arrest and Apoptosis in Human Prostate Cancer Cells via Spindle Assembly Checkpoint Activation**

Z.R. Zhang, M.A. Zaharna, M. M. K. Wong, S.K. Chiu, H.Y. Cheung

*PLoS ONE* 8(1): e54577, Published: January 28, 2013

#### Abstract

Andrographolide (Andro) suppresses proliferation and triggers apoptosis in many types of cancer cells. Taxifolin (Taxi) has been proposed to prevent cancer development similar to other dietary flavonoids. In the present study, the cytotoxic and apoptotic effects of the addition of Andro alone and Andro and Taxi together on human prostate carcinoma DU145 cells were assessed. Andro inhibited prostate cancer cell proliferation by mitotic arrest and activation of the intrinsic apoptotic pathway. Although the effect of Taxi alone on DU145 cell proliferation was not significant, the combined use of Taxi with Andro significantly potentiated the anti-proliferative effect of increased

mitotic arrest and apoptosis by enhancing the cleavage of poly(ADP-ribose) polymerase, and caspases-7 and -9. Andro together with Taxi enhanced microtubule polymerization in vitro, and they induced the formation of twisted and elongated spindles in the cancer cells, thus leading to mitotic arrest. In addition, we showed that depletion of MAD2, a component in the spindle assembly checkpoint (SAC), alleviated the mitotic block induced by the two compounds, suggesting that they trigger mitotic arrest by SAC activation. This study suggests that the anti-cancer activity of Andro can be significantly enhanced in combination with Taxi by disrupting microtubule dynamics and activating the SAC.

### **34. Cell type-dependent effects of andrographolide on human cancer cell lines**

M.T.W. Cheung, R. Ramalingam, K.K.K. Lau, M.W.L. Chiang, S.K. Chiu, H.Y. Cheung, Y.W. Lam

*Life Sciences*, 2012, 91, 751-760

#### Abstract

##### *Aims*

*Andrographolide (ANDRO) is emerging as a promising anti-tumour compound. While it causes apoptosis in most cancer cells, andrographolide induces cell cycle arrest in hepatocellular cancer lines. In this study, we studied the effect of andrographolide on hepatocellular cancers and other cancer types, and elucidated the possible hepatoma-specific features of andrographolide toxicity.*

##### *Main methods*

*We compared the responses of a panel of human cell lines to andrographolide treatment by using flow cytometry, cell synchronisation and time-lapse microscopy. We have also examined their expression of cell cycle-related proteins and proteome changes after andrographolide treatment.*

##### *Key findings*

*Andrographolide exerts its effect on hepatocellular cancer cells through cell cycle arrest and not apoptosis. In HepG2 cells, it blocks G2 cells from entering mitosis and prevents mitosis from completion. This might be due to the disruption of mitotic spindle during metaphase. Despite the dramatic differences in their responses to andrographolide, HepG2 and HeLa cells display similar biochemical consequences. Andrographolide induces DNA damages, as indicated by the expression of phospho-H2AX in both cell lines. Proteomic experiments show that heme oxygenase 1 and heat shock protein 70 are among the proteins induced by andrographolide, which indicate the possible role of oxidative stress in the anti-cancer mechanism of this drug.*

##### *Significance*

*Andrographolide can invoke different cellular responses depending on the biochemical and*

*physiological context in different cell and cancer types, and reveal an additional dimension of the therapeutic applications of this compound.*

### **35. Development of HPLC fingerprint for species differentiation and quality assessment of Rhizome Smilicis Glabrae**

Q. F. Zhang, H. Y. Cheung, L. B. Zeng

*Journal of Natural Medicines*, 2013, 67, 207-211

#### Abstract

*Rhizoma Smilacis Glabrae (RSG) is a commonly used herbal material in functional food and Traditional Chinese Medicine. A HPLC chromatographic fingerprint was developed for its quality control and species differentiation. Nine peaks were found in the chromatogram of RSG and all these peaks were identified by diode array detection and electrospray ionization-MS/MS: 5-O-caffeoylshikimic acid, taxifolin, engeletin, isoengeletin, trans-resveratrol, astilbin and its three stereoisomers. Six of these constituents were consistently found in 18 batches of samples. The standard fingerprint of RSG was generated by mean simulation of all tested samples. Using the standard fingerprint, RSG could be easily differentiated from Rhizoma Smilacis Chinae and Rhizoma Heterosmilacis, the two species that can be confused with RSG.*

