

1. Oligosaccharide-marker approach for qualitative and quantitative analysis of specific polysaccharide in herb formula by ultra-high-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry: *Dendrobium officinale*, a case study

T.L. Wong, L.F. Li, J.X. Zhang, S.P. Bai, L. S. Zhou, H.Y. Fung, Q.W. Zhang, D.L. Ma, C.H. Leung, Z.Z. Zhao, Q.B. Han

Journal of Chromatography A, 2019, In press

Abstract

Qualitative and quantitative analysis of polysaccharides in herb formula remain challenge due to the limited choices of analytical methods concerning the intrinsic characteristics of large molecular mass. Herein, an oligosaccharide-marker approach was newly developed for quality assessment of polysaccharides in herbal materials, using *Dendrobium officinale* as a case study. This method involved partial acid hydrolysis of *D. officinale* polysaccharide (DOP) followed by *p*-aminobenzoic ethyl ester (ABEE) derivatization. Two ABEE-labeled oligosaccharides namely, Te-Man-ABEE and Pen-Man-ABEE, were selected as chemical markers due to their high specificity in herb formula. The linear relationship between the content of these two markers and the content of DOP was then successfully established respectively. The linear relationship was further transformed to that between peak area of chemical markers and DOP content so that chemical markers were not necessary to be isolated for analysis. This linear relationship was systemically validated in terms of precision and accuracy. The results showed that these two oligosaccharide-markers presented a good linear relationship with DOP ($R^2 \geq 0.997$) in the range of 0.68–16.02 μg . These markers also demonstrated satisfactory precision ($RSD < 7.0\%$), and recovery (91.41%–118.30%) in real sample determination. Additionally, there was no significant difference between the results given by the two chemical markers as the RSD values were not more than 7.0%. While concerning the results given by the oligosaccharide-markers and the previously-published polysaccharide marker, the RSD value was not more than 6.4%. These suggest that the oligosaccharide-marker approach is a simple, quick, and reliable method to qualitatively and quantitatively determine of specific polysaccharide in herb formula.

2. Recent progress in nanomaterial-based assay for the detection of phytotoxins in foods

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Food Chemistry, 2019, 277, 162-178

Abstract

Phytotoxins refers to toxic chemicals derived from plants. They include both secondary metabolites that are dose-dependently toxic and allergens that can cause anaphylactic shock in sensitive individuals. Detecting phytotoxins in foods is increasingly important. Conventional methods for detecting phytotoxins lack sufficient sensitivity and operational convenience. Nanomaterial-based determination assays show great competence in fast and accurate sensing of trace substances. In the present review, representative phytotoxin categories of alkaloids, cyanides, and proteins are discussed. Application of notable nanomaterials, e.g. carbon nanotubes, graphene oxide, magnetic

nanoparticles, metal-based nanotools, and quantum dots, in specific sensing strategies to fit the physiochemical properties of the target toxins are summarized. Nanomaterials mainly play four roles in phytotoxin detection: 1) analyte enricher; 2) sensor structure mediator; 3) target recognizer or reactant; 4) signaling agent. Great achievements have been made in the detection of trace plant-derived toxins in food matrices, yet there are still challenges awaiting further investigation.

3. Characterization of Chemical Component Variations in Different Growth Years and Tissues of *Morindae Officinalis Radix* by Integrating Metabolomics and Glycomics

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Journal of Agricultural and Food Chemistry, 2019, 26, 7304-7314

Abstract

Morindae Officinalis Radix (MOR), the dried root of *Morinda officinalis* F.C. How (Rubiaceae), is a popular food supplement in southeastern China for bone protection, andrological, and gynecological healthcare. In clinical use, 3-4 year old MOR is commonly used and the xylem is sometimes removed. However, there is no scientific rationale for these practices so far. In this study, metabolomics and glycomics were integrated using multiple chromatographic and mass spectrometric techniques coupled with multivariate statistical analysis to investigate the qualitative and quantitative variations of secondary metabolome and glycome in different growth years (1-7 years) and tissues (xylem and cortex) of MOR. The results showed that various types of bioactive components reached a maximum between 3 and 4 years of growth and that the xylem contained more potentially toxic constituents but less bioactive components than the cortex. This study provides the chemical basis for the common practice of using 3-4 year old MOR with the xylem removed.

4. Comparative quality of the forms of decoction pieces evaluated by multidimensional chemical analysis and chemometrics: *Poria cocos*, a pilot study

L.X. Zhu, J. Xu, L.F. Su, K.Y.C. Lam, E.R. Qi, X.P. Dong, H.B. Chen, Y.D. Liu, Z.Z. Zhao

Journal of Food and Drug Analysis, 2019, 27, 766-777

Abstract

Many Chinese medicinal materials (CMMs) are parts of plants or fungi that have been processed into different physical forms, termed decoction pieces, that are typically boiled in water for consumption. One CMM may have several decoction pieces forms, e.g., slices, small cubes (dice), or grains. The specifications that have different morphological parameters (shape, size and thickness) for these various decoction pieces have been developed over, in some cases, centuries of practice. Nevertheless, whether and how the form of decoction pieces affects the extraction (decoction) dynamics, and quality stability during storage has not been studied. Here, we investigated *Poria cocos* (PC) as a pilot study; we explore how the form of PC decoction pieces affects its chemistry using multidimensional chemical evaluation such as ultra-performance liquid chromatography-photodiode array-quadrupole time-of-flight mass spectrometry (UHPLC-PDA-QTOF-MS/MS), ultra-performance liquid chromatography-triple quadrupole mass spectrometry

(UHPLC-QqQ-MS/MS) and high performance gel permeation chromatography coupled with charged aerosol detector (HPGPC-CAD), combined with analysis of variance (ANOVA), principal component analysis (PCA), factor analysis (FA) and hierarchical cluster analysis (HCA). The results indicated that different specifications had significant differences, and these specifications could be divided into four groups. The comprehensive results of the chemical analyses undertaken here indicate that the highest potentially available quality of PC decoction pieces was in the forms of curl, ultra-small grains and small grains, followed by thin slices. This information not only is conducive to promoting the standardization of the specification/form of PC decoction pieces and maximizing the benefits from its utilization, but also provide a promising strategy for assessing other CMM decoction pieces in different forms.

5. Stronger anti-obesity effect of white ginseng over red ginseng and the potential mechanisms involving chemically structural/compositional specificity to gut microbiota

S.S. Zhou, K.K.W. Auyeung, K.M. Yip, R. Ye, Z.Z. Zhao, Q. Mao, J. Xu, H.B. Chen, S.L. Li.

Phytomedicine, 2018, In press

Abstract

Background: Ginseng has therapeutic potential for treating obesity and the associated gut microbiota dysbiosis. However, whether white ginseng and red ginseng, the two kinds of commonly used processed ginseng, possess different anti-obesity effects remains unknown. *Purpose:* Anti-obesity effects of water extracts of white ginseng and red ginseng (WEWG and WERG) were compared, and the potential mechanisms were discussed.

Methods: Chemical profiles of WEWG and WERG were characterized by ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS) and high performance liquid chromatography coupled with evaporative light scattering detector (HPLC-ELSD). Anti-obesity effects of WEWG/WERG were examined by determining fat accumulation, systemic inflammation, enteric metabolic disorders and gut microbiota dysbiosis in high-fat diet (HFD)-fed obese mice.

Results: Both WEWG and WERG exerted anti-obesity effects, with WEWG stronger than WERG. Compared to WERG, WEWG contained less contents of carbohydrates (polysaccharides, oligosaccharides, free monosaccharides) and ginsenosides, but chemical structures or compositions of these components in WEWG were characteristic, i.e. narrower molecular weight distribution and higher molar ratios of glucose residues of polysaccharides; higher content ratios of oligosaccharides DP2–3 (di-/tri-saccharides)-to-oligosaccharides DP4–7 (tetra-/penta-/hexa-/hepta-saccharides), sucrose-to-melibiose, maltose-to-trehalose and high-polar-to-low-polar ginsenosides. WEWG better ameliorated fat accumulation, enteric metabolic disorders and gut microbiota dysbiosis in HFD-fed obese mice than WERG. *Conclusion:* The stronger anti-obesity effect of white ginseng appears to correlate with differences in its chemical profile as compared to red ginseng. The carbohydrates and ginsenosides in WEWG potentially present more structural and compositional specificity to the obesity-associated gut bacteria, allowing more beneficial effects of WEWG on the gut microbiota dysbiosis. This consequently better alleviates the enteric metabolic disorders and systemic inflammation, thereby contributing to the stronger anti-obesity effect of WEWG as compared to WERG.

6. Less SO₂ residue may not indicate higher quality, better efficacy and weaker toxicity of sulfur-fumigated herbs: Ginseng, a pilot study

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Journal of Hazardous Materials, 2019, 364, 376-387

Abstract

Sulfur dioxide (SO₂) is a hazardous residue in sulfur-fumigated herbs. Standards limiting SO₂ content have been adopted worldwide for quality control of sulfur-fumigated herbs, and herbs with less SO₂ are believed to be better. However, the standards are based only on the safe dose of SO₂ and may not characterize changes in herbal quality, thereby the efficacy and toxicity, resulting from sulfur fumigation. To confirm this, here the correlation of residual SO₂ content with the quality/efficacy/toxicity of sulfur-fumigated herb was investigated, and ginseng was selected as a pilot study object. Four sulfur-fumigated ginseng samples with different SO₂ contents were systemically compared regarding their quality, anti-inflammatory, anti-shock and anti-stress efficacies, as well as acute and chronic toxicities. The results demonstrated that the SO₂ content did not correlate with the quality, efficacy and toxicity changes of ginseng; more specifically, less SO₂ residue did not indicate higher quality, better efficacy nor weaker toxicity. This fact suggests that SO₂ content cannot characterize the variations in quality, efficacy and toxicity of sulfur-fumigated herbs. Therefore, the standard limiting SO₂ content alone may be inadequate for quality control of sulfur-fumigated herbs, and new standards including other indicators that can exactly reflect herbal efficacy and safety are necessary.

7. Astragalus Polysaccharide RAP Induces Macrophage Phenotype Polarization to M1 via the Notch Signaling Pathway

W. Wei, Z.P. Li, Z.X. Bian, Q.B. Han

Molecules, 2019, 24, 2016

Abstract

Macrophages occur in polarized phenotypes, whose characteristics determine the role they play in tumor growth. The M1 phenotype macrophages promote tumoricidal responses and suppress tumor growth. Our previous study showed that a polysaccharide isolated from Radix Astragali, named RAP, was itself non-cytotoxic but induced RAW264.7 cells' cytotoxicity against cancer cells. The current study was undertaken to determine its mechanism. Series studies was conducted to show that RAP is able to induce much higher gene expression of M1 markers, including iNOS, IL-6, TNF- α , and CXCL10, compared with the control group. When RAP-induced BMDMs were transplanted together with 4T1 tumor cells in BALB/c mice, both tumor volume and tumor weight decreased. Further studies indicated that RAP induces the Notch signaling pathway in RAW264.7 cells. The function of Notch signaling in macrophage polarization was confirmed by using γ -secretase inhibitor. These results suggested that Astragalus polysaccharide RAP induces macrophage's polarization to M1 phenotype via the Notch signaling pathway.

8. **Astragalus polysaccharide RAP selectively attenuates paclitaxel-induced cytotoxicity toward RAW 264.7 cells by reversing cell cycle arrest and apoptosis**

W.R. Bao, Z.P. Li, Q.W. Zhang, L.F. Li, H.B. Liu, D.L. Ma, C.H. Leung, A.P. Lu, Z.X. Bian, Q.B. Han

Frontiers in Pharmacology, 2019, 9,1580

Abstract

Purpose: The purpose of this study was to determine if an Astragalus polysaccharide (RAP) can protect immune cells from the toxic side effects of paclitaxel (Taxol), a powerful anti-tumor drug whose equally powerful side effects limit its clinical use.

Methods: We hypothesized that RAP can reduce the toxic effects induced by Taxol. To test this hypothesis, we conducted a series of studies in vivo and in vitro. First, we confirmed RAP's effects in vivo utilizing BALB/c mice inoculated with 4T1 mouse breast cancer cells as the tumor model. Mice were treated with RAP and/or Taxol, and the differences in the life spans were recorded. Second, a co-culture cell model was used to study the protective effect of RAP on cells vis-a-vis Taxol. The cell cycle and apoptosis of RAW 264.7 cells that were treated with RAP with/without Taxol were checked by flow cytometry and Hoechst staining. Proteins involved in the cell cycle and apoptosis were also tested by Western blot to reveal the probable mechanism.

Results: RAP prolonged the life span of tumor-bearing mice treated with Taxol. The in vitro experiments showed that Taxol suppressed the proliferation of RAW 264.7 cells while RAP protected the RAW 264.7 cells from Taxol-induced suppression. The protection is selective because RAP had no effect on 4T1 cells. Furthermore, Taxol clearly led to cell cycle arrest mainly at the G2/M phase and generated cytotoxicity against RAW 264.7 cells, while RAP blocked cell cycle arrest and protected cells from apoptosis. Taxol up-regulated the protein levels of P-H2A, PARP, Chk1, p53, and p21 and down-regulated Bcl-XI and Mcl-1, and RAP reversed the expression of all these proteins.

Conclusion: These results suggested that RAP can protect immune cells from Taxol-induced toxicity, by changing the cell cycle and apoptosis.

9. **Clarifying the origin of Houzao**

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Chinese Medicine, 2018, 13, 25

Abstract

BACKGROUND: Houzao (bezoar) is a valuable imported Chinese medicine that is commonly used as a pediatric medicine to transform phlegm. There are mainly two types of Houzao, "Southeast Asian Houzao" and "Indian Houzao". "Indian Houzao" is the dominant commercial product accounts for over 95% of the actual utilization in the market. However, its origin, formation, composition, efficacy and pharmacology remain unclear. Therefore, we have conducted on-site investigation to clarify the origin of Indian Houzao. We have dissected one male and one female domestic Indian goats in the pastoral areas of Telangana province in south-central India. Our results show: Indian Houzao originates from Indian goats rather than from macaques; it comes from goats and not sheep, and is not limited to female goats. The exact location of the bezoar is in the caecum and not stomach or intestines. Acacia seeds serve as the primer to induce the formation of bezoar in the caecum. The

formation and development of the bezoar are closely related to the special local ecosystem and food chain. These goats eat the shoots of *Acacia nilotica*, and also other local plants in the families of Euphorbiaceae, Rutaceae, Combretaceae, etc. It takes around 120 days for the bezoar to be fully developed inside the goat. Many goats are slaughtered in the Indian festival Dusserah from October to December. Indian Houzao is the bezoar from the caecum of Indian goats, formed in response to pathological stimulation, and is the dominant commercial form of "Houzao" on the market. It has been used historically. It has natural supply source. Producers can guarantee a sustainable supply of the bezoars for the market. The usage of bezoar as medicine is also acceptable from the perspective of animal protection. Many patients and people in the Chinese medicine field do not know Indian Houzao comes from Indian goats but from other unsustainable animal sources, which has a negative influence on its actual use and scientific research potential.

CONCLUSION: Our study has clarified the origin of Indian Houzao, which can help to further develop Indian Houzao for the treatment of diseases.

10. Tissue-specific analysis of secondary metabolites creates a reliable morphological criterion for quality grading of polygoni multiflori radix

L. Liang, J. Xu, Z.T. Liang, X.P. Dong, H.B. Chen, Z.Z. Zhao

Molecules, 2018, 23, E1115

Abstract

In commercial herbal markets, Polygoni Multiflori Radix (PMR, the tuberous roots of Polygonum multiflorum Thunb.), a commonly-used Chinese medicinal material, is divided into different grades based on morphological features of size and weight. While more weight and larger size command a higher price, there is no scientific data confirming that the more expensive roots are in fact of better quality. To assess the inherent quality of various grades and of various tissues in PMR and to find reliable morphological indicators of quality, a method combining laser microdissection (LMD) and ultra-performance liquid chromatography triple-quadrupole mass spectrometry (UPLC-QqQ-MS/MS) was applied. Twelve major chemical components were quantitatively determined in both whole material and different tissues of PMR. Determination of the whole material revealed that traditional commercial grades based on size and weight of PRM did not correspond to any significant differences in chemical content. Instead, tissue-specific analysis indicated that the morphological features could be linked with quality in a new way. That is, PMR with broader cork and phloem, as seen in a transverse section, were typically of better quality as these parts are where the bioactive components accumulate. The tissue-specific analysis of secondary metabolites creates a reliable morphological criterion for quality grading of PMR.