### **36. Qualitative Evaluation and Bioactivity Assessment of Polygonum bistorta**

Z. F. Zhang, Q. Shen, H. Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2012, 19, 166-169

#### Abstract

*Polygonum bistorta L. is rich in starch and has been roasted and consumed by people as vegetable. The rhizome of this plant is also an important herbal drug. It is listed in the Pharmacopoeia of the People's Republic of China, and is frequently dispensed in many Traditional Chinese Medicines. It has been reported that the alcoholic extract of the rhizome exhibited some antibacterial, anti-inflammatory and anti-mutation activities. This herb has been used for the treatment of dysentery with bloody stools, diarrhoea in acute gastroenteritis, acute respiratory infection with cough; carbuncles, scrofula, aphthous ulcer, haematemesis, epistaxis, haemorrhoidal bleeding and venomous snake bite. Several triterpenoids, flavones and phenolic acids in the extract of P. Bistorta have been isolated and structurally determined.*

### 37. Bioactive Substances and Medicinal Effects of Lycii Cortex

Z.F. Zhang, Y.Y. Li, J.T. Baibado, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2012, 19, 118-123

#### Abstract

*Lycium barbarum* and *L. chinese* are two important species of the genus *Lycium* (Solanaceae) that have been used as herbal medicine. The root bark of these herbs is recorded as Lycii Cortex in the Pharmacopoeia of the People's Republic of China. It is frequently used in clearing heat and cooling blood agent. A great number of secondary compounds including alkaloids, amides, peptides, flavonoids, coumarins, lignans, terpenoids, organic acids, sterols and steroids have been isolated and structurally identified from the root bark of *Lycium chinense* Mill. and *Lycium barbarum* L. Modern pharmacological studies revealed that extracts or some compounds isolated from Lycii cortex have many biological activities including antihypertensive, anti-diabetic, anti-inflammatory, antioxidant, antibiotic, and anti-parasitic. Hence, it is worthwhile to develop and explore this Chinese Materia Medica.

### 38. Preparative Separation, Characterization, and Determination of Major Compounds in *Gynostemma pentaphyllum* Using Two-dimensional Counter-current Chromatography and Information-dependant Acquisition Mediated UPLC-MS/MS

Y.X. Wang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2012, 19, 112-117

#### Abstract

A method for preparative separation, characterization and determination of rutin, isorhamnetin-3-O-[6"-rhamnosyl(1 → 6)] glucopyranoside, isorhamnetin and cirsiolol from *G. pentaphyllum* was successfully established in this paper. The extracts, using 50% ethanol as extractant, were then purified by elution extrusion counter-current chromatography (EECCC) with *n*-hexane/ethyl acetate/methanol/water (5:6:5:6, v/v/v/v) as the first dimension and ethyl acetate/*n*-butanol/water (4:1:5, v/v/v) as the second dimension. The compounds obtained can be used as reference substances for chromatographic purposes and each compound was characterized and determined by information-dependent acquisition mediated ultra-performance liquid chromatography tandem mass spectrometry (IDA-UPLC-MS/MS). Quantitative performance was evaluated. Satisfactory average correlation coefficients (0.9964~0.9994) were obtained. The intraday repeatability (RSD) ranged from 1.52 to 5.64% and the inter-day repeatability was lower than 6.85% for all analytes. The LOD was ranging from 0.010 to 0.032 ng•mL<sup>-1</sup> while the LOQ was ranging from 0.064 to 0.301 ng•mL<sup>-1</sup> range from 81.09 to 94.37% in three matrices with RSD values lower than 7.8%. All the results showed that EECCC coupling with IDA-UPLC-MS/MS is

an efficient way for separation, characterization and determination of major compounds from natural sources.