11. Interference of Quercetin on Astragalus Polysaccharide-Induced Macrophage Activation

Z.P. Li, H.B. Liu, Q.W. Zhang, L.F. Li, W.R. Bao, D.L. Ma, C.H. Leung, Z.X. Bian, A.P. Lu, Q.B. Han

Molecules, 2018, 23, E1563

Abstract

Polysaccharides, which exert immunoregulatory effects, are becoming more and more popular as food supplements; however, certain components of ordinary foods could be reducing the

polysaccharides beneficial effects. Quercetin, a flavonoid found in common fruits and vegetables, is one such component. This study investigated the effects of quercetin on *Astragalus* polysaccharide RAP induced-macrophage activation. The results show quercetin decreases the NO production and iNOS gene expression in RAW264.7 cells, and it inhibits the production of cytokines in RAW264.7 cells and peritoneal macrophages. Western blot analysis results suggest that quercetin inhibits the phosphorylation of Akt/mTORC1, MAPKs, and TBK1, but has no effect on NF- κ B in RAP-induced RAW264.7 cells. Taken together, the results show that quercetin partly inhibits macrophage activation by the *Astragalus* polysaccharide RAP. This study demonstrates that quercetin-containing foods may interfere with the immune-enhancing effects of *Astragalus* polysaccharide RAP to a certain extent.

12. Comprehensive comparison of polysaccharides from *Ganoderma lucidum* and *G. sinense*: chemical, antitumor, immunomodulating and gut-microbiota modulatory properties

L.F. Li, H.B. Liu, Q.W. Zhang, Z.P. Li, T.L. Wong, H.Y. Fung, J.X. Zhang, S.P. Bai, A.P. Lu, Q.B. Han

Scientific Reports, 2018, 8: 6172

Abstract

Both *Ganoderma lucidum* (GL) and *G. sinense* (GS) are used as Lingzhi in China. Their functions are assumed to mainly derive from triterpenes and polysaccharides; however, the two species have very different triterpenes profiles, if this was the case, then the bioactivity of these two species should differ. Instead, could the polysaccharides be similar, contributing to the shared therapeutic basis? In this study, two main polysaccharide fractions from different batches of GL and GS were systematically compared by a series of chemical and biological experiments. The results showed that the polysaccharides from two species shared the same structural features in terms of mono-/oligo-saccharide profiles, molecular size, sugar linkages, and IR/NMR spectra. In addition, these polysaccharides showed similar tumor-suppressive activity in mice. Further study on RAW264.7 cells indicated that these polysaccharides exhibited similar inducing effects to macrophages, as evaluated in the phagocytosis function, NO/cytokines production, inhibition against the viability and migration of cancer cells. Mechanistic investigation revealed the identical activation via TLR-4 related MAPK/NF- κ B signaling pathway and gut-microbiota modulatory effects. In summary, GL and GS polysaccharides presented similar chemical features, antitumor/immunomodulating activities and mechanism; this establishes polysaccharides as the active principles and supports the official use of both species as Lingzhi.

13. Tissue-based metabolite profiling and qualitative comparison of two species of *Achyranthes* roots by use of UHPLC-QTOF MS and laser micro-dissection

Y. Jaiswal, Z.T. Liang, A. Ho, H.B. Chen, L. Williams, Z.Z. Zhao

Journal of Pharmaceutical Analysis, 2018, 8, 10-19

Abstract

Achyranthes bidentata and *Achyranthes aspera* are saponin and steroid rich medicinal plants, used extensively for therapeutic treatments in Traditional Chinese Medicine (TCM) and Ayurveda. A.

bidentata is reported to be one of the rare and extensively exploited medicinal plant species that face the issue of being endangered. Finding qualitative substitute with identical phyto-constituents contributing to similar composition and pharmacological benefits will help in reducing the burden of exploitation of the natural habitats of such plants. In the present study, a comparative metabolite analysis of the whole drug and specific tissues isolated by laser micro-dissection (LMD) was carried out for both the selected species, by use of ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS). The results of the study indicate that the cortex and the medullary ray tissues are rich in their content of steroidal and saponin constituents such as (25S)-inokosterone-20,22-acetonide, ginsenoside Ro, bidentatoside II and achyranthoside B. Metabolite profiling of the whole tissues of both the species indicates presence of identical constituents. Thus, it is inferred that *A. bidentata* and *A. aspera* can be used as qualitative substitutes for each other.

14. Qualitatively and quantitatively comparing secondary metabolites in three medicinal parts derived from *Poria cocos* (Schw.) Wolf using UHPLC-QTOF-MS/MS-based chemical profiling

L. Zhu, J. Xu, S. Zhang, R. Wang, Q. Huang, H.B. Chen, X. Dong, Z.Z. Zhao

Journal of Pharmaceutical and Biomedical Analysis, 2018, 18, 278-286

Abstract

Poria cocos (Schw.) Wolf, is a fungus that grows on dead pine roots, producing a solid white sclerotium (fruiting body). Three parts of the sclerotium are commonly used in traditional Chinese medicine, namely, the epidermis, known as *Poria Cutis* or *fulingpi* in Chinese; the middle part, known as *Poria* or *baifuling*; and the sclerotium with some host attached, known as *fushen*. For *fushen*, the hostwood is the inner part, known as *fushenmu*. The epidermis, middle part and middle-plus-inner parts have long been used for different syndromes or diseases, but the differences in their chemistry have not been established. In this study, ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UHPLC-QTOF-MS/MS) was used to explore the differences of secondary metabolites in these three botanical parts of *Poria cocos*. In 13 batches of samples, a total of 147 chromatographic peaks were unequivocally or tentatively identified. Of these, 15 peaks were common to all three parts (the epidermis, middle, and inner part), while the rest were found in only one or two of the parts. Meanwhile, dissimilarities among the chemical compositions of three botanical parts were further investigated by principal component analysis (PCA), and supervised orthogonal partial least squares-discriminate analysis (OPLS-DA). Eight major bioactive triterpene acids that played important roles in differentiating the epidermis, middle and inner part were simultaneously quantified using the extracting ion mode of UHPLC-QTOF-MS/MS. The quantitative results showed that the analytes accumulated mostly in the epidermis and inner part, with the least accumulation in the middle part. This systematic information on the differences in secondary metabolites in the three botanical regions of *Poria cocos* provides a factual chemical basis not only for discrimination in therapeutic practice but also for quality evaluation of *Poria cocos*.

15. Oolong tea: A critical review of processing methods, chemical composition, health effects, and risk

K.W. Ng, Z.J. Cao, H.B. Chen, Z.Z. Zhao, L. Zhu, T. Yi

Critical Reviews in Food Science and Nutrition, 2018, 58, 2957-2980

Abstract

Oolong tea (OT) is a traditional Chinese tea (*Camellia sinensis*) and is especially popular in south China. This review is to comprehensively summarize the miscellaneous research that has been done towards to the processing, phytochemistry, health benefit, and risk of OT. These literatures were carried out not only from different electronic databases but also from text books written in English, Japanese, and Chinese, including those traditional records tracing back to the Tang Dynasty (A.D. 618–907). The full process OT producing is depicted below in this review. The phytochemistry of OT has been comprehensively investigated. More than 100 chemical compositions have been isolated and identified. In health benefit, OT performs outstandingly in reducing obesity and controlling diabetes explained by modern pharmacological studies. (–)-Epigallocatechin-3-gallate (6) in OT prevention of cancerous cells developing. OT can also improve and reduce on heart and vascular disease, protect teeth and bone, function as anti-oxidative and antibacterial agents. This review also mentioned the risk, summarized briefly on various forms of toxicity and harmful associated with OT. In short, this review can provided a natural product library of OT, gave inspirations for further new garden systems, designed idea on quality, bioactivity-oriented screening. In addition, it is suggested more scientists and education is necessary to guarantee the stability and safety of drinking OT.

16. Laser microdissection hyphenated with high performance gel permeation chromatography-charged aerosol detector and ultra performance liquid chromatography-triple quadrupole mass spectrometry for histochemical analysis of polysaccharides in herbal medicine: Ginseng, a case study

Q.L. Chen, Y.J. Chen, S.S. Zhou, K.M. Yip, J. Xu, H.B. Chen, Z.Z. Zhao

International Journal of Biological Macromolecules, 2018, 107, 332-342

Abstract

This study establishes a new combinatorial approach for histochemical analysis of polysaccharides in herbal medicines using laser microdissection followed by high performance gel permeation chromatography coupled with charged aerosol detector and ultra-performance liquid chromatography hyphenated with triple quadrupole mass spectrometry. Ginseng was employed as a study model. Tissue-specific qualitative and quantitative characterization of ginseng polysaccharides was performed by determining their molar masses and monosaccharide compositions in three macro-dissected parts (rhizome, main and branched roots) and five micro-dissected tissues (cork, cortex, xylem, phloem and resin canal). The results showed that ginseng “flesh” (xylem, phloem and resin canal) contained more polysaccharides with larger molecular weights and higher ratios of glucose residue, whereas ginseng “skin” (cork and cortex) had fewer polysaccharides with smaller molecular weights and higher ratios of non-glucose constituents (e.g. galacturonic acid, galactose, arabinose and rhamnose). These findings suggested that the polysaccharides of the “flesh” were predominantly starch-like glucans, while those of the “skin” were

of a higher proportion of acidic pectins. The revealed histologic distribution and accumulation pattern of ginseng polysaccharides contributes to the scientific understanding of ginseng regarding the biosynthesis and transportation of polysaccharides, medicinal quality evaluation as well as empirical clinical application.

17. Comparison of the chemical profiles and inflammatory mediator-inhibitory effects of three *Siegesbeckia* herbs used as Herba *Siegesbeckiae* (Xixiancao)

H. Guo, Y. Zhang, B.C.Y. Cheng, M.Y. Lau, X.Q. Fu, T. Li, T. Su, P.L. Zhu, Y.C. Chan, A.K.W. Tse, T. Yi, H.B. Chen, Z.L. Yu

BMC Complementary and Alternative Medicine, 2018, 18, 141

Abstract

Background: *Herba Siegesbeckiae* (HS, Xixiancao in Chinese) is a commonly used traditional Chinese medicinal herb for soothing joints. In ancient materia medica books, HS is recorded to be the aerial part of *Siegesbeckia pubescens* Makino (SP) which is also the only origin of HS in the 1963 edition of the Chinese Pharmacopeia (ChP). The aerial parts of *Siegesbeckia orientalis* L. (SO) and *Siegesbeckia glabrescens* Makino (SG) have been included as two additional origins for HS in each edition of ChP since 1977. However, chemical and pharmacological comparisons among these three species have not been conducted.

Methods: An HPLC with diode array detector (HPLC-DAD) method combined with similarity analysis, hierarchical cluster analysis (HCA) and principal component analysis (PCA) was developed for comparing the fingerprint chromatograms of the three species. The inhibitory effects of the three species on NO production and IL-6 secretion in LPS-stimulated RAW264.7 macrophages were compared.

Results: Fingerprint chromatograms of the three species showed different profiles, but had 13 common peaks. Results from HCA and PCA of the common peaks demonstrated that all 14 herbal samples of the three species tended to be grouped and separated species dependently. The extents of inhibition on NO production and IL-6 secretion of the three species were different, with SG being the most and SP the least potent.

Conclusions: Both chemical profiles and inflammatory mediator-inhibitory effects of the three species were different. These findings provide a chemical and pharmacological basis for determining whether the three species can all serve as the origins of HS.

18. Corni Fructus: A review of chemical constituents and pharmacological activities

Y. Dong, Z.L. Feng, H.B. Chen, F.S. Wang, J.H. Lu

Chinese Medicine, 2018, 13, 34

Abstract

Cornus officinalis Sieb. et Zucc. is part of the genus *Cornus* of the family Cornaceae. Ripening and dry fruits (Corni Fructus) are recognized as an essential herb medicine in the traditional Chinese medicine (TCM) and have been widely used for over 2000 years. This review provides a comprehensive summary of Corni Fructus (CF), including the botany, phytochemistry, traditional

use, and current pharmacological activities. According to the basic theory of TCM, CF usually participates in various Chinese medicinal formulae to exert the essential roles in replenishing liver and kidney, arresting seminal emission and sweat. Based on modern pharmacological studies, about 90 compounds have been isolated and identified from CF. In vivo and in vitro experimental studies indicate that CF exhibits extensive pharmacological activities including hypoglycemic, antioxidant, anti-inflammatory, anticancer, neuroprotective, hepatoprotective, and nephroprotective activities. However, only about 18% of chemical constituents in CF were tested. It means the potential pharmacological activities and clinical values of CF need to be further investigated.

19. Comprehensive quality evaluation and comparison of *Angelica sinensis* radix and *Angelica acutiloba* radix by integrated metabolomics and glycomics

S.S. Zhou, J. Xu, C.K. Tsang, K.M. Yip, W.P. Yeung, Z.Z. Zhao, S. Zhu, H. Fushimi, H.Y. Chang, H.B. Chen

Journal of Food and Drug Analysis, 2018, 26, 1122-1137

Abstract

Angelica radix (Danggui in Chinese) used in China and Japan is derived from two species of *Angelica*, namely *Angelica sinensis* and *Angelica acutiloba*, respectively. The differences in quality between *A. sinensis* radix (ASR) and *A. acutiloba* radix (AAR) should be therefore investigated to guide the medicinal and dietary applications of these two species. Secondary metabolites and carbohydrates have been demonstrated to be the two major kinds of bioactive components of Danggui. However, previously, quality comparison between ASR and AAR intensively concerned secondary metabolites but largely overlooked carbohydrates, thus failing to include or take into consideration an important aspect of the holistic quality of Danggui. In this study, untargeted/targeted metabolomics and glycomics were integrated by multiple chromatography-based analytical techniques for qualitative and quantitative characterization of secondary metabolites and carbohydrates in Danggui so as to comprehensively evaluate and compare the quality of ASR and AAR. The results revealed that not only secondary metabolites but also carbohydrates in ASR and AAR were different in type and amount, which should collectively contribute to their quality difference. By providing more comprehensive chemical information, the research results highlighted the need to assess characteristics of both carbohydrates and secondary metabolites for overall quality evaluation and comparison of ASR and AAR.

20. Integrating targeted and untargeted metabolomics to investigate the processing chemistry of *Polygoni multiflori* radix

L. Liang, J. Xu, W.W. Zhou, E. Brand, H.B. Chen, Z.Z. Zhao

Frontiers in Pharmacology, 2018, 9, 934

Abstract

Polygoni Multiflori Radix (PMR, Heshouwu in Chinese), derived from the tuberous roots of *Polygonum multiflorum* Thunb., is a widely-used Chinese medicinal material. For traditional clinical use, raw PMR (RPMR) is processed by nine cycles of steaming and drying to generate processed

PMR (PPMR); RPMR and PPMR have distinct medicinal purposes based on the theory of traditional Chinese medicine. While PMR has been processed for hundreds of years, including the present, the chemistry of that processing has not been well studied. In this study, targeted and untargeted metabolomics analyses using ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UPLC-QTOF-MS/MS) and ultra-performance liquid chromatography-quadrupole/triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS) were integrated to investigate the processing chemistry of PMR. The results demonstrate that processing by nine cycles of steaming and drying qualitatively and quantitatively alters the chemical profile of PMR. Several mechanisms, namely hydrolysis, dehydration, isomerization, and Maillard reaction appear to be involved in the chemical transformation that occurs. The qualitative and quantitative data further suggest that nine cycles might be necessary for the preparation of PPMR, as PPMR that has been processed nine times shows significant differences in its chemical profile.

21. Correlation between quality and geographical origins of *Poria cocos* revealed by qualitative fingerprint profiling and quantitative determination of triterpenoid acids

L.X. Zhu, J. Xu, R.J. Wang, H.X. Li, Y.Z. Tan, H.B. Chen, X.P. Dong, Z.Z. Zhao

Molecules, 2018, 23, 2200

Abstract

Poria cocos (Schw.) Wolf (PC) is a well-known saprophytic fungus, and its sclerotium without the epidermis (PCS) is widely used in traditional Chinese medicine and as a functional food in many countries. PCS is normally collected from multiple geographical regions, but whether and how the quality of PCS correlates with where it grows have not been determined. This correlation could be significant both for quality control and optimum utilization of PCS as a natural resource. In this study, a qualitative fingerprint profiling method performed by ultra-performance liquid chromatography (UHPLC) with diode array detection (DAD) combining quadrupole time-of-flight-mass spectrometry (QTOF-MS/MS) and a quantitative UHPLC coupled with triple quadrupole mass spectrometry (QqQ-MS/MS) approach were established to investigate whether and how the quality of PCS correlates with its collection location. A standard fingerprint of PCS was generated by median simulation of 25 tested samples collected from four main producing areas of China, and similarity analysis was applied to evaluate the similarities between the fingerprints of samples and the standard fingerprint. Twenty three common peaks occurring in the fingerprint were unequivocally or tentatively identified by UHPLC-QTOF-MS/MS. Meanwhile, principal component analysis (PCA), supervised orthogonal partial least squares-discriminate analysis (OPLS-DA) and hierarchical cluster analysis (HCA) were employed to classify 25 batches of PCS samples into four groups, which were highly consistent with the four geographical regions. Ten compounds were screened out as potential markers to distinguish the quality of PCS. Nine triterpene acids, including five compounds that played important roles in the clusters between different samples collected from the four collection locations, were simultaneously quantified by using the multiple reaction monitoring (MRM) mode of UHPLC-QqQ-MS/MS. The current strategy not only clearly expounded the correlation between quality and geographical origins of PCS, but also provided a fast, accurate and comprehensive qualitative and quantitative method for assessing the quality of PCS.

22. Anti-inflammatory and antiproliferative prenylated chalcones from *Hedysarum gmelinii*

Y. Liu, J. Zhang, R. Wen, G.Z. Tu, H.B. Chen, H. Liang, Y.Y. Zhao

Journal of Asian Natural Products Research, 2018, 20, 1009-1018

Abstract

Five new prenylated chalcones hedysarumines C-G (1–5), along with eight known chalcones (6–13) all of which were isolated from the genus *Hedysarum* for the first time, were isolated from the roots of *Hedysarum gmelinii* by chromatographic methods. Their structures were determined by extensive spectroscopic techniques. The isolated chalcones (2–13) and previously isolated prenylated chalcones (14–16) were evaluated for antiproliferative activity against five human cancer cell lines (HepG2, A549, Du145, BGC823, and HCT116) and in vitro anti-inflammatory activity. Compounds 5, 10, and 15 inhibited NO production induced by lipopolysaccharide in BV-2 cells, with IC₅₀ values ranging from 3.25 to 8.48 μ M. Compounds 4 and 11 showed moderate antiproliferative activity to selective human cancer cell lines, with the IC₅₀ values of 4 and 11 against A549 cell line being 7.79 and 9.67 μ M, respectively, and the IC₅₀ value of 11 against HCT116 cell line being 8.85 μ M.

23. Synchronous characterization of carbohydrates and ginsenosides yields deeper insights into the processing chemistry of ginseng

S.S. Zhou, J. Xu, M. Kong, K.M. Yip, J.D. Xu, H. Shen, Z.Z. Zhao, S.L. Li, H.B. Chen

Journal of Pharmaceutical and Biomedical Analysis, 2017, 145, 59-70

Abstract

Carbohydrates and ginsenosides in ginseng are biologically interrelated. Their synchronous analysis is therefore essential in chemical research on ginseng to characterize its “holistic” quality. Here we investigated the processing chemistry of red ginseng (RG), a ginseng product processed by water-steaming, for which both carbohydrates and ginsenosides were qualitatively and quantitatively determined through multiple analytical techniques. Results revealed that the steam-processing not only qualitatively and quantitatively altered the ginsenosides but also affected the polymeric carbohydrates via changing their physiochemical parameters, i.e. water-solubility, molecular size, types and ratios of constituent monosaccharides. Potential mechanisms involved in the transformation of ginseng chemicals are proposed and discussed, including hydrolysis (deglycosylation, demalonylation, deacetylation), dehydration, polymerization, volatilization, reduction and the Maillard reaction. The study strengthens the research on the processing chemistry of RG, and therefore should be helpful for elucidating the scientific basis of RG preparation and application.

24. Identification of polar constituents in the decoction of *Juglans mandshurica* and in the medicated egg prepared with the decoction by HPLC-Q-TOF MS²

T.M. Wang, Y. Fu, W.J. Yu, C. Chen, X. Di, H. Zhang, Y.J. Zhai, Z.Y. Chu, T.G. Kang, H.B. Chen

Molecules, 2017, 22, E1452

Abstract

As a folk medicinal plant, *Juglans mandshurica* has been used for the treatment of cancer in China and Korea. Traditionally, *J. mandshurica* is decocted together with chicken eggs. Both the decoction and medicated eggs possess anti-tumor properties. Clarifying the constituents of the decoction and absorbed by the medicated eggs is essential for the investigation of the active principles of *J. mandshurica*. Herein, the medicated eggs were prepared by decocting raw chicken eggs, having unbroken shells, with the decoction of *J. mandshurica*. A systematic investigation of the chemical profile of the *J. mandshurica* decoction and the medicated egg extraction was conducted by HPLC-Q-TOF-MS2. In total, 93 peaks, including 45 tannins, 14 naphthalene derivatives, 17 organic acids, 3 diarylheptanoids, 4 lignans, 3 anthraquinones, 1 flavonoid glycoside, 3 amino acids, and 3 nitrogenous compounds, were tentatively identified in the decoction. In the medicated egg extraction, 44 peaks including 11 organic acids, 3 amino acids, 3 nitrogenous compounds, 8 naphthalene derivatives, 3 diarylheptanoids, 15 tannins, and 1 lignan were tentatively identified. The chemical profile presented provided a detailed overview of the polar chemical constituents in *J. mandshurica* and useful information for the research of bioactive compounds of this plant.

25. Multiconstituent identification in root, branch, and leaf extracts of *Juglans mandshurica* using ultra high performance liquid chromatography with quadrupole time-of-flight mass spectrometry

T.M. Wang, J. Liu, T. Yi, Y.J. Zhai, H. Zhang, H.B. Chen, S.Q. Cai, T.G. Kang, Z.Z. Zhao

Journal of Separation Science, 2017, 40, 3440-3452

Abstract

As a traditional medicinal plant, *Juglans mandshurica* has been used for the treatment of cancer. Different organs of this plant showed anti-tumor activity in clinic and laboratory. Comparative identification of constituents in different plant organs is essential for investigation of the relationship between chemical constituents and pharmacological activities. For this aim, the roots, branches, and leaves of *J. mandshurica* were extracted with 50% v/v methanol and then subjected to ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry analysis conducted under low and high energy. As a result, we have to date identified 111 compounds consisting of 56 tannins, 29 flavonoids, 13 organic acids, 8 naphthalene derivatives, and 5 anthracenes. Five compounds, namely, diquercetin trihydroxy-truxinoyl-glucoside, two quercetin kaempferol dihydroxy-truxinoyl-glucosides, syringoyl-tri-galloyl-O-glucose, and dihydroxy-naphthalene syringoyl-glucoside, were tentatively identified as new compounds. Of the compounds identified, 76 were found in the root extract, 67 in the branch extract, and 37 in the leaf extract. Only six compounds including four organic acids and two tannins were found in all three extracts. We developed a rapid and sensitive ultra high performance liquid chromatography with quadrupole time-of-flight mass spectrometry approach to identify multiple constituents of complex extracts without separation and ion selection. The results presented provide useful information on further research of the bioactive compounds of *J. mandshurica*.

26. Comparison of chemical profiles between the root and aerial parts from three *Bupleurum* species based on a UHPLC-QTOF-MS metabolomics approach

L. Zhu, Z.T. Liang, T. Yi, Y. Ma, Z.Z. Zhao, B.L. Guo, J.Y. Zhang, H.B. Chen

BMC Complementary and Alternative Medicine, 2017, 17, 305

Abstract

Background: *Bupleuri Radix* (Chaihu) represents one of the most successful and widely used herbal medicines in Asia for the treatment of many diseases such as inflammatory disorders and infectious diseases over the past 2000 years. In the Chinese Pharmacopoeia, Chaihu is recorded as the dried roots of *Bupleurum chinense* DC. and *B. scorzonifolium* Willd. (Umbelliferae). However, the widespread demand for the herb has tended to far outstrip the supply. Whether the aerial parts, which account for 70 ~ 85% of the dry weights of *Bupleurum* species, could be used as an alternative for the root has become an important scientific issue for the sustainable utilization of *Bupleurum* species. On the other hand, in some areas including the southeast of China as well as in Spain, the aerial parts of *Bupleurum* species have already been used in the folk medications. Therefore, to clarify whether the root and aerial parts of *Bupleurum* species are "equivalent" in the types and quantities of chemical constituents which subsequently influence their biological activities and therapeutic effects is of great importance for both the rational and sustainable use of this herb. *Methods:* In the present study, the chemical profiles between the root and aerial parts of *Bupleurum* species from different species and collected from various locations were analyzed and compared by the ultra-high performance liquid chromatography quadrupole/time of flight-mass spectrometry (UHPLC-QTOF-MS).

Results: A total of 56 peaks were identified in the root and/or aerial parts from different batches of *Bupleurum* species, by comparison of references standards or with those reported in the literature. Principal Component Analysis (PCA) was conducted for displaying the differentiating clustering between these two parts.

Conclusion: The results disclosed the distinct variations between them, which indicated that the aerial parts could not be used as an alternative of root from a chemodiversity perspective. The differentiating markers resulted from the PCA analysis could also be utilized for the differentiation between them. Further validation of their biological differences is anticipated in the future study.

27. Comprehensive Quantitative Analysis of 32 Chemical Ingredients of a Chinese Patented Drug Sanhuang Tablet

H.Y. Fung, Y. Lang, H.M. Ho, T.L. Wong, D.L. Ma, C.H. Leung, Q.B. Han.

Molecules, 2017, 22, 111

Abstract

Sanhuang Tablet (SHT) is a Chinese patented drug commonly used for the treatment of inflammations of the respiratory tract, gastrointestinal tract, and skin. It contains a special medicinal composition including the single compound berberine hydrochloride, extracts of *Scutellariae Radix* and *Rhei Radix et Rhizoma*, as well as the powder of *Rhei Radix et Rhizoma*. Despite advances in analytical techniques, quantitative evaluation of a Chinese patented drug like SHT remains a challenge due to the complexity of its chemical profile. In this study, ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC-Q-TOF-MS)

was used to simultaneously quantify 29 non-sugar small molecule components of SHT (11 flavonoids, two isoflavonoids, one flavanone, five anthraquinones, two dianthranones, five alkaloids, two organic acids and one stilbene). Three major saccharide components, namely fructose, glucose, and sucrose, were also quantitatively determined using high performance liquid chromatography-charged aerosol detector (HPLC-CAD) on an Asahipak NH2P-50 4E amino column. The established methods were validated in terms of linearity, sensitivity, precision, accuracy, and stability, and then successfully applied to analyze 27 batches of commercial SHT products. A total of up to 57.61% (w/w) of SHT could be quantified, in which the contents of the determined non-saccharide small molecules varied from 5.91% to 16.83% (w/w) and three saccharides accounted for 4.41% to 48.05% (w/w). The results showed that the quality of the commercial products was inconsistent, and only four of those met Chinese Pharmacopoeia criteria.

28. A modified formulation of Huanglian-Jie-Du-Tang reduces memory impairments and β -amyloid plaques in a triple transgenic mouse model of Alzheimer's disease

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Scientific Reports, 2017, 7: 6238

Abstract

Alzheimer's disease (AD) is a degenerative disorder typified by progressive deterioration of memory and the appearance of β -amyloid peptide ($A\beta$)-rich senile plaques. Recently we have identified a novel function of a patented formulation of modified Huanglian-Jie-Tu-Tang (HLJDT-M), a Chinese herbal medicine, in treating AD in in vitro studies (US patent No. 9,375,457). HLJDT-M is a formulation composed of *Rhizoma Coptidis*, *Cortex Phellodendri* and *Fructus Gardeniae* without *Radix Scutellariae*. Here, we assessed the efficacy of HLJDT-M on a triple transgenic mouse model of AD (3XTg-AD). Oral administration of HLJDT-M ameliorated the cognitive dysfunction of 3XTg-AD mice and lessened the plaque burden. In addition, biochemical assays revealed a significant decrease in levels of detergent-soluble and acid-soluble $A\beta$ via decreasing the levels of full length amyloid- β precursor protein (FL-APP) and C-terminal fragments of APP (CTFs) in brain lysates of HLJDT-M-treated mice. HLJDT-M treatment also significantly reduced the levels of FL-APP and CTFs in N2a/SweAPP cells. In contrast, treatment using the classical formula HLJDT did not reduce the memory impairment of 3XTg-AD mice and, rather, increased the $A\beta$ /FL-APP/CTFs in both animal and cell culture studies. Altogether, our study indicates that HLJDT-M is a promising herbal formulation to prevent and/or cure AD.

29. Anti-fatigue effects of the unique polysaccharide marker of *Dendrobium officinale* on BALB/c mice

W. Wei, Z.P. Li, T. Zhu, H.Y. Fung, T.L. Wong, X. Wen, D.L. Ma, C.H. Leung, Q.B. Han..

Molecules, 2017, 22, 155

Abstract

Dendrobium officinale extract shows potent anti-fatigue effects; however, the active substance responsible for these effects remains undetermined. A glucomannan with a huge molecular size of 730 kDa, called DOP, was identified as the unique authentication marker of this expensive herb. DOP exhibited immunomodulating effects on macrophages and lymphocytes in our previous study. Clinical reports also showed that people with fatigue syndrome have a disturbed immune system. Because DOP is the unique and dominant component of *D. officinale*, we hypothesize that DOP may also have anti-fatigue activity. The present study aims to evaluate the anti-fatigue activity of DOP on BALB/c mice, with *Rhodiola rosea* extract as a positive control. DOP and *Rhodiola rosea* extract were orally administered at doses of 50 mg/kg and 100 mg/kg, respectively, for four weeks, and the anti-fatigue activity of DOP on BALB/c mice was evaluated using the weight-loaded swimming test. The contents of lactic dehydrogenase (LDH), creatine phosphokinase (CK), triglyceride (TG), blood urea nitrogen (BUN), superoxide dismutase (SOD), malondialdehyde (MDA), lactic acid (LD), and glutathione peroxidase (GSH-Px) in serum, glycogen of liver and gastrocnemius muscle were also determined. Their effects on variability of T cells and B cells were determined by using tetrazolium compound (MTS) method. The weight-loaded swimming exercise caused fatigue syndrome, mainly including the decreases of serum SOD/GSH-Px and gastrocnemius glycogen, as well as the increases of LDH, BUN, MDA, CK, TG, and LD in serum. All of these indicators of fatigue were inhibited to a certain extent by both DOP and *Rhodiola rosea* extract; however, the effects of DOP were much stronger than those of *Rhodiola rosea* extract. Compared to the positive control, mice dosed with DOP showed increases in endurance, body weight, and food intake. Furthermore, DOP-feeding mice significantly increased the cell variability of T lymphocytes and B lymphocytes, compared with that of mice in control group. This study indicates that the unique and dominant polysaccharide DOP of *D. officinale* has stronger anti-fatigue activity than *Rhodiola rosea* extract. As such, DOP has promising potential for pharmaceutical development into health products to reduce fatigue.

30. A natural product-like JAK2/STAT3 inhibitor induces apoptosis of malignant melanoma cells

K.J. Wu, J.M. Huang, H.J. Zhong, Z.Z. Dong, K. Vellaisamy, J.J. Lu, X.P. Chen, P. Chiu, D.W.J. Kwong, Q.B. Han, D.L. Ma, C.H. Leung.

PLoS One, 2017, 12 (6):e0177123. Published: June 1, 2017

Abstract

The JAK2/STAT3 signaling pathway plays a critical role in tumorigenesis, and has been suggested as a potential molecular target for anti-melanoma therapeutics. However, few JAK2 inhibitors were being tested for melanoma therapy. In this study, eight amentoflavone analogues were evaluated for their activity against human malignant melanoma cells. The most potent analogue, compound 1, inhibited the phosphorylation of JAK2 and STAT3 in human melanoma cells, but had no discernible effect on total JAK2 and STAT3 levels. A cellular thermal shift assay was performed to identify that JAK2 is engaged by 1 in cell lysates. Moreover, compound 1 showed higher antiproliferative activity against human melanoma A375 cells compared to a panel of cancer and normal cell lines. Compound 1 also activated caspase-3 and cleaved PARP, which are markers of apoptosis, and suppressed the anti-apoptotic Bcl-2 level. Finally, compound 1 induced apoptosis in

80% of treated melanoma cells. To our knowledge, compound 1 is the first amentoflavone-based JAK2 inhibitor to be investigated for use as an anti-melanoma agent.