### **39. Absence of Chlorogenic Acid in Ginkgo biloba Leaf Samples and Their Over-the-Counter Products**

L.X. Zhu, J.C. Gao, J.T. Baibado, Y.S. Cheng; K.K.C. Chan, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2012, 19, 108-111

#### Abstract

*Ginkgo biloba* leaves (also called *Ginkgo Folia*, GF) and their extracts are popular herbal products or nutritional supplements used worldwide. In both the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP), chlorogenic acid and rutin are suggested as reference markers for the TLC identification of *Ginkgo* leaves. We have found from both TLC assays and HPLC analysis that another compound which is present in *Ginkgo Folia* exhibits similar TLC behavior after heating to chlorogenic acid, and is in fact the real marker in TLC analysis. Further LC/MS/MS analysis of the compound, previously mistaken to be chlorogenic acid, revealed that it is 6-hydroxykynurenic acid, which is one of the many ingredients reported in *Ginkgo* leaves. Hence, we recommend that 6-hydroxykynurenic acid, instead of chlorogenic acid, should be used as the marker for quick TLC identification of the *Ginkgo* leaf and its derived functional food. (Note: The discovery of this research work has been endorsed in year 2009 by the Scientific Committee and the International Advisory Board of the Hong Kong Chinese Materia Medica Standards.)

### **40. Biochemical- and Biological-Based Studies of Abri Herba**

M. Yang, S.H. Cho, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2012, 19, 73-84

#### Abstract

*Abri Herba* is derived from the dried plants of *Abrus* species. *Abrus cantoniensis* Hance is the main constituent of a popular herbal tea in southern China, which contains alkaloid, hydroxylanthraquinone, saponin, saponins and other bioactive compounds. Biologically, the herb is hepatoprotective, and acts as an anti-cancer, anti-oxidative, anti-mutagenic, anti-inflammatory, anti-diabetic, anti-hyperlipidemia, anti-bacterial agent and has immunoprotective effects. Among the compounds that are isolated and identified from this herb are saponin compounds such as soyasaponin I and kaikasaponin III that are regarded as the main constituents responsible for the biological effects listed above. The toxic protein abrin, which has potent antitumor effects, is mainly concentrated in the seeds and can be easily removed during processing of the herbal material. According to clinical trials, extract of *A. cantoniensis* has also been shown to have

*positive effects on acute hepatitis and ABO incompatibility patients. Given this, the therapeutic potential of A. cantoniensis in treating hepatic-related diseases is envisioned in the future.*

#### **41. Method Development for the Colorimetric Determination of Total Alkaloids in Herbal Medicine**

H.Y. Cheung, J.T. Baibado, G.L. Chan, Z.F. Zhang

*Hong Kong Pharmaceutical Journal, 2012, 19, 28-32*

##### Abstract

*Herbal plants contain many bioactive components that are responsible for their bioactivities. These compounds are very complex and closely related in structure making them difficult to isolate qualitatively and quantitatively. Numerous studies have demonstrated that isosteroidal alkaloids are the major active components in medicinal plants. However, the content of individual alkaloid components vary among species and the content of any potential marker is present at very low level (less than 0.01%) so that it cannot be quantified by High Performance Liquid Chromatography (HPLC). It was found that using a specific marker for the assay is not an ideal choice for many herbs. Hence, the total alkaloid content was used as an index for the quality control of the herb. The aim of this study was to optimize and acid-dye colorimetric method for the quantitative determination of the total alkaloids in herbal medicines. The method developed shows good reproducibility and high reliability. This quantitative method is useful for the quality control of herbal medicine with low alkaloid content.*

#### **42. Turtle shell extract as a functional food and its component-based comparison among different species**

H.Y. Cheung, L.Q. LI

*Hong Kong Pharmaceutical Journal, 2012, 19, 33-37*

##### Abstract

*Turtle shell is a traditional Chinese medicine, which has been used for thousands of years by the Chinese. Plastron has been prescribed to nourish yin and suppress sthenic yang. It benefits kidney, invigorates the bone, relieves metrorragia and can regulate menstruation. In recent decades, many studies have been done to ensure that diverse species of turtle could be used as material medica. In this review article, sources, identification and chemical composition of some turtle shells, as well as their biological effects and medical uses are reviewed. Its addition to a few popular functional foods by the Chinese herbalist are also described.*