31. Cinnamon induces browning in subcutaneous adipocytes

H.Y. Kwan, J. Wu , T. Su, X.J. Chao, B. Liu, X. Fu, C.L. Chan, R.H.Y. Lau, A.K.W. Tse, Q.B. Han, W.F. Fong, Z.L. Yu.

Scientific Reports, 2017, 7, 2447

Abstract

Browning is the process of increasing the number of brite cells, which helps to increase energy expenditure and reduce obesity. Consumption of natural and non-toxic herbal extracts that possess the browning effect is an attractive anti-obesity strategy. In this study, we examined the browning effect of cinnamon extract. We found that cinnamon extract (CE) induced typical brown adipocyte multiocular phenotype in 3T3-L1 adipocytes. The treatment also increased brown adipocytes markers and reduced white adipocytes markers in the 3T3-L1 adipocytes. In ex vivo studies, we found that CE increased brown adipocytes markers in the subcutaneous adipocytes isolated from db/db mice and diet-induced obesity (DIO) mice. However, CE did not significantly affect UCP1 expression in the adipocytes isolated from perinephric adipose tissue and epididymal adipose tissue. β 3-adrenergic receptor (β 3-AR) antagonist reduced the CE-enhanced UCP1 expression, suggesting an involvement of the β 3-AR activity. Oral administration of CE significantly increased UCP1 expression in the subcutaneous adipose tissue in vivo and reduced the body weight of the DIO mice. Taken together, our data suggest that CE has a browning effect in subcutaneous adipocytes. Our study suggests a natural non-toxic herbal remedy to reduce obesity.

32. Understanding the Molecular Mechanisms of the Interplay Between Herbal Medicines and Gut Microbiota

J. Xu, H.B. Chen, S.L. Li

Medicinal Research Reviews, 2017, 37, 1140-1185

Abstract

Herbal medicines (HMs) are much appreciated for their significant contribution to human survival and reproduction by remedial and prophylactic management of diseases. Defining the scientific basis of HMs will substantiate their value and promote their modernization. Ever-increasing evidence suggests that gut microbiota plays a crucial role in HM therapy by complicated interplay with HM components. This interplay includes such activities as: gut microbiota biotransforming HM chemicals into metabolites that harbor different bioavailability and bioactivity/toxicity from their precursors; HM chemicals improving the composition of gut microbiota, consequently ameliorating its dysfunction as well as associated pathological conditions; and gut microbiota mediating the interactions (synergistic and antagonistic) between the multiple chemicals in HMs. More advanced experimental designs are recommended for future study, such as overall chemical characterization of gut microbiota-metabolized HMs, direct microbial analysis of HM-targeted gut microbiota, and precise gut microbiota research model development. The outcomes of such research can further elucidate the interactions between HMs and gut microbiota, thereby opening a new window for

defining the scientific basis of HMs and for guiding HM-based drug discovery.

33. Bioactivity, toxicity and detoxification assessment of *Dioscorea bulbifera* L.: a comprehensive review

X.R. Guan, L. Zhu, Z.G. Xiao, Y.L. Zhang, H.B. Chen, T. Yi

Phytochemistry Reviews, 2017, **16**, 573-601

Abstract

Dioscorea bulbifera L. (Chinese: Huangdu, common name: air potato) is a traditional herbal medicine in China, and it is also one of the most widely consumed yam species, especially in West Africa. Studies have verified that *D. bulbifera* is effective in treating a wide range of diseases, such as pharyngitis, goitre, pyogenic skin infections, orchitis and cancer. However, more and more studies have also reported liver and kidney damage caused by *D. bulbifera*. To promote understanding of the bioactivity, toxicity and methods for detoxification of this medicinal and edible plant, the present article reviews the most valuable recent reports on its phytochemistry and pharmacological effects. The possible reasons for its toxicity include the toxic effects of diosbulbin B and D on hepatocytes, the inhibition of antioxidant enzymes in liver mitochondria, and inhibition of enzymes that ordinarily metabolize the herb's components. Synergistic compatibility detoxification may help to reduce toxic effects and improve therapeutic effects. More clinical trials are also required to fully achieve its therapeutic potential.

34. Qualitative and quantitative characterization of secondary metabolites and carbohydrates in Bai-Hu-Tang using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and ultraperformance liquid chromatography coupled with photodiode array detector

W.F. Zhong, W.S. Tong, S.S. Zhou, K.M. Yip, S.L. Li, Z.Z. Zhao, J. Xu, H.B. Chen

Journal of Food and Drug Analysis, 2017, 1-14

Abstract

Bai-Hu-Tang (BHT), a classic traditional Chinese medicine (TCM) formula used for clearing heat and promoting body fluid, consists of four traditional Chinese medicines, i.e., Gypsum Fibrosum (Shigao), *Anemarrhenae Rhizoma* (Zhimu), *Glycyrrhizae Radix et Rhizoma Praeparata cum Melle* (Zhigancao), and nonglutinous rice (Jingmi). The chemical composition of BHT still remains largely elusive thus far. To qualitatively and quantitatively characterize secondary metabolites and carbohydrates in BHT, here a combination of analytical approaches using ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and ultraperformance liquid chromatography coupled with photodiode array detector was developed and validated. A total of 42 secondary metabolites in BHT were tentatively or definitely identified, of which 10 major chemicals were quantified by the extracting ion mode of quadrupole time-of-flight mass spectrometry. Meanwhile, polysaccharides, oligosaccharides, and monosaccharides in BHT were also characterized via sample pretreatment followed by sugar composition analysis. The quantitative results indicated that the determined chemicals accounted for 35.76% of the total extract of BHT, which demonstrated that the study could be instrumental in chemical dissection and

quality control of BHT. The research deliverables not only laid the root for further chemical and biological evaluation of BHT, but also provided a comprehensive analytical strategy for chemical characterization of secondary metabolites and carbohydrates in traditional Chinese medicine formulas.

35. Economic botany collections: A source of material evidence for exploring historical changes in Chinese medicinal materials

E. Brand, C. Leon, M. Nesbitt, P. Guo, R. Huang, H.B. Chen, L. Liang, Z.Z. Zhao

Journal of Ethnopharmacology, 2017, 200, 209-227

Abstract

Many Chinese medicinal materials (CMMs) have changed over centuries of use, particularly in terms of their botanical identity and processing methods. In some cases, these changes have important implications for safety and efficacy in modern clinical practice. As most previous research has focused on clarifying the evolution of CMMs by analyzing traditional Chinese materia medica ("bencao") literature, assessments of historical collections are needed to validate these conclusions with material evidence. Historical collections of Chinese medicines reveal the market materials in circulation at a given moment in time, and represent an underexploited resource for analyzing the evolution of Chinese herbal medicines. This study compares specimens from a rare collection of CMMs from the 1920s with contemporary market materials; by highlighting examples of changes in botanical identity and processing that remain relevant for safe clinical practice in the modern era, this work aims to stimulate further research into previously unexplored historical collections of Chinese medicines. 620 specimens of CMMs that were collected from Chinese pharmacies in the Malay peninsula in the 1920s were examined macroscopically and compared with current pharmacopoeia specifications and authentic contemporary samples. These historical specimens, which are stored in the UK in the Economic Botany Collections (EBC) of Royal Botanic Gardens Kew, were morphologically examined, photographed, and compared to authentic CMMs stored at the Bank of China (Hong Kong) Chinese Medicines Center at Hong Kong Baptist University, as well as authentic herbarium-vouchered specimens from the Leon Collection (LC) at the Kew EBC. Case studies were selected to illustrate examples of historical changes in botanical identity, used plant parts, and processing methods. This investigation confirmed that confusion due to shared common names and regional variations in the botanical identity of certain CMMs has been a persistent issue over time. Additionally, historical changes in processing methods and the plant parts used were observed for some CMMs. In some cases, these changes have direct implications for the safe clinical practice of Chinese medicine. This preliminary assessment illustrated the significant potential of collections for clarifying historical changes in CMMs. More research is needed to investigate pre-modern collections of CMMs, including a more comprehensive assessment of the holdings in the Kew EBC and other European collections that have not yet been explored from the perspective of Chinese medicine

36. Effects of boiling duration in processing of White Paeony Root on its overall quality evaluated by ultra-high performance liquid chromatography quadrupole/time-of-flight mass spectrometry based metabolomics analysis and high performance liquid chromatography quantification

K. Ming, J. Xu, H.H. Liu, J.D. Xu, X.Y. Li, M. Lu, C.R. Wang, H.B. Chen, S.L. Li

Chinese Journal of Natural Medicines, 2017, 15, 62-70

Abstract

Boiling processing is commonly used in post-harvest handling of White Paeony Root (WPR), in order to whiten the herbal materials and preserve the bright color, since such WPR is empirically considered to possess a higher quality. The present study was designed to investigate whether and how the boiling processing affects overall quality of WPR. First, an ultra-high performance liquid chromatography quadrupole/time-of-flight mass spectrometry-based metabolomics approach coupled with multivariate statistical analysis was developed to compare the holistic quality of boiled and un-boiled WPR samples. Second, ten major components in WPR samples boiled for different durations were quantitatively determined using high performance liquid chromatography to further explore the effects of boiling time on the holistic quality of WPR, meanwhile the appearance of the processed herbal materials was observed. The results suggested that the boiling processing conspicuously affected the holistic quality of WPR by simultaneously and inconsistently altering the chemical compositions and that short-time boiling processing between 2 and 10 min could both make the WPR bright-colored and improve the contents of major bioactive components, which were not achieved either without boiling or with prolonged boiling. In conclusion, short-term boiling (2–10 min) is recommended for post-harvest handling of WPR.

37. Two new prenylated isoflavones from Hedysarum multijugum

W. Wang, Y.Y. Zhao, H.B. Chen, Q.Y. Zhang, H. Liang

Journal of Asian Natural Products Research, 2017, 19, 444-447

Abstract

Chemical investigation on the ethyl acetate extract of the roots of Hedysarum multijugum resulted in the isolation of two new prenylated isoflavonoids: 5, 7, 2'-trihydroxy-4'-methoxy-3'-(γ, γ-dimethylallyl) isoflavone (hedysarimisoiflavone A, 1) and 5, 7, 2'-trihydroxy-4'-methoxy-6, 8-di-(γ, γ-dimethylallyl) isoflavone (hedysarimisoiflavone B, 2). Their structures were elucidated on the basis of spectroscopic analyses, especially, ¹H and ¹³C NMR spectra in conjunction with their 2D experiments, ¹H-¹H COSY, HMQC, HMBC, and NOESY.

38. Euphorbia factor L2 induces apoptosis in A549 cells through the mitochondrial pathway

M.T. Lin, S.L. Tang, C. Zhang, H.B. Chen, W.J. Huang, Y. Liu, J.Y. Zhang

Acta Pharmaceutica Sinica B, 2017, 7, 59-64

Abstract

Euphorbia factor L2, a lathyrane diterpenoid isolated from caper euphorbia seed (the seeds of Euphorbia lathyris L.), has been traditionally applied to treat cancer. This article focuses on the cytotoxic activity of Euphorbia factor L2 against lung carcinoma A549 cells and the mechanism by

which apoptosis is induced. We analyzed the cytotoxicity and related mechanism of Euphorbia factor L2 with an MTT assay, an annexin V-FITC/PI test, a colorimetric assay, and immunoblotting. Euphorbia factor L2 showed potent cytotoxicity to A549 cells. Euphorbia factor L2 led to an increase in reactive oxygen species (ROS) generation, a loss of mitochondrial electrochemical potential, release of cytochrome c, activation of caspase-9 and caspase-3, and cleavage of poly(ADP-ribose) polymerase, suggesting that Euphorbia factor L2 induced apoptosis through a mitochondrial pathway. The cytotoxic activity of Euphorbia factor L2 in A549 cells and the related mechanisms of apoptotic induction provide support for the further investigation of caper euphorbia seeds.

39. Determination of ginsenosides in Asian and American Ginsengs by liquid chromatography-quadrupole/time-of-flight MS: assessing variations based on morphological characteristics

Y.J. Chen, Z.Z. Zhao, H.B. Chen, E. Brand, T. Yi, M.J. Qin, Z.T. Liang

Journal of Ginseng Research, 2017, 41, 10-22

Abstract

Background

Asian ginseng and American ginseng are functional foods that share a close genetic relationship and are well-known worldwide. This article aims to investigate the correlation between morphological characteristics and the inherent quality of Asian and American ginsengs.

Methods

In this study, an ultra-HPLC–quadrupole/time-of-flight MS (UHPLC-Q/TOF-MS) method was established for the quantitative analysis of 45 ginseng samples. The method developed for determination was precise and accurate.

Results

The results showed that Asian ginseng samples with the same growing time (with the same or similar number of stem scars) that had a thinner main root, a longer rhizome and more branch roots contained greater amounts of ginsenosides. For American ginseng, two tendencies were observed in the relationship between the diameter of the main root and contents of ginsenosides. One tendency was that samples with thinner main roots tended to contain higher levels of ginsenosides, which was observed in the samples sold under the commercial name pao-shen. Another tendency was that samples with thicker main roots contained higher contents of ginsenosides, which was observed in the samples sold under the commercial name pao-mian, as well as in samples of American ginseng cultivated in Jilin, China.

Conclusion

An approach using ultra-HPLC–quadrupole/time-of-flight MS was successfully established to link morphology and active components for evaluating the quality of Asian and American ginsengs. Clear correlation between visible morphological features and quality of Asian and American ginsengs was found. People can see the difference; this means consumers and vendors can evaluate ginseng by themselves.

40. Rapid differentiation of Xihuangcao from the three *Isodon* species by UPLC-ESI-QTOF-MS/MS and chemometrics analysis

L.L. Wong, Z.T. Liang, H.B. Chen, Z.Z. Zhao

Chinese Medicine, 2016, 11: 48, DOI 10.1186/s13020-016-0120-y

Abstract

Background

Isodon lophanthoides, *I. lophanthoides* var. *graciliflorus* and *I. serra* are the three botanical sources of Xihuangcao, which are often used indiscriminately in herbal products. The aim of this study was to develop a rapid and accurate analytical method to identify the three different botanical sources of Xihuangcao by combining UPLC-ESI-QTOF-MS with chemometrics analysis.

Methods

Fifteen batches of plants were collected as reference materials and their chemical profiles were analyzed by UPLC-ESI-QTOF-MS. These data were subsequently processed by statistical methods, including principal component analysis (PCA), hierarchical cluster analysis (HCA) and orthogonal partial least squared discriminant analysis (OPLS-DA). An automated sample class prediction model was also built using Naive Bayes as a class prediction algorithm to rapidly determine the source species of twenty-seven batches of commercial Xihuangcao samples.

Results

The base peak chromatograms of the three authenticated species showed different patterns and twenty-seven peaks were chosen, including six diterpenoids, one phenolic acid and two glycosides to distinguish among these three species. The results showed good differentiation among the three species by PCA, HCA and OPLS-DA. *Isodon lophanthoides* var. *graciliflorus* was found to be the major botanical source of the commercial samples.

Conclusion

UPLC-ESI-QTOF-MS and subsequent chemometrics analysis were demonstrated effective to differentiate among the three different species of plants used as Xihuangcao.

41. *Astragalus membranaceus*: A review of its protection against inflammation and gastrointestinal cancers

K.K. Auyeung, Q.B. Han, J.K. Ko

The American Journal of Chinese Medicine, 2016, 44:1-22

Abstract

Astragalus membranaceus is a major medicinal herb commonly used in many herbal formulations in the practice of traditional Chinese medicine (TCM) to treat a wide variety of diseases and body disorders. Among its diversified clinical applications, the potential use of this herb and its chemical constituents in treatments of inflammatory diseases and cancers has been actively investigated in recent years. *Astragalus*-based treatments have demonstrated significant amelioration of the toxicity induced by other concurrently administered orthodox drugs (e.g., immunosuppressants and cancer chemotherapeutics). The major components of *Astragalus membranaceus* are polysaccharides, flavonoids, and saponins. Contemporary use of *Astragalus membranaceus* mainly focuses on its immunomodulating, anti-oxidant, and anti-inflammatory, as well as anticancer effects. In this paper, we summarize the properties of *Astragalus membranaceus* and its major constituents in the biological system based on experimental and clinical studies. The

antitumorigenic mechanisms of a novel Astragalus saponins extract called AST in treating various gastrointestinal cancers are highlighted. We discuss in detail how the Astragalus herb and AST influence the immune system, modulate various cancer signaling pathways, and interact with specific transcription molecules during protection against gastrointestinal inflammation and cancers. This information could help clinicians and scientists develop novel target-specific and effective therapeutic agents that are deprived of major systemic side effects, so as to establish a better treatment regimen in the battle against inflammatory diseases and cancers of the gut.