#### **43. Development of capillary electrophoresis fingerprint for quality control of *Rhizoma Smilacis Glabrae***

Q.F. Zhang, H.Y. Cheung

*Phytochemical Analysis*, 2011, 22, 18-25

##### Abstract

*Introduction* -- *Rhizoma Smilacis Glabrae* (RSG) is a Chinese herbal medicine used for detoxication and as a diuretic. However, in some regions of China, RSG is used confusedly with some other herbs. *Objective* -- To develop a capillary electrophoresis (CE)-DAD fingerprint method for quality evaluation, species differentiation and product identification of RSG. *Methodology* -- The CE separation conditions and extraction procedure were optimised. Eighteen batches of RSG samples were analysed and the standard fingerprint used for authentication was simulated by the average of all tested samples. *Results* -- The optimal CE separation conditions were developed with running buffer of 20 mM borax containing 3 mM  $\beta$ -cyclodextrin at pH 9.4, voltage of 25 kV and temperature of 25°C. The separation could be completed within 8 min. Nine peaks were found in the electropherogram of RSG and five peaks were identified as astilbin, taxifolin, 5-Ocaffeoylshikimic acid, shikimic acid and trans-resveratrol, respectively. Methanol and sonication were recommended for the sample preparation. All RSG samples showed similar chromatographic profile and six 'held in common' peaks were found. By the standard fingerprint, RSG could be well distinguished from its two confusable species, *Rhizoma Smilacis Chinae* and *Rhizoma Hetero Smilacis*. *Conclusion* -- A CE-DAD fingerprint analysis method was developed for the quality control of RSG. The standard fingerprint could represent the chemical profile of RSG and be used for its authentication.

#### **44. Macroscopic and Microscopic Identification of the Two Species of *Atractylodis***

L.W. Lam, Z.F. Zhang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2010, 17, 112-114

##### Abstract

*Atractylodis Rhizoma*, also known as Cangzhu (蒼術), is a traditional Chinese medicinal herb. It has been used for the treatment of abdominal distention, diarrhea and edema. This herb, however, has two species; namely *Atractylodes lancea* (Thunb) DC (茅蒼術) and *Atractylodes chinesis* Koidz (北蒼術). Both species look alike and are not easily distinguishable by most people based on their appearance. To distinguish the two species and ensure their safety and efficacy, microscopic characteristics of rhizomes, including transverse sections, as well as the crude drug powder, were examined. The fixed, sectioned, and stained plant materials, as well as the crude powder, were

studied using a light microscope according to the usual microscopic techniques. The results of the microscopic features were systematically and comparatively described and illustrated. The two species have distinct microscopic characteristic differences, thus allowing us to distinguish between the species and a comparative chart of the key authentication parameters based on these macroscopic and anatomic characteristics was drawn up and is presented for the *Atractylodes* species studied. The study indicated that light microscopy and related techniques provide a method that is convenient, feasible, and can be unambiguously applied to the authentication of species of *Atractylodes*.

#### **45. Simultaneous Determination of Flavonoids, Phenolic Compounds and Triterpenes in Herb by Capillary Zonal Electrophoresis Can be Enhanced by the Addition of $\beta$ -Cyclodextrin**

Q.F. Zhang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2010, 17, 28-33

##### Abstract

A cyclodextrin-modified capillary zonal electrophoresis (CD-CZE) method was established for the separation and determination of three isomeric compounds (ursolic acid, oleanolic acid and betulinic acid), caffeic acid, p-courmaric acid, rosmarinic acid, rutin and quercetin. These eight components, representing some common flavonoids, phenolic compounds and triterpenes in herb, were well separated from each other within 20 min with a borax running buffer (40 mM of borax, pH 9.4) containing 2 mM  $\beta$ -Cyclodextrin and 4% methanol (V/V) at the voltage of 25 kV, temperature of 25°C and detection wavelength of 210 nm. The relative standard deviation (RSD) of migration time ranged from 0.25 to 0.74% while those of the peak area ratios ranged from 2.17 to 4.59% for six determinations of the analytes at concentration of 25  $\mu$ g/mL. The correlation coefficients of the calibration curves of analytes were all  $>0.998$ , and the recoveries were from 96.8 to 103.6%. The method was successfully applied to determine these bioactive components in the extracts of *Prunella vulgaris* L. and its beverage drink products. Our results reveal that in all beverage drinks analyzed, only the isomeric compounds and rosmarinic acid were found.

#### **46. The content of astilbin and taxifolin in concentrated extract of *Rhizoma Smilacis Glabrae* and turtle jelly vary significantly**

Q.F. Zhang, H.Y. Cheung

*Food Chemistry*, 2010, 119, 907-912

##### Abstract

Extract of *Rhizoma Smilacis Glabrae* (RSG) is one of the main ingredients in turtle jelly, which is a

traditional functional food in Southern China and Hong Kong. A capillary electrophoresis method was successfully developed for determination of astilbin and taxifolin in turtle jelly and RSG concentrated extract samples. For six determinations of astilbin and taxifolin at concentrations of  $20 \mu\text{g ml}^{-1}$ , the relative standard deviations of migration time were 0.62% and 0.87%, while those of the peak area ratios were 2.17% and 3.62%, respectively. Eighteen turtle jelly samples manufactured in mainland China and Hong Kong were collected for analysis. The results show that astilbin and taxifolin were all from the RSG ingredient. The contents of astilbin and taxifolin in turtle jelly were distinctly different between brands, and some were found not to contain these substances.

#### **47. The Morphological Features, Chemical Constituents and Biological Effects of *Fritillaria Hupehensis* Bulbus**

Z.F. Zhang, Y.J. Xu, L.W. Lam, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2010, 17, 36-38

##### Abstract

*Hubei-Beimu*, as a traditional Chinese medicinal herb, is derived from the dry bulbs of *Fritillaria hupehensis* Hsiao et K. C. hsia, which is a perennial plant found in Hubei province, China by folk people for a long time and is recorded in Chinese pharmacopeia. It is an important medicinal bulb available in the market along with two other species of the Genus, namely *Fritillaria cirrhosa* D. Don and *Fritillaria thunbergii* Miq. It contains at least 10 steroidal alkaloids, 2 diterpenes and 3 diterpenoid dimmers. Many chemical and pharmacological studies demonstrated that the alkaloids are responsible for its antitussive and expectorant activity. The biological effect based on modern research of this herb coincides with its traditional uses by the Chinese. However, further research on its molecular pharmacology is necessary in order to maximize its uses and exploit even further the resource of *Hubei-Beimu*.

#### **48. $\beta$ -Cyclodextrin facilitates simultaneous analysis of six bioactive compounds in *Rhizoma Smilacis Glabrae* by capillary zone electrophoresis**

Q.F. Zhang, S.C. Li, W.P. Lai, H.Y. Cheung

*Food Chemistry*, 2009, 113, 684-691

##### Abstract

A cyclodextrin-modified capillary zone electrophoretic method was developed for the separation and determination of trans-resveratrol, astilbin, taxifolin, shikimic acid, syringic acid and ferulic acid in *Rhizoma Smilacis Glabrae*. A running buffer comprising 20 mM borax and 2 mM  $\beta$ -cyclodextrin

at pH 9.46 was used. These six components were well separated from each other within 8 min at a voltage of 25 kV, temperature of 25 °C and detection wavelength of 214 nm. The relative standard deviations of migration time ranged from 0.07% to 0.30% while those of the peak area ratios ranged from 2.66% to 4.12% for six determinations of analytes at the concentration of 5 and 25 µg mL<sup>-1</sup>. The correlation coefficients of the calibration curves of analytes were all >0.9990, and the recoveries were from 97.5% to 108.3%. The method was successfully applied to determine the bioactive components in 12 samples of *Rhizoma Smilacis Glabrae* collected from China.

#### **49. Differences between *Peucedanum praeruptorum* Dunn and *Peucedanum decursivum* Maxim—the Two Commonly Mixed Species of *Radix Peucedani* (前胡)**

C.K. Yip, E.J. Lau, Z.F. Zhang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2009, 16, 106-110

##### Abstract

According to the Chinese Pharmacopoeia, *Qianhu* (前胡) is the root of *Peucedanum praeruptorum* Dunn (白花前胡) or *Peucedanum decursivum* Maxim (紫花前胡). It is commonly used for relieving lung heat and resolving phlegm-heat. The two species of genus *Peucedanum*, however, are different and could be distinguished by a variety of methods. In this brief review, differences between the two species are highlighted and compared in many aspects, namely, the morphological appearance, characteristic of pulverized powder, genomic profiles, major bioactive components, and pharmacological activities. It is concluded that these two species have their own unique features and functions. Hence, it is suggested that they should not be used interchangeable.