42. Comparative evaluation of chemical profiles of three representative 'snow lotus' herbs by UPLC-DAD-QTOF-MS combined with principal component and hierarchical cluster analyses

Q.L. Chen, L. Zhu, Y.N. Tang, H.Y. Kwan, Z.Z. Zhao, H.B. Chen, T. Yi

Drug Testing and Analysis, 2017, 9:1105-1115

Abstract

Herbal healthcare products are used worldwide as relatively safe and effective alternatives to allopathic drugs. Saussurea laniceps Hand.-Mazz. (SL), S. medusa Maxim. (SM) and S. involucrata (Kar. et Kir.) Sch.Bip. (SI) are three sources of the renowned 'snow lotus', Chinese materia medica for treating inflammatory diseases. The three species have different therapeutic effects, among which SL has been proved to be the most potent, but they are frequently confused on the market and in the academic community. An ultra-high performance liquid chromatography-diode array detector-quadrupole time of flight-mass spectrometry (UPLC-DAD-QTOF-MS) method was developed and used to analyze 49 herbal samples for species analysis and overall quality evaluation. With 25 simultaneously identified constituents, of which 12 were quantified, the three herbs showed different chemical profiles. Four-dimensional principle component analysis (4D-PCA) and orthogonal hierarchical cluster analysis (2D-HCA) results illustrated that SL should be grouped away from SM and SI, contradicting the botanical record in Flora of China. The present chemical determination and pattern recognition results directly explain the therapeutic potency of SL and distinguish the three confused snow lotus herbs. Furthermore, the findings suggest a possible extensive quality evaluation model for multi-origin medicinal plants and help monitor falsification of snow lotus herbal products on the market, contributing to a more regulated pharmaceutical industry.

43. Ultrasound-Assisted Extraction May Not Be a Better Alternative Approach than Conventional Boiling for Extracting Polysaccharides from Herbal Medicines

K.M. Yip, J. Xu, W.S. Tong, S.S. Zhou, T. Yi, Z.Z. Zhao, H.B. Chen

Molecules, 2016, 21, 1569

Abstract

In clinical practice polysaccharides from herbal medicines are conventionally prepared by boiling water extraction (BWE), while ultrasound-assisted extraction (UAE) has often been used instead employed in laboratory research due to its strong extraction ability and efficiency. However, if and how the polysaccharides obtained by UAE and BWE are comparable, and hence whether the UAE-based research is instructive for the actual usage of herbal polysaccharides still requires further

evaluation. To address this issue, here we chemically analyzed and compared the UAE- and BWE-obtained polysaccharides from three herbal medicines, i.e., *Ginseng Radix*, *Astragali Radix* and *Dendrobii Officinalis Caulis*. Then, the spike recovery of two series of standard dextran and pullulan by UAE and BWE was tested. The results showed that the polysaccharides from the herbal medicines by UAE were quantitatively and qualitatively different with those by BWE. The powerful extraction ability and polysaccharide degradation caused by ultrasound collectively contributed to these differences. It was then revealed that not only the UAE conditions but also the polysaccharide structures could affect the extraction ability and polysaccharide degradation. Given these, we highly recommended that the effects of UAE on polysaccharides from herbal medicines should be first carefully considered before employing it in relevant chemical and pharmacological analysis.

44. Comprehensive Quantitative Analysis of SQ Injection Using Multiple Chromatographic Technologies

S.L. Chau, Z.B. Huang, Y.G. Song, R.Q. Yue, A. Ho, C.Z. Lin, W.H. Huang, Q.B. Han

Molecules, 2016, 21(8), 1092

Abstract

Quality control of Chinese medicine injections remains a challenge due to our poor knowledge of their complex chemical profile. This study aims to investigate the chemical composition of one of the best-selling injections, Shenqi Fuzheng (SQ) injection (SQI), via a full component quantitative analysis. A total of 15 representative small molecular components of SQI were simultaneously determined using ultra-high performance liquid chromatography (UHPLC) coupled with quadrupole tandem time-of-flight mass spectrometry (Q-TOF-MS); saccharide composition of SQI was also quantitatively determined by high performance liquid chromatography (HPLC) with evaporative light scattering detector (ELSD) on an amino column before and after acid hydrolysis. The existence of polysaccharides was also examined on a gel permeation chromatography column. The method was well validated in terms of linearity, sensitivity, precision, accuracy and stability, and was successfully applied to analyze 13 SQI samples. The results demonstrate that up to 94.69% (w/w) of this injection product are quantitatively determined, in which small molecules and monosaccharide/sucrose account for 0.18%–0.21%, and 53.49%–58.2%, respectively. The quantitative information contributes to accumulating scientific evidence to better understand the therapy efficacy and safety of complex Chinese medicine injections.

45. UPLC-QTOF-MS based metabolomics coupled with the diagnostic ion exploration strategy for rapidly evaluating sulfur-fumigation caused holistic quality variation in medicinal herbs, Moutan Cortex as an example

X.Y. Li, J.D. Xu, J. Xu, M. Kong, S.S. Zhou, Q. Mao, E. Brand, H.B. Chen, H.Q. Liu, S.L. Li

Analytical Methods, 2016, 8, 1034-1043

Abstract

In the present study, a new strategy using UPLC-QTOF-MS based metabolomics coupled with diagnostic ion exploration for rapidly evaluating sulfur-fumigation caused holistic quality variation in medicinal herbs was proposed and validated by employing Moutan Cortex (MC), a commonly used traditional Chinese medicinal herb, as an example. First, the UPLC-QTOF-MS data of MC and sulfur-fumigated MC (S-MC) were subjected to unsupervised segregation principal component

analysis (PCA) and supervised orthogonal partial least squares discriminant analysis (OPLS-DA), three chemical markers in S-MC were rapidly found and structurally elucidated to be pinane monoterpene glucoside sulfonates; then, after exploring the MS fragmentation pattern of these chemical markers, a common sulfur-containing ion m/z 259 was selected as the diagnostic ion, and additional seven pinane monoterpene glucoside sulfonates were detected and identified in S-MS with the diagnostic ion extraction; finally, the holistic quality variation in MC was further dissected by dynamic determination of these ten characteristic components at different durations of sulfur-fumigation. All the results indicated that sulfur-fumigation can induce chemical transformation of pinane monoterpene glucosides in MC, and the duration of sulfur-fumigation was a decisive factor in the holistic quality variation in S-MC, and that the proposed strategy should be applicable for rapid evaluation of sulfur-fumigation caused holistic quality variation in other medicinal herbs.

46. Comprehensive quantitative analysis of Chinese patent drug YinHuang drop pill by ultra high-performance liquid chromatography quadrupole time of flight mass spectrometry

T.L. Wong, Y.Q. An, B.C. Yan, R.Q. Yue, T.B. Zhang, H.M. Ho, T.J. Ren, H.Y. Fung, D.L. Ma, C.H. Leung, Z.L. Liu, J.X. Pu, Q.B. Han, H.D. Sun

Journal of Pharmaceutical and Biomedical Analysis, 2016, 125, 415-426

Abstract

YinHuang drop pill (YHDP) is a new preparation, derived from the traditional YinHuang (YH) decoction. Since drop pills are one of the newly developed forms of Chinese patent drugs, not much research has been done regarding the quality and efficacy. This study aims to establish a comprehensive quantitative analysis of the chemical profile of YHDP. ultra high-performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-Q-TOF-MS/MS) was used to identify 34 non-sugar small molecules including 15 flavonoids, 9 phenolic acids, 5 saponins, 1 iridoid, and 4 iridoid glycosides in YHDP samples, and 26 of them were quantitatively determined. Sugar composition of YHDP in terms of fructose, glucose and sucrose was examined via a high performance liquid chromatography-evaporative light scattering detector on an amide column (HPLC-NH₂P-ELSD). Macromolecules were examined by high performance gel permeation chromatography coupled with ELSD (HPGPC-ELSD). The content of the drop pill's skeleton component PEG-4000 was also quantified via ultra-high performance liquid chromatography coupled with charged aerosol detector (UHPLC-CAD). The results showed that up to 73% (w/w) of YHDP could be quantitatively determined. Small molecules accounted for approximately 5%, PEG-4000 represented 68%, while no sugars or macromolecules were found. Furthermore, YHDP showed no significant differences in terms of daily dosage, compared to YinHuang granules and YinHuang oral liquid; however, it has a higher small molecules content compared to YinHuang lozenge.

47. HSCCC-based strategy for preparative separation of in vivo metabolites after administration of an herbal medicine: *Saussurea laniceps*, a case study

T. Yi, L. Zhu, G.Y. Zhu, Y.N. Tang, J. Xu, J.Y. Fan, Z.Z. Zhao, H.B. Chen

Scientific Reports, 2016, 6, 33036

Abstract

This paper reports a novel strategy based on high-speed counter-current chromatography (HSCCC) technique to separate in vivo metabolites from refined extract of urine after administration of an herbal medicine. Saussurea laniceps (SL) was chosen as a model herbal medicine to be used to test the feasibility of our proposed strategy. This strategy succeeded in the case of separating four in vivo metabolites of SL from the urine of rats. Briefly, after oral administration of SL extract to three rats for ten days (2.0 g/kg/d), 269.1 mg of umbelliferone glucuronide (M1, purity, 92.5%), 432.5 mg of scopoletin glucuronide (M2, purity, 93.2%), 221.4 mg of scopoletin glucuronide (M3, purity, 92.9%) and 319.0 mg of scopoletin glucuronide (M4, purity, 90.4%) were separated from 420 mL of the rat urine by HSCCC using a two-phase solvent system composed of methyl tert-butyl ether–n-butanol–acetonitrile–water (MTBE–n-BuOH–ACN–H₂O) at a volume ratio of 10:30:11:49. The chemical structures of the four metabolites, M1 to M4, were confirmed by MS and ¹H, ¹³C NMR. As far as we know, this is the first report of the successful separation of in vivo metabolites by HSCCC after administration of an herbal medicine.

48. Full component analysis of *Tianma-Gouteng-Yin*

Y.Y. Huang, L.F. Liu, R.Q. Yue, J. Xu, A. Ho, M. Li, Q.B. Han

Chinese Medicine, 2016, 11:44

Abstract

Background: Tianma-Gouteng-Yin (TGY), which is common Chinese medicine formulation consisting of 11 different herbs and being used in China for the treatment of Parkinson's disease, inflammatory conditions and cardiovascular diseases, was selected for full component analysis. The aim of this study was to quantitatively analyze the chemical profiles of ten commercial TGY samples and one sample produced in our laboratory.

Methods: Ultra-high performance liquid chromatography (UHPLC) coupled with quadrupole-tandem time-of-flight mass spectrometry (Q-TOF-MS) was used to analyze the non-saccharide small molecule components of the different TGY samples. The established method was validated in terms of its linearity, sensitivity, precision, accuracy and stability. High performance liquid chromatography coupled with evaporative light scattering detection (HPLC-ELSD) was also used to quantify three major saccharides (fructose, glucose and sucrose).

Results: The relative standard deviations for the precision, repeatability and stability of these compounds were less than 5 %, while the accuracy of the method was 95–105 %. Twenty-eight of the compounds found in TGY were successfully identified, with 20 being quantified. The macromolecules present in these samples were also identified using an ethanol precipitation method, representing 294.68–696.64 mg/g of the total material depending on the batch. Notably, the components identified using this method represented up to 78 % of the total weight of the TGY samples.

Conclusions: The developed UHPLC/Q-TOF-MS and HPLC-ELSD methods successfully identified 28 of the complex compounds found in TGY.

49. Rapid Fingerprint Analysis of Flos Carthami by Ultra-Performance Liquid Chromatography and Similarity Evaluation

S. Yu, L. Zhu, Z.G Xiao, J. Shen, J. Li, H.S. Lai, J.J. Li, H.B. Chen, Z.Z. Zhao, T. Yi

Journal of Chromatographic Science, 2016, 54, 1619–1624

Abstract

Flos Carthami (FC), the dried flower of Carthamus tinctorius L., has widely been used for the treatment of cardiovascular diseases in China. A rapid fingerprint analysis method of FC was established by ultra-performance liquid chromatography (UPLC) for the quality control. The analysis time was shortened by 45 min, from 75 min on conventional high-performance liquid chromatography (HPLC) to 30 min on UPLC. Ten batches of FC samples from various sources were analyzed by the UPLC method. Coexisting peaks in FC chromatograms were chosen to calculate their relative retention time (RRT) and relative peak area (RPA). Thirteen characteristic peaks were illustrated on UPLC fingerprints. In the 24-h stability test, the relative standard deviation (RSD) for the RRT and RPA was <0.08% and 4.41%, respectively. The RSD for precision of the RRT and RPA was <0.05% and 4.26%, respectively. And the RSD for repeatability was <0.05% for the RRT and 3.91% for the RPA. The similarities of the 10 batches of FC were ranged from 0.938 to 0.995. Compared with the HPLC method, UPLC provided shorter analysis time, higher resolution and better separation performance, which is feasible for the quality control of FC.

50. Establishment of Platforms to Facilitate the Inheritance and Innovation of Chinese Medicinal Authentication

Z.Z. Zhao, H.B. Chen, P. Guo, Z.T. Liang, E. Brand, L.L. Wong

The Journal of Japanese Botany, 2016, 91, 217–222

Abstract

Chinese medicinal authentication is the first and most fundamental step for standardizing Chinese medicinals for global markets and research. Many issues related to authentication have remained unresolved since ancient times. Determining the authenticity and quality of Chinese medicinals remains as much a frontier as it is an essential science in guaranteeing the safety and efficacy of Chinese medicinals in clinical use. In this review, a path of inheritance and innovation of macro-/microscopic identification techniques is explored, featuring the establishment of the dynamic Chinese medicinal database and the research Centre for Standardization of Chinese Medicines (CSCM).

51. Assessing the quality of Smilacis Glabrae Rhizoma (Tufuling) by colormetrics and UPLC-Q-TOF-MS

X.C. He, T. Yi, Y.N. Tang, J. Xu, J.Y. Zhang, Y.Z. Zhang, L.S. Dong, H.B. Chen

Chinese Medicine, 2016, 11: 33, DOI 10.1186/s13020-016-0104-y

Abstract

Background

The quality of the materials used in Chinese medicine (CM) is generally assessed based on an analysis of their chemical components (e.g., chromatographic fingerprint analysis). However, there

is a growing interest in the use of color metrics as an indicator of quality in CM. The aim of this study was to investigate the accuracy and feasibility of using color metrics and chemical fingerprint analysis to determine the quality of *Smilacis Glabrae Rhizoma* (Tufuling) (SGR). The SGR samples were divided into two categories based on their cross-sectional coloration, including red SGR (R-SGR) and white SGR (W-SGR).

Methods

Forty-three samples of SGR were collected and their colors were quantized based on an RGB color model using the Photoshop software. An ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/QTOF MS) system was used for chromatographic fingerprint analysis to evaluate the quality of the different SGR samples. Hierarchical cluster analysis and dimensional reduction were used to evaluate the data generated from the different samples. Pearson correlation coefficient was used to evaluate the relationship between the color metrics and the chemical compositions of R-SGR and W-SGR.

Results

The SGR samples were divided into two different groups based on their cross-sectional color, including color A (CLA) and B (CLB), as well as being into two separate classes based on their chemical composition, including chemical A (CHA) and B (CHB). Standard fingerprint chromatograms were for CHA and CHB. Statistical analysis revealed a significant correlation (Pearson's $r = -0.769$, $P < 0.001$) between the color metrics and the results of the chemical fingerprint analysis.

Conclusions

The SGR samples were divided into two major clusters, and the variations in the colors of these samples reflected differences in the quality of the SGR material. Furthermore, we observed a statistically significant correlation between the color metrics and the quality of the SGR material.