#### **50. The Mysterious Veil of the Biological Functions of *Panax ginseng* C.A. Meyer – What Ginsenosides Can Do for Our Health?**

Z.G. Yu, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2009, 16, 151-156

##### Abstract

The main medical application of *Panax ginseng* C.A. Meyer is the maintenance of homeostasis of the body. The most important bioactive components that are responsible for the pharmacological effects of *P. ginseng* that have been identified, all belongs to ginseng saponins (GS). Their effects include activities against cancers, enhancement of immune system function and improvement of brain function. Some of the effects are summarized and reviewed in this article. Bioactive substances, such as G-Rb1, GRg1 and G-Rg2 have been successfully isolated and characterized.

They are the main pharmacologically-active components responsible for improving the cerebral functions, while G-Rh2 and G-Rg1 are ingredients responsible for enhancing the immune system, inflammation and allergy. Studies reveal that the anticancer effects of ginseng are attributed to the presence of G-R3, G-Rv2, G-Rg3, G-Rh2, G-Rp1 and GS. Non-saponin compounds, such as polyacetylene play an important role in the pharmacological function against cancer. The angiogenesis of ginseng is attributed to G-Rg1 and GRe. Nevertheless, the exact pharmacological function of GS has not been completely confirmed, and further research is required in order to unveil the complete biological functions of ginseng.

## **51. Use of *Herba Lobellae Chinensis* (半邊蓮) is Attributed to the Bronchodilation and Diuretic Effects of Lobeline and its Derivatives**

Z.R. Zhang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2009, 16, 26-32

### Abstract

*Lobelia is a fall wild flower commonly growing in China. It reduces swelling and has good anti-inflammatory effects. The herb is used by the Chinese for cooling the blood in what they called fire toxin, such as tonsillitis and for asthma treatment and reducing the toxicity of insect stings and snakebites. It has significant and prolonged diuretic effect. Modern TCM practitioners use lobelia for the treatment of ascites in the later stages of schistosomiasis, a parasitic disease. Lobeline is one of the main alkaloids present in the herb. It has both temperature-dependent and independent neuroprotective effects against methamphetamine toxicity.*

## **52. Enhanced analysis of triterpenes, flavonoids and phenolic compounds in *Prunella vulgaris* L. by capillary zone electrophoresis with the addition of running buffer modifiers**

H.Y. Cheung, Q.F. Zhang

*Journal of Chromatography A*, 2008, 1213, 231-238.

### Abstract

*A cyclodextrin-modified capillary zone electrophoresis method was developed for the separation and determination of three isomeric compounds (ursolic acid, oleanolic acid and betulinic acid), caffeic acid, p-coumaric acid, rosmarinic acid, rutin and quercetin. Without the addition of  $\beta$ -cyclodextrin ( $\beta$ -CD) and methanol, the separation of these analytes was poorly resolved. These eight compounds, however, were well separated from each other within 20 min with a borax running buffer (40 mM of borax, pH 9.4) containing 2 mM  $\beta$ -CD and 4% (v/v) methanol at the voltage of 25 kV, temperature of 25 °C and detection wavelength of 210 nm. The relative standard*

deviations (RSDs) of migration time ranged from 0.16 to 0.74% while those of the peak area ratios ranged from 2.17 to 4.61% for six determinations of the analytes at concentration of 10 and 25 µg mL<sup>-1</sup>. The correlation coefficients of the calibration curves of the analytes were all >0.998, and the recoveries were from 96.8 to 103.6%. The method was successfully applied to determine these bioactive components in the samples of *Prunella vulgaris* L. and its beverage drink products. Our results reveal that only the isomeric compounds and rosmarinic acid could be detected in the spikes of *P. vulgaris* L.; other components were either too low to be detected or not present while only rosmarinic acid was detected in the beverage products.