52. The variation in the major constituents of the dried rhizome of *Ligusticum chuanxiong* (Chuanxiong) after herbal processing

T. Yi, J.Y. Fang, L. Zhu, Y.N. Tang, H. Ji, Y.Z. Zhang, J.C. Yu, X.J. Zhang, Z.L. Yu, Z.Z. Zhao, H.B. Chen

Chinese Medicine, 2016, 11: 26, DOI 10.1186/s13020-016-0098-5

Abstract

Background: *Rhizoma Chuanxiong* (RC; Chuanxiong), which is the dried rhizome of *Ligusticum chuanxiong* (Umbel- liferae), is commonly used in Chinese medicine (CM) for improving blood circulation and dispersing blood stasis. RC is usually processed before use in clinical practice to enhance its therapeutic efficacy. This study aimed to investigate the temporal variations of the major constituents of RC by HPLC-DAD-MS during herbal processing to investigate the effects of an adjuvant (e.g., wine), steaming vs stir-frying and the optimal processing time. **Methods:** An HPLC-DAD-MS method was developed to determine the major constituents of the RC processed by one of the four processing methods, i.e., stir-frying, steaming, stir-frying with rice wine and steaming with rice wine. Processing was conducted over 60 min. Six major compounds, namely ferulic acid, senkyunolide I, senkyunolide H, senkyunolide A, Z-ligustilide and levistolide A, were selected as markers to analyze the effects on the markers' levels of the different processing methods and optimize the processing time.

Results: The results indicated that (a) processing with wine had no discernible impact on the amounts of the six chemical markers in RC; (b) the amounts of the major constituents of RC subjected to steam processing were higher than those of the RC subjected to stir-fry processing. Conclusion: Among the four different methods evaluated for RC processing, steaming was better and the optimal time for steaming RC was 40 min.

53. Bruceine D Induces Apoptosis in Human Chronic Myeloid Leukemia K562 Cells via Mitochondrial Pathway

J.Y. Zhang, M.T. Lin, H.Y. Tung, S.L. Tang, T. Yi, Y.Z. Zhang, Y.N. Tang, Z.Z. Zhao, H.B. Chen

American Journal of Cancer Research, 2016, 6, 819-826

Abstract

Chronic myeloid leukemia (CML), an acquired malignant myeloproliferative disorder of hematopoietic stem cells, is one of the three most common forms of leukemia. In this study, we investigated the effects of bruceine D, which have been isolated from Brucea javanica (L.) Merr. on human chronic myeloid leukemia K562 cells. MTT assay was used to evaluate cell growth inhibition. Flow cytometry was performed to analyze mitochondrial membrane potential ($\Delta\Psi_m$). Western blot was applied to detect expression of cytochrome c, caspases-9, -3, PARP and other proteins. Bruceine D exhibited potent cytotoxicity to K562 cells with IC₅₀ of $6.37 \pm 0.39 \mu\text{M}$. It led to loss of $\Delta\Psi_m$, release of cytochrome c, activation of caspases-9, -3 and cleavage of PARP, which suggested that bruceine D induced apoptosis of K562 cells through mitochondrial pathway. In addition, bruceine D inhibited the phosphorylation of AKT and ERK. It's indicative that the potent anticancer activity of bruceine D be related to MAPK and PI3K pathways.

54. Gut microbiota-involved mechanisms in enhancing systemic exposure of ginsenosides by coexisting polysaccharides in ginseng decoction

S.S. Zhou, J. Xu, H. Zhu, J. Wu, J.D. Xu, R. Yan, X.Y. Li, H.H. Liu, S.M. Duan, Z. Wang, H.B. Chen, H. Shen, S.L. Li

Scientific Reports, 2016, 6, 22474

Abstract

*Oral decoctions of traditional Chinese medicines (TCMs) serve for therapeutic and prophylactic management of diseases for centuries. Small molecules and polysaccharides are the dominant chemicals co-occurred in the TCM decoction. Small molecules are well-studied by multidisciplinary elaborations, whereas the role of polysaccharides remains largely elusive. Here we explore a gut microbiota-involved mechanism by which TCM polysaccharides restore the homeostasis of gut microbiota and consequently promote the systemic exposure of concomitant small molecules in the decoction. As a case study, ginseng polysaccharides and ginsenosides in Du-Shen-Tang, the decoction of ginseng, were investigated on an over-fatigue and acute cold stress model. The results indicated that ginseng polysaccharides improved intestinal metabolism and absorption of certain ginsenosides, meanwhile reinstated the perturbed holistic gut microbiota, and particularly enhanced the growth of *Lactobacillus* spp. and *Bacteroides* spp., two major metabolic bacteria of ginsenosides. By exploring the synergistic actions of polysaccharides with small molecules, these*

findings shed new light on scientization and rationalization of the classic TCM decoctions in human health care.

55. A targeted strategy to analyze untargeted mass spectral data: Rapid chemical profiling of *Scutellaria baicalensis* using ultra-high performance liquid chromatography coupled with hybrid quadrupole orbitrap mass spectrometry and key ion filtering

X. Qiao, R. Li, W. Song, W.J. Miao, J. Liu, H.B. Chen, D.A. Guo, M. Ye

Journal of Chromatography A, 2016, 1441, 83-95

Abstract

Structural identification of natural products by tandem mass spectrometry requires laborious spectral analysis. Herein, we report a targeted post-acquisition data processing strategy, key ion filtering (KIF), to analyze untargeted mass spectral data. This strategy includes four steps: (1) untargeted data acquisition by ultra-high performance liquid chromatography coupled with hybrid quadrupole orbitrap mass spectrometry (UHPLC/orbitrap-MS); (2) construction of a key ion database according to diagnostic MS/MS fragmentations and conservative substructures of natural compounds; (3) high-resolution key ion filtering of the acquired data to recognize substructures; and (4) structural identification of target compounds by analyzing their MS/MS spectra. The herbal medicine Huang-Qin (*Scutellaria baicalensis* Georgi) was used to illustrate this strategy. Its extract was separated within 20min on a C18 column (1.8 μ m, 2.1 \times 150mm) eluted with acetonitrile, methanol, and water containing 0.1% formic acid. The compounds were detected in the (-)-ESI mode, and their MS/MS spectra were recorded in the untargeted manner. Key ions were then filtered from the LC/MS data to recognize flavones, flavanones, O-/C-glycosides, and phenylethanoid glycosides. Finally, a total of 132 compounds were identified from Huang-Qin, and 59 of them were reported for the first time. This study provides an efficient data processing strategy to rapidly profile the chemical constituents of complicated herbal extracts.

56. Astragalus saponins Inhibits Lipopolysaccharide-Induced Inflammation in Mouse Macrophages

Y. Wang, T.J. Ren, L.C. Zheng, H.B. Chen, J. K.S. Ko and K. K.W. Auyeung

American Journal of Chinese Medicine, 2016, 44, 579-593

Abstract

Excessive nitric oxide (NO) and pro-inflammatory cytokines are produced during the pathogenesis of inflammatory diseases and cancer. It has been demonstrated that anti-inflammation contributes *Astragalus membranaceus* saponins (AST)'s beneficial effects in combination of conventional anticancer drugs. However, the immunomodulating property of AST has not been well characterized. In this study, we found that AST suppressed lipopolysaccharide (LPS)-induced generation of NO without causing cytotoxicity in the mouse macrophage RAW264.7. The gene and protein overexpression of inducible NO synthase (iNOS) as well as the production of tumor necrosis factor-[Formula: see text], evoked by LPS, was consistently down-regulated by AST. AST also inhibited the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and suppressed nuclear factor (NF)-[Formula: see text]B activation and the associated I[Formula: see text]

text]B[Formula: see text] degradation during LPS insult. Furthermore, AST induced growth inhibition in promyelocytic leukemic HL-60 cells and T-lymphocyte leukemic Jurkat cells, but exerted no cytotoxic effects in normal human peripheral blood mononuclear cells (PBMC). It is known that the chemotherapeutic drug 5-FU can suppress the immune system, which can be identified by a reduced white blood cell count and decreased hematocrit, while the combination of AST and 5-FU can reverse the above hematologic toxicities. To summarize, non-cytotoxic concentrations of AST suppress LPS-induced inflammatory responses via the modulation of p38 MAPK signaling and the inhibition of NO and cytokine release. Importantly, AST can alleviate the hematologic side effects of current chemotherapeutic agents. These findings can facilitate the establishment of AST in the treatment of inflammatory diseases and inflammation-mediated tumor development.

57. Preparation and *in vitro* evaluation of ampelopsin-loaded nanomicelles

R.J. Huang, X.L. Yan, H.B. Chen

China Journal of Chinese Materia Medica, 2016, 41, 65-69

Abstract

To improve the solubility and antitumor activity of ampelopsin, ampelopsin-loaded nanomicelles from the mixture of pluronic F127 and D- α -tocopheryl polyethylene glycol 1000 succinate (TPGS1000) were prepared by film-thin hydration method, in order to optimize the process conditions and physicochemical properties. The antitumor activities against MCF-7 cells between ampelopsin and nanomicelles were compared by MTT method, respectively. The results showed that the optimal nanomicelles were round with the nanometric size of (22.6 ± 0.5) nm, encapsulation efficiency rate of $(80.42 \pm 1.13)\%$, and drug-loading rate of $(4.41 \pm 0.26)\%$. The solubility of ampelopsin in mixed nanomicelles significantly increased by 16 times. In different release media, the mixed nanomicelles could release more than 90% of drug in 8 h, and showed stronger cytotoxicity and inhibition against MCF-7 cells ($P < 0.01$). The mixed nanomicelles can be used as new drug delivery system of ampelopsin.

58. Review on *Saussurea laniceps*, a potent medicinal plant known as “Snow Lotus”: botany, phytochemistry and bioactivities

Q.L. Chen, X.Y. Chen, L. Zhu, H.B. Chen, H.M. Ho, W.P. Yeung, Z.Z. Zhao, T. Yi.

Phytochemistry Reviews, 2016, 15, 537–565

Abstract

Saussurea laniceps (Compositae), commonly known as “cotton-headed snow lotus”, is the most effective “snow lotus” used in both Tibetan and Chinese folk medicine. It performs outstandingly in treating rheumatoid arthritis, which mainly is credited for its anti-inflammatory and anti-nociceptive efficacy, as explained by modern pharmacological studies. Extracts of the herb, including umbelliferone and scopoletin, exert such effects in various *in vivo* and *in vitro* studies. Besides the two chemicals above, more than 100 organic compounds have been found in *S. laniceps*, and 58 of them are presented here in molecular structure, including cynaropicrin, mokko lactone, apigenin, acacetin, and luteolin, all contributing to different bioactivities, such as analgesic, antioxidant, immunomodulatory, anti-microbial and anticancer effects. We provide a natural product library of *S. laniceps*, giving inspirations for structure modification and bioactivity-oriented screening, enabling

sustainable use of this valuable plant. The ethnomedical applications and pharmacological discoveries are compared and crosslinked, revealing modern evidence for traditional usages. Despite that *S. lanicep* is a representative “snow lotus” herb, its material medica records and clinical applications are complicated; there is considerable confusion with the different snow lotuses in the academic community and on the market. This review also aims at clearing such confusion, and improving quality assessment and control of the herb. To better utilize the valuable plant, further comparison among the chemical constitutions, pharmacological activities and therapeutic mechanisms of different snow lotuses are needed.

59. Structure Characterization and Immunomodulating Effects of Polysaccharides Isolated from *Dendrobium officinale*

W. Wei, L. Feng, W.R. Bao, D.L. Ma, C.H. Leung, S.P. Nie, Q.B. Han

Journal of Agricultural and Food Chemistry, 2016, 64, 881-889

Abstract

A crude polysaccharide fraction (cDOP) has been determined to be the characteristic marker of *Dendrobium officinale*, an expensive tea material in Asia, but its chemistry and bioactivity have not been studied. In work reported here, cDOP was destarched (DOP, 90% yield) and separated into two subfraction polysaccharides, DOPa and DOPb, which were characterized by monosaccharide composition and methylation analyses and spectral analyses (FT-IR and ¹H and ¹³C NMR). Both are composed of mannose and glucose at similar ratios and have a similar structure with a backbone of 1,4-linked β-d-mannopyranosyl and β-d-glucopyranosyl residues. Significant differences were observed only in their molecular weights. Bioassay using mouse macrophage cell line RAW264.7 indicated that DOP and its two subfractions enhance cell proliferation, TNF-α secretion, and phagocytosis in a dose-dependent manner. They also induced the proliferation of lymphocytes alone and with mitogens. DOPa and DOPb are thus proven to be major, active polysaccharide markers of *D. officinale*.

60. TLR-4 may mediate signaling pathways of Astragalus polysaccharide RAP induced cytokine expression of RAW264.7 cells

W. Wei, H. T. Xiao, W.R. Bao, D.L. Ma, C.H. Leung, X.Q. Han, C.H. Ko, C.B.S. Lau, C.K. Wong, K. P. Fung, P.C. Leung, Z. X. Bian, Q.B. Han

Journal of Ethnopharmacology, 2016, 179, 243-252

Abstract

ETHNOPHARMACOLOGICAL RELEVANCE:

Polysaccharides of *Radix Astragali* (*Astragalus membranaceus* (Fisch) Bge.; Huangqi) are able to induce cytokine production of macrophages and are considered the main active ingredient for the immune-enhancing effect of this commonly used medicinal herb.

AIM OF THE REVIEW:

To investigate the molecular mechanism of immunomodulating activities of a reported *Astragalus* polysaccharide, RAP, which is a hyperbranched heteroglycan with average molecular weight of 1334 kDa.

Materials and methods:

The cytokine production of RAW264.7 cells were analyzed by using ELISA assays while cell viability was assessed by MTT method. Western blot analysis was used for determining protein contents of mitogen-activated protein kinases (MAPKs). In addition, the level of IL-6, iNOS, and TNF- α mRNA was determined by RT-PCR.

RESULTS:

It has been found that RAP itself did not have any cytotoxic effect on mouse mammary carcinoma 4T1 cells, but it significantly enhanced cytotoxicity of the supernatant of RAW264.7 cells on 4T1 cells. Furthermore, RAP enhanced the production of NO and cytokines in RAW264.7 cells, and significantly up-regulated gene expressions of TNF- α , IL-6, iNOS. All these bioactivities were blocked by the inhibitor of TLR4 (Toll-like receptor 4), suggesting that TLR4 is a receptor of RAP and mediates its immunomodulating activity. Further analyses demonstrated that RAP rapidly activated TLR4-related MAPKs, including phosphorylated ERK, phosphorylated JNK, and phosphorylated p38, and induced translocation of NF- κ B as well as degradation of I κ B- α . These results are helpful to better understand the immunomodulating effects of Radix Astragali.

CONCLUSION:

RAP may induce cytokine production of RAW264.7 cells through TLR4-mediated activation of MAPKs and NF- κ B.

61. Sulfur dioxide residue in sulfur-fumigated edible herbs: the fewer, the safer?

S.M. Duan, J. Xu, Y.J. Bai, Y. Ding, M. Kong, H.H. Liu, X.Y. Li, Q.S. Zhang, H.B. Chen, L.F. Liu, S.L. Li.

Food Chemistry, 2016, 192, 119-124.

Abstract

The residual content of sulfur dioxide is frequently regarded as the exclusive indicator in the safety evaluation of sulfur-fumigated edible herbs. To examine the feasibility of such assessment criteria, here the variations in residual sulfur dioxide content during sulfur-fumigation and the potential mechanisms involved were investigated, using *Angelicae Sinensis Radix* (ASR) as a model herb. The residual sulfur dioxide content and ten major bioactive components in sulfur-fumigated ASR samples were dynamically examined at 13 successive time points within 72 h sulfur-fumigation. The relationship between the content variation tendency of sulfur dioxide and the ten chemicals was discussed. The results suggested that sulfur dioxide-involved chemical transformation of the original components in ASR might cause large consumption of residual sulfur dioxide during sulfur-fumigation. It implies that without considering the induced chemical transformation of bioactive components, the residual sulfur dioxide content alone might be inadequate to comprehensively evaluate the safety of sulfur-fumigated herbs.

62. Distributive and quantitative analysis of the main active saponins in *Panax notoginseng* by UHPLC-QTOF/MS combining with fluorescence microscopy and laser microdissection

Q.L. Chen, Z.T. Liang, E. Brand, H.B. Chen, Z.Z. Zhao.

Planta Medica, 2016, 82, 263-272.