### **53. Antioxidant activity of *Rhizoma Smilacis Glabrae* extracts and its key constituent-astilbin**

Q.F. Zhang, Z.R. Zhang, H.Y. Cheung

*Food Chemistry*, 2008, 115, 297-303

#### Abstract

*Rhizoma Smilacis Glabrae* is widely consumed by Chinese as functional food and in folk medicine for its medicinal properties. In this study, methanol and water extracts of *Rhizoma Smilacis Glabrae* were prepared. The water extract was further divided into polysaccharide and supernatant fractions. Constituents in different extracts were analysed by capillary electrophoresis, and levels of total phenolics were also determined using the Folin-Ciocalteu method. Astilbin, the main constituent in the herb, was isolated and purified. Different antioxidant tests were employed to evaluate the antioxidant activities of the extracts and the isolated astilbin, and the results were compared with two commonly used synthetic antioxidants-butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Methanol, water extract and supernatant fraction showed concentration dependent antioxidant activity while polysaccharide didn't show any antioxidant activity. Purified astilbin showed the strongest antioxidant activity in comparison to any other extracts.

### **54. Literature Review of *Rhizoma Smilacis Glabrae* (土茯苓)**

Q.F. Zhang, Z.R. Zhang, H.Y. Cheung

*Hong Kong Pharmaceutical Journal*, 2008, 15, 65-71

#### Abstract

*Rhizoma Smilacis Glabrae*, also called Tufuling in Chinese, is commonly used for detoxication, relieving dampness and as a diuretic in traditional Chinese medicine. It is also one of the main ingredients in Guling gao; an old traditional functional food in Southern China and Hong Kong. To facilitate the research about this herbal medicine, different aspects e.g. confusable species, compounds isolation, quality control and bioactivity of this herb had been reviewed in this article.

## **55. Reversal of multidrug resistance in cancer cells by Rhizoma Alismatis extract**

W.F. Fong, C. Wang, G.Y. Zhu, C.H. Leung, M.S. Yang, H.Y. Cheung

*Phytomedicine*, 2007, 14, 160-165

### Abstract

*Prolonged chemotherapy may lead to the selective proliferation of multidrug resistant (MDR) cancer cells. In MDR HepG2-DR and K562-DR cells that over-expressed P-glycoprotein (Pgp), the extract of the rhizomes of Alisma orientalis (Sam) Juzep. showed a synergistic growth inhibitory effect with cancer drugs that are Pgp substrates including actinomycin D, puromycin, paclitaxel, vinblastine and doxorubicin. At the same toxicity levels the herbal extract was more effective than verapamil, a standard Pgp inhibitor, in enhancing cellular doxorubicin accumulation and preventing the efflux of rhodamin-123 from the MDR cells. The extract restored the effect of vinblastine on the induction of G2/M arrest in MDR cells. Our data suggest that A. orientalis may contain components that are effective inhibitors of Pgp.*

## **56. Simultaneous determination of key bioactive components in Hedyotis diffusa by capillary electrophoresis**

H.Y. Cheung, S.H. Cheung, M.L. Law, W.P. Lai

*Journal of Chromatography B*, 2006, 834, 195-198

### Abstract

*A capillary zone electrophoresis (CZE) method based on systematic one-variable-at-a time approach was developed for the analysis of four important bioactive components (geniposidic acid, ursolic acid, quercetin and p-coumaric acid) in the extract of Hedyotis diffusa (HD). Separations were carried out in a fused-silica capillary tube with peak detection at 214 nm. Good separation was achieved using a 20 mM borate buffer containing 5% acetonitrile as organic modifier and pH adjusted to 10.0. Operating voltage was 15 kV and temperature was maintained at 25 °C while hydrodynamic injection was 5 s. A good linearity, with correlation coefficients in the ranges of 0.997–0.999 was obtained in the calibration curves of each standard. Relative standard deviation (R.S.D.) of migration time was between 0.32 and 0.70% and deviation of corrected peak area was between 8.84 and 11.99%. These results indicate that this method could be used for rapid and simultaneous analysis of the bioactive components in HD and other herbal products.*