Abstract

The distribution of the secondary metabolites in different tissues of *Panax notoginseng* has not yet been investigated. Furthermore, there is no scientific evidence available for the quality assessment of *P. notoginseng*. This is the first study on the tissue-specific chemicals to identify and determinate the main secondary metabolite profiling of *P. notoginseng* in order to provide more information for quality evaluation. In this study, the ultrahigh-performance liquid chromatography quadrupole time-of-flight mass spectrometry approach combined with fluorescence microscopy and laser microdissection was developed and validated for distributive and quantitative analyses of the main active saponins of different tissues from *P. notoginseng*. The results showed that the total content of notoginsenoside R1, ginsenoside Rg1, ginsenoside Rb1, and ginsenoside Rd in the xylem were higher than those in the cork, phloem, and cortex. There was no significant difference in the distribution of saponins between the main roots and the branch roots of the fresh unprocessed materials, nor was there a significant difference in their distribution between the main roots from the fresh unprocessed vs. the dried processed commercial materials. This method illustrated the distribution pattern of the main saponins in the tissues of *P. notoginseng*, which could help to explain the relationship between its anatomical structures, morphological characteristics, and quality. In summary, this study has significance for the procurement, collection, cultivation, effective management, and quality control of *P. notoginseng*.

63. A novel coumarin, (+)-3'-angeloxyloxy-4'-keto-3',4'-dihydroseselin, isolated from *Bupleurum malconense* (Chaihu) inhibited NF-κB activity

H.X. Mu, C.Y. Lin, L.F. Huang, D.J. Yang, A.P. Lu, Q.B. Han, Z.X. Bian.

Chinese Medicine, 2016, 11:5, DOI: 10.1186/s13020-016-0077-x

Abstract

Background

This study aims to identify the major anti-inflammatory components in the petroleum ether extract of *Bupleurum malconense* (Chaihu), by bioassay-guided fractionation, and to investigate the anti-inflammatory mechanisms of active components in lipopolysaccharide (LPS)-stimulated murine macrophage RAW-Blue cells.

Methods

A QUANTI-Blue assay was used to guide fractionation of *B. malconense* root extract. The petroleum ether extract which exerted significant secreted embryonic alkaline phosphatase (SEAP) inhibition effect was purified by silica gel column chromatography and assisted with reverse phase HPLC. The major bioactive compound which significantly inhibited SEAP activity was obtained and its anti-inflammatory effects in LPS-induced RAW-Blue cells were measured by the overproduction of NO (Griess method), gene expression of *Il-1β*, *Tnf-α* and *iNos* (real-time PCR). In parallel, protein expressions of COX-2, *iNOS* and *IκB-α* were determined by western blot.

Results

In bioassay-guided fractionation using LPS-stimulated mouse macrophage RAW-Blue cells, (+)-3'-angeloxyloxy-4'-keto-3',4'-dihydroseselin (Pd-Ib) was identified by MS and NMR spectral analyses. Pd-Ib (5, 10, 20 μg/mL) suppressed the gene expression of *Il-1β* ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ for three respective concentrations), *Tnf-α* ($P = 0.006$, $P = 0.001$, $P < 0.0001$ for three respective concentrations) and *iNos* ($P = 0.009$, $P < 0.0001$, $P < 0.0001$ for three respective concentrations) in LPS-stimulated macrophages. The production of cyclooxygenase-2 ($P = 0.019$,

$P = 0.002$, $P < 0.0001$), iNOS ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ for three respective concentrations) and NO ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ for three respective concentrations) significantly decreased when macrophages were treated with Pd-Ib (5, 10, 20 $\mu\text{g/mL}$) in the presence of LPS. Pd-Ib (5, 10, 20 $\mu\text{g/mL}$) suppressed the nuclear activation of NF- κB while it up-regulated the I $\kappa\text{B}-\alpha$ level ($P = 0.028$, $P = 0.013$, $P = 0.005$ for three respective concentrations) in LPS-stimulated macrophages.

Conclusions

Pd-Ib isolated from *B. malconense* suppressed LPS-induced inflammatory responses in macrophages by inhibiting NF- κB activity and reducing the expression of iNOS, COX-2 as well as pro-inflammatory cytokines.

64. Tissue - specific metabolite profiling and quantitative analysis of ginsenosides in *Panax quinquefolium* using laser microdissection and liquid chromatography–quadrupole/time of flight-mass spectrometry

Y.J. Chen, L. Xu, Y.C. Zhao, Z.Z. Zhao, H.B. Chen, T. Yi, M.J. Qin, Z.T. Liang

Chemistry Central Journal, 2015, 9: 66

Abstract

BACKGROUND:

The root of *Panax quinquefolium* L., famous as American ginseng all over the world, is one of the most widely-used medicinal or edible materials. Ginsenosides are recognized as the main bioactive chemical components responsible for various functions of American ginseng. In this study, tissue-specific chemicals of *P. quinquefolium* were analyzed by laser microdissection and ultra-high performance liquid chromatography- quadrupole/time-of-flight-mass spectrometry (UHPLC-Q/TOF-MS) to elucidate the distribution pattern of ginsenosides in tissues. The contents of ginsenosides in various tissues were also compared.

RESULTS:

A total of 34 peaks were identified or temporarily identified in the chromatograms of tissue extractions. The cork, primary xylem or cortex contained higher contents of ginsenosides than phloem, secondary xylem and cambium. Thus, it would be reasonable to deduce that the ratio of total areas of cork, primary xylem and the cortex to the area of the whole transection could help to judge the quality of American ginseng by microscopic characteristics.

CONCLUSION:

This study sheds new light on the role of microscopic research in quality evaluation, and provides useful information for probing the biochemical pathways of ginsenosides. Graphical abstract.

65. Tianma Gouteng Yin, a Traditional Chinese Medicine decoction, exerts neuroprotective effects in animal and cellular models of Parkinson's disease

L.F. Liu, J.X. Song, J.H. Lu, Y.Y. Huang, Y. Zeng, L.L. Chen, S.S. Durairajan, Q.B. Han, M. Li
Scientific Reports, 2015, 5, 16862.

Abstract

Tianma Gouteng Yin (TGY) is a traditional Chinese medicine (TCM) decoction widely used to treat symptoms associated with typical Parkinson's disease (PD). In this study, the neuroprotective effects of water extract of TGY were tested on rotenone-intoxicated and human α -synuclein

transgenic *Drosophila* PD models. In addition, the neuroprotective effect of TGY was also evaluated in the human dopaminergic neuroblastoma SH-SY5Y cell line treated with rotenone and the rotenone intoxicated hemi-parkinsonian rats. In rotenone-induced PD models, TGY improved survival rate, alleviated impaired locomotor function of *Drosophila*, mitigated the loss of dopaminergic neurons in hemi-parkinsonian rats and alleviated apoptotic cell death in SH-SY5Y cells; in α -synuclein transgenic *Drosophila*, TGY reduced the level of α -synuclein and prevented degeneration of dopaminergic neurons. Conclusively, TGY is neuroprotective in PD models both in vivo and in vitro.

66. Chemical analysis of the *Hedysarum multijugum* root by HPLC fingerprinting

Y. Liu, W. Wang, Y.Y. Zhao, H.B. Chen, H. Liang, Q.Y. Zhang.

Journal of Chinese Pharmaceutical Sciences, 2015, 24, 654-659.

Abstract

The root of *Hedysarum multijugum* (RHM) is recorded as a folk herbal medicine in China and is sometimes used as a substitute for *Hedysari Radix*, which is a famous traditional Chinese medicine derived from the roots of *Hedysarum polybotrys*. In the present study, a sensible, reliable, and reproducible HPLC-DAD fingerprint analysis method for RHM was developed and then subsequently applied to analyze RHM samples from different origins. The chemical constituents of the RHM samples were generally consistent, although it was slightly affected by the local environment of the plant. In addition, the chemical constituency of RHM was shown to be significantly different from that of *Hedysari Radix*, suggesting that RHM is not suitable as a substitute for *Hedysari Radix*, at least from the chemical point of view.

67. Inheritance and innovation of traditional Chinese medicinal authentication

Z.Z. Zhao, H.B. Chen, P.G. Xiao, P. Guo, Z.T. Liang, F. Hung, L.L. Wong, E. Brand, J. Liu.

China Journal of Chinese Materia Medica, 2015, 40, 3385-3390. (in Chinese)

Abstract

Chinese medicinal authentication is fundamental for the standardization and globalization of Chinese medicine. The discipline of authentication addresses difficult issues that have remained unresolved for thousands of years, and is essential for preserving safety. Chinese medicinal authentication has both scientific and traditional cultural connotations; the use of scientific methods to elucidate traditional experience-based differentiation carries the legacy of Chinese medicine forward, and offers immediate practical significance and long-term scientific value. In this paper, a path of inheritance and innovation is explored through the scientific exposition of Chinese medicinal authentication, featuring a review of specialized publications, the establishment of a Chinese medicine specimen center and Chinese medicinal image databases, the expansion of authentication technologies, and the formation of a cultural project, dedicated to the Compendium of *Materia Medica*.

68. Cardioprotective effect of total saponins from three medicinal species of *Dioscorea* against isoprenaline-induced myocardial ischemia

Y.N. Tang, X.C. He, M. Ye, H. Huang, H.L. Chen, W.L. Peng, Z.Z. Zhao, T. Yi, H.B. Chen.

Journal of Ethnopharmacology, 2015, 175, 451–455

Abstract

ETHNOPHARMACOLOGICAL RELEVANCE

As folk medicines used in China since 1950s, *Dioscorea nipponica* Makino (DN), *D. panthaica* Prain et Burkill (DP), and *D. zingiberensis* C. H. Wright (DZ) are regarded as having more or less the same traditional therapeutic actions, such as activating blood, relieving pain, and dispersing swelling. It is noteworthy that, of the 49 species of the genus *Dioscorea* distributed in China, based on such traditional efficacies, only these three have been further developed as effective single-herb medicines for treating cardiovascular diseases by the modern pharmaceutical industry. In our previous study, it was found that the chemical compositions of DN and DP were similar, and both were distinct from that of DZ. Hence, whether their different chemical profiles support their anti-IHD (ischemic heart disease) activity in common still needs to be answered. So far it is still unknown whether the efficacies of these three herbs act via similar mechanism and whether they possess comparable therapeutic efficacy for experimental myocardial ischemia (MI).

AIM OF THE STUDY

The present study aimed to further investigate the underlying mechanisms with respect to anti-oxidative stress activity by which these *Dioscorea* spp. attenuate MI, and to compare the therapeutic effect of total saponins from these three species on myocardial antioxidant levels and myocardial histology.

Material and methods

The serum levels of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), total superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPx), the total antioxidant capacity (T-AOC), and malondialdehyde (MDA), as well as myocardial histology, were compared among rat groups administered with total saponins (TS) of DN, DP or DZ (abbreviated as DNTS, DPTS and DZTS, respectively). The rats experienced myocardial ischemia induced by isoprenaline (ISO) injection; the test solutions (DNTS, DPTS, DZTS) were administered either after the ISO injection, or both before and after.

RESULTS

Compared with the model group (ISO injection only), TS groups exhibited significantly reduced activities of CK, LDH and AST, lowered level of MDA, and increased activities of SOD, CAT, GPx and T-AOC; heart tissues from TS groups revealed less severe histological damage. The cardioprotective efficacy of these three *Dioscorea* TS for rat MI was closely comparable based on the above observations.

CONCLUSION

The findings of the present study provide evidence that the anti-MI effect of DNTS, DPTS and DZTS can be attributed to the increase of myocardial antioxidant levels and decrease of lipid peroxidation formation, and the closely comparable results observed from these three *Dioscorea* saponins thereby explains the similarity in their clinical efficacy as anti-MI drugs.

69. *Saussurea involucrata*: A review of the botany, phytochemistry and ethnopharmacology of a rare traditional herbal medicine

W.I. Chik, L. Zhu, L.L. Fan, T. Yi, G.Y. Zhu, X.J. Gou, Y.N. Tang, J. Xu, W.P. Yeung, Z.Z. Zhao, Z.L. Yu, H.B. Chen.

Journal of Ethnopharmacology, 2015, 172, 44-60.

Abstract

ETHNOPHARMACOLOGICAL RELEVANCE:

Saussurea involucrata Matsum. & Koidz. is an endangered species of the Asteraceae family, growing in the high mountains of central Asia. It has been, and is, widely used in traditional Uyghur, Mongolian and Kazakhstan medicine as well as in Traditional Chinese Medicine as Tianshan Snow Lotus (Chinese:). In traditional medical theory, *S. involucrata* can promote blood circulation, thereby alleviating all symptoms associated with poor circulation. It also reputedly eliminates cold and dampness from the body, diminishes inflammation, invigorates, and strengthens Yin and Yang. It has long been used to treat rheumatoid arthritis, cough with cold, stomach ache, dysmenorrhea, and altitude sickness in Uyghur and Chinese medicine.

AIM OF THE REVIEW:

To comprehensively summarize the miscellaneous research that has been done regarding the botany, ethnopharmacology, phytochemistry, biological activity, and toxicology of *S. involucrata*.

METHOD:

An extensive review of the literature was carried out. Apart from different electronic databases including SciFinder, Chinese National Knowledge Infrastructure (CNKI), ScienceDirect that were sourced for information, abstracts, full-text articles and books written in English and Chinese, including those traditional records tracing back to the Qing Dynasty. Pharmacopoeia of China and other local herbal records in Uighur, Mongolian and Kazakhstan ethnomedicines were investigated and compared for pertinent information.

RESULTS:

The phytochemistry of *S. involucrata* has been comprehensively investigated. More than 70 compounds have been isolated and identified; they include phenylpropanoids, flavonoids, coumarins, lignans, sesquiterpenes, steroids, ceramides, polysaccharides. Scientific studies on the biological activity of *S. involucrata* are equally numerous. The herb has been shown to have anti-neoplastic, anti-inflammatory, analgesic, anti-oxidative, anti-fatigue, anti-aging, anti-hypoxic, neuroprotective and immunomodulating effects. Many have shown correlations to the traditional clinical applications in Traditional Chinese Medicine and medicines. The possible mechanisms of *S. involucrata* in treating various cancers are revealed in the article, these include inhibition of cancer cells by affecting their growth, adhesion, migration, aggregation and invasion, inhibition of epidermal growth factor receptor signaling in cancer cells, hindrance of cancer cell proliferation, causing cytotoxicity to cancer cells and promoting expression of tumor suppressor genes. Dosage efficacy is found to be generally concentration- and time-dependent. However, studies on the correlation between particular chemical constituents and specific bioactivities are limited.

CONCLUSION:

In this review, we have documented the existing traditional uses of *S. involucrata* and summarized recent research into the phytochemistry and pharmacology of *S. involucrata*. Many of the traditional uses have been validated by phytochemical and modern pharmacological studies but there are still

some areas where the current knowledge could be improved. Although studies have confirmed that *S. involucrata* has a broad range of bioactivities, further in-depth studies on the exact bioactive molecules and the mechanism of action are expected. Whether we should use this herb independently or in combination deserves to be clarified. The exact quality control as well as the toxicology studies is necessary to guarantee the stability and safety of the clinic use. The sustainable use of this endangered resource was also addressed. In conclusion, this review was anticipated to highlight the importance of *S. involucrata* and provides some directions for the future development of this plant.

70. Metabolite Profiling of Tissues of *Acorus calamus* and *Acorus tatarinowii* Rhizomes by Using LMD, UHPLC-QTOF MS, and GC-MS

Y. Jaiswal, Z.T. Liang, A. Ho, H.B. Chen, Z.Z. Zhao

Planta Medica, 2015, 81, 333-341

Abstract

Acorus calamus and its related species are of significant importance to the food and fragrance industries due to their varied applications. They are also a cause of critical concern due to their toxic β -asarone content. Several toxicity cases have occurred due to high β -asarone compositions in food products. Hence, limits for their use are strictly regulated by the Food and Drug Administration, the European Union, and legislations of different countries. The identification of species with a lower β -asarone content is of great significance. In this report, the metabolite profiles and essential oil content of *A. calamus* and *Acorus tatarinowii* rhizomes were analysed and compared using UHPLC-QTOF-MS and GC-MS techniques. The metabolite profiles were similar; however, β -asarone content was higher in *A. calamus* rhizomes. The developed methods can be applied for microscopic and macroscopic identification, and quality control of food products containing β -asarone.

71. UPLC and HPLC analysis on contents of astilbin and engeletin in dong medicine “sunl gaems” of Guizhou origin by QAMS

H.Z. Du, X.C. He, H. Nong, L.S. Dong, H.B. Chen, J. Cai, M. Li.

China Journal of Chinese Materia Medica, 2015, 40, 27-33. (in Chinese)

Abstract

This study aimed to simultaneously determine the contents of astilbin and engeletin in dong medicine “sunl gaems” of Guizhou origin by quantitative analysis of multi-components by single marker (QAMS), with astilbin as the internal standard substance. On UPLC and HPLC chromatograms, different models of instruments were used to investigate relative correction factors (RCF), in order to discuss the interoperability of RCFs established in different chromatographic systems. The engeletin content was calculated based on the established RCFs and compared by the one point external standard method and the external standard working curve method, in order to verify the accuracy of QAMS. According to the result, in different chromatograms, the ratios between RCF and retention time of engeletin and astilbin had a good reproducibility, with RSD between 2.0% and 1.8%, both were less than 3%. The relative differences among results of QAMS, the external standard working curve method of dong medicine “sunl gaems” ranged between 1.6%

and 3.9%, with RSD between 2.02%-0.80% in line with relevant requirements and Pearson correlation coefficient at 0.999 8 ($P < 0.01$). The findings showed that QAMS was an accurate, reliable and highly reproducible method to determine the contents of astilbin and engeletin in dong medicine "sunl gaems" of Guizhou origin and so could be used to control the inherent quality of the herb.

72. Quality evaluation of Smilacis Glabrae Rhizoma based on standard fingerprint by UPLC-Q-TOF-MS

X.C. He, H.Z. Du, H.B. Chen, L.S. Dong, H. Nong.

Chinese Journal of Pharmaceutical Analysis, 2015, 35, 120-125. (in Chinese)

Abstract

Objective: To establish the standard fingerprint of Smilacis Glabrae Rhizoma in Guizhou province by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS). Methods: The analysis was performed on an Acquity UPLC, and the mass spectrum data was recorded with negative ion electrospray ionization (ESI) modes. The complete original data was parsed using MS toolbox in MATLAB, and linear interpolation calculation was employed to get global correction chromatogram by relative time. Then, standard fingerprint was generated through the average method, and similarity evaluation was carried out based on the global chromatogram. Results: Standard fingerprint of Smilacis Glabrae Rhizoma was established preliminarily by mass. Consistent results were observed from the two similarity parameters with similarity evaluation based on the 28 chromatographic peaks. Conclusion: Standard fingerprint of MS based on internal standard calibration is accurate, reliable, and convenient to use, which can well reflect the inner quality of Smilacis Glabrae Rhizoma in Guizhou province.

73. Combinational Treatment of Curcumin and Quercetin against Gastric Cancer MGC-803 cells in Vitro

J.Y. Zhang, M.T. Lin, M.J. Zhou, T. Yi, Y.N. Tang, S.L. Tang, Z.J. Yang, Z.Z. Zhao, H.B. Chen.

Molecules, 2015, 20, 11524-11534.

Abstract

Gastric cancer remains a major health problem worldwide. Natural products, with stronger antitumor activity and fewer side effects, are potential candidates for pharmaceutical development as anticancer agents. In this study, quercetin and curcumin were chosen for testing and were applied separately and in combination to human gastric cancer MGC-803 cells. The MTT assay was used to evaluate cell growth inhibition. Annexin V-FITC/PI was carried out to measure apoptosis rate. Flow cytometry was performed to analyze mitochondrial membrane potential levels. Western blots were applied to detect expression of cytochrome c, total and phosphorylated ERK and AKT. Combined treatment with curcumin and quercetin resulted in significant inhibition of cell proliferation, accompanied by loss of mitochondrial membrane potential ($\Delta\Psi_m$), release of cytochrome c and decreased phosphorylation of AKT and ERK. These results indicate that the combination of curcumin and quercetin induces apoptosis through the mitochondrial pathway. Notably, effect of combined treatment with curcumin and quercetin on gastric cancer MGC-803 cells is stronger than

that of individual treatment, indicating that curcumin and quercetin combinations have potential as anti-gastric cancer drugs for further development.

74. *Saussurea medusa*, source of the medicinal herb snow lotus: a review of its botany, phytochemistry, pharmacology and toxicology

J.Y. Fan, H.B. Chen, L. Zhu, H.L. Chen, Z.Z. Zhao, T. Yi.

Phytochemistry Reviews, 2015, 14, 353-366.

Abstract

Saussurea medusa Maxim. is a plant source of the traditional medicine known as “snow lotus” which has been recorded in many ethnopharmacy books, including: Herbal Communications, Flora Tibet, National Herbal Compendium, and Crystal beads Materia Medica. This plant is also a medicinal plant source of Gaode-Subage, a Mongolian folk medicine. The review summarizes the traditional uses and current knowledge concerning the botany, phytochemistry, pharmacological effects, toxicology studies and clinical applications of *S. medusa*. These could guide the priorities and direction of the future research. Information on *S. medusa* was gathered from e-literature found on the internet using SCI Finder, Google Scholar, Web of science, PubMed, CNKI, and the Wanfang database, and from printed material (books, theses, etc.) found in libraries. Additionally, information was also obtained from some local herb gatherers, herbal peddler and herbalist. More than 70 chemical compounds have been isolated, including apigenin, quercetin, rutin, arctigenin, saussureoside A, and saussureoside B. Modern pharmacological studies have found that *S. medusa* has anti-inflammatory, analgesic, anti-fungal, anti-tumor, anti-anoxia, anti-oxidation, and anti-fatigue effects. This plant also benefits the uterus, cardiovascular system, intestines, tracheal smooth muscle, and immune system. *S. medusa* is an important traditional herbal medicine with a broad spectrum of therapeutic efficacies. It has the reputation of non-toxic. Due to the low source of the original plant, experiment of cell culture of *S. medusa* can improve the output of the medicine. Further studies can lead to the development of new drugs and therapeutics from this medical plant for various diseases.

75. Ingredient authentication of commercial Xihuangcao herbal tea by a microscopic technique combined with UPLC-ESI-QTOF-MS/MS

L.L. Wong, Z.T. Liang, H.B. Chen, Z.Z. Zhao

Analytical methods, 2015, 7, 4257-4268

Abstract

Herbal tea is a widely consumed beverage in the world, numerous materials can be used as their ingredients. As they are not regarded as drugs, the ingredients in the tea are proposed to be safe. To ensure the safe use and quality of herbal tea, an easy and practical method should be built to identify the ingredients in commercial herbal tea. In this study, a combined method which used microscopic technique and UPLC-ESI-QTOF-MS/MS analysis was applied in the identification of commercial Xihuangcao herbal tea bag containing multiple herbs. Three original plants of Xihuangcao were first collected and studied as reference materials, and the ingredients in eight Xihuangcao herbal tea samples were identified under microscopy. The herbs that appeared in high frequency were further verified by UPLC-ESI-QTOF-MS with representative peaks in extracted ion

chromatograms. Result showed that Xihuangcao can be found in 7 out of 8 batches of tea bags, which *Isodon serra* is the major species. Some herbs which are not listed as ingredients were also found in the samples. This is the first study that uses a combined microscopic and mass spectrometric method to identify mixed herbal tea samples with unknown ingredients, which proved to be a fast, accurate and practical method that can be applied in routine laboratories.

76. Fingerprint analysis of processed Rhizoma Chuanxiong by high-performance liquid chromatography coupled with diode array detection

J.Y. Fang, L. Zhu, Y. Tao, J.Y. Zhang, Y. Ling, Z.T. Liang, L. Xia, J.F. Feng, J. Xu, Y.N. Tang, Z.Z. Zhao, H.B. Chen

Chinese Medicine, 2015, 10:2, DOI: 10.1186/s13020-015-0031-3

Abstract

Background

Rhizoma Chuanxiong (RC) is the dried rhizome of Ligusticum chuanxiong Hort., and various types of processed Rhizoma Chuanxiong (PRC) are widely used in China. However, quality assurance and quality control of these processed medicines remain challenging. This study aims to investigate the chemical compositions of various PRC preparations by a high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) method.

Methods

A HPLC-DAD method with validation was developed for PRC samples. Seven batches of plant samples from two processing methods, stir-frying and steaming, were analyzed by the HPLC-DAD method. Common peaks in PRC chromatograms were chosen to calculate their relative retention time (RRT) and relative peak area (RPA), and similarity analyses of the chromatographic fingerprints were conducted by Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine software (Version 2004 A).

Results

In the 24-h stability test, the relative standard deviation for the RRT and RPA was less than 0.07% and 2.57%, respectively. The precision was less than 0.08% for the RRT and 2.48% for the RPA. The repeatability for the RRT and RPA was less than 0.03% and 2.64%, respectively. The similarities between the seven PRC batches were range from 0.956 to 0.990. After stir-frying or steaming, the amount of ferulic acid in PRC was much higher than that in the raw material.

Conclusions

The fingerprint analysis of PRC by different processing methods was feasible by HPLC-DAD.

77. Comprehensive quantitative analysis of Shuang-Huang-Lian oral liquid using UHPLC–Q-TOF-MS and HPLC-ELSD

T.B. Zhang, R.Q. Yue, J. Xu, H.M. Ho, D.L. Ma, C.H. Leung, S.L. Chau, Z.Z. Zhao, H.B. Chen, Q.B. Han

Journal of Pharmaceutical and Biomedical Analysis, 2015, 102, 1–8

Abstract

Shuang-Huang-Lian oral liquid (SHL) is a well-known Chinese patent drug containing three herbal medicines: Radix Scutellariae, Flos Lonicerae Japonicae and Fructus Forsythiae. It is usually used to treat acute upper respiratory tract infection caused by virus or bacteria. Although the licensing of botanical drug Veregen approved by FDA has indicated the importance of quantitative analysis in quality control of herbal medicines, quantitative evaluation of a Chinese patent drug like SHL remains a challenge due to the complex chemical profile. In this study, 15 small molecular components of SHL (four flavonoids, six quinic acid derivatives, three saponins and two phenylethanoid glycosides) were simultaneously determined using ultra-high performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (UHPLC–Q-TOF-MS). The contents of the three major saccharides, namely fructose, glucose and sucrose were quantified using high performance liquid chromatography-evaporative light scattering detector on an amino column (HPLC-ELSD). The macromolecules were quantified by precipitating in 80% ethanol, drying the precipitate, and then weighing. The established methods were validated in terms of linearity, sensitivity, precision, accuracy and stability and then successfully applied to analyze 12 batches of commercial products of SHL produced by four different manufacturers. The results indicated that 57.52–78.11% (w/w) of SHL could be quantitatively determined (non-saccharide small molecules: 1.77–3.75%, monosaccharides: 0.93–20.93%, macromolecules: 2.63–5.76% and sucrose: 49.20–65.94%). This study may provide a useful way to comprehensively evaluate the quality of SHL.

78. Chemical diversity investigation of hepatotoxic pyrrolizidine alkaloids in Qianliguang (*Senecio scandens*) and related species by UHPLC-QTOF-MS1

L. Zhu, N. Li, J.Q. Ruan, P.P. Fu, Z.Z. Zhao, G. Lin

Modern Research on Chinese Materia Medica, 2015, 1(2), 1-11

Abstract

Objective:

*Qianliguang (*Senecio scandens*) is a common Chinese medicinal herb. Qianliguang-containing herbal proprietary products are registered as over-the-counter remedies in China and exported to Western countries. The presence of hepatotoxic pyrrolizidine alkaloids (PAs) has raised concerns about the safety of using Qianliguang and its products. The present study aims at investigation of different types of PAs present in Qianliguang collected from representative locations in China.*

Methods:

*In this study, a simple but specific UHPLC-QTOF-MS method for the determination of toxic PAs was developed, based on the characteristic fragment ions specific to different types of PAs. It was successfully applied for the identification and distinguishing of PAs present in Qianliguang and related *Senecio* species growing in different locations of China.*

Results:

Significant diversity of the PA types and quantities were revealed among the samples tested. The estimated total amounts of toxic PAs in three of the samples exceed the toxic limits of PA intake restricted by WHO, demonstrating the timely and highly demand for regulating both types and quantities of PAs present in Qianliguang.

Conclusions:

This study provides the methodology for simultaneous identification and quantification of PAs

present in herbs without requiring corresponding standards, which could be further used for more systematic investigations of the PA distribution in Qianliguang and other PA-containing herbs.

79. Whole transverse section and specific-tissue analysis of secondary metabolites in seven different grades of root of *Paeonia lactiflora* using laser microdissection and liquid chromatography-quadrupole/time of flight-mass spectrometry

Q.L. Wang, Z.T. Liang, Y. Peng, J.L. Hou, S.L. Wei, Z.Z. Zhao, W.Q. Wang

Journal of Pharmaceutical and Biomedical Analysis, 2015, 103, 7-16

Abstract

*The root of *Paeonia lactiflora* Pall. is widely used in the pharmaceutical, food and cosmetic industries. For these purposes, roots are graded according to diameter, with larger roots considered to be of better quality. To assess the inherent quality of different grades and of different tissues in roots of *P. lactiflora*, here laser microdissection coupled with UPLC-Q/TOF-MS was applied. The results show the quantity of pharmaceutically important components decreased with increase in root diameter from 0.3cm to 0.7cm. Above 0.7cm of diameter, quantity and diversity of these components increased proportionally with increase in root diameter. The tissue-specific study indicated that the high content of paeoniflorin and albiflorin are mainly distributed in the cork and cortex. According to the results of this study, the roots of *P. lactiflora* greater than 1.7cm in diameter are of better quality medicinal use than smaller, and the commercial grades chose was best cutoff points. The fine roots and the outer bank of roots, which besides the commercial grades, contain such significant amounts of chemical components too. This study provides a new and practical method for evaluating the different grades of *P. lactiflora*.*

80. Tissue-specific metabolite profiling of Turmeric by using laser microdissection, ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry and liquid chromatography- tandem mass spectrometry

Y. Jaiswal, Z.T. Liang, A. Ho, H.B. Chen, Z.Z. Zhao

European Journal of Mass Spectrometry 2014, 20, 383-393

Abstract

Curcuma longa L. is recognized for its therapeutic and culinary uses both in Ayurveda and traditional Chinese medicine and is considered to be a boon to mankind. It has been extensively studied for its benefits and still continues to be an important drug with continued potential for further exploration and research. We studied the tissue-specific distribution of secondary metabolites to establish the validity of the use of rhizome samples from India and China, as substitutes for each other, based upon their metabolite profiles and curcumin contents. Laser microdissection was used for the isolation of microscopic tissues, such as cork, cortex and leaf-trace vascular bundles from rhizomes. Metabolite profiling was carried out by ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry and curcumin content was estimated by a method validated as per the Harmonized Tripartite Guidelines. The cortex and cork revealed the presence of a higher number of secondary metabolites than in the leaf-trace vascular bundles. The curcumin

contents in rhizome samples from both the countries, estimated with the help of a precise and accurate validated method, were found to be comparable. Based on the results, we conclude that turmeric rhizomes grown in India and China are qualitatively and quantitatively indistinguishable and therefore can be used as substitutes. The developed method can be widely applied for microscopic identification, authentication and analysis of the distribution of phytoconstituents in other botanical species of interest or of species with a significant commercial and therapeutic value.

81. Distribution of toxic alkaloids in tissues from three herbal medicine Aconitum species using laser micro-dissection, UHPLC-QTOF MS and LC-MS/MS techniques.

Y. Jaiswal, Z.T. Liang, A. Ho, L.L.Wong, P. Yong, H.B. Chen, Z.Z. Zhao

Phytochemistry, 2014, 107, 155-174

Abstract

Aconite poisoning continues to be a major type of poisoning caused by herbal drugs in many countries. Nevertheless, despite its toxic characteristics, aconite is used because of its valuable therapeutic benefits. The aim of the present study was to determine the distribution of toxic alkaloids in tissues of aconite roots through chemical profiling. Three species were studied, all being used in traditional Chinese Medicine (TCM) and traditional Indian medicine (Ayurveda), namely: Aconitum carmichaelii, Aconitum kusnezoffii and Aconitum heterophyllum. Laser micro-dissection was used for isolation of target microscopic tissues, such as the metaderm, cortex, xylem, pith, and phloem, with ultra-high performance liquid chromatography equipped with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) employed for detection of metabolites. Using a multi-targeted approach through auto and targeted LC-MS/MS, 48 known compounds were identified and the presence of aconitine, mesaconitine and hypaconitine that are the biomarkers of this plant was confirmed in the tissues. These results suggest that the three selected toxic alkaloids were exclusively found in A. carmichaelii and A. kusnezoffii. The most toxic components were found in large A. carmichaelii roots with more lateral root projections, and specifically in the metaderm, cork and vascular bundle tissues. The results from metabolite profiling were correlated with morphological features to predict the tissue specific distribution of toxic components and toxicity differences among the selected species. By careful exclusion of tissues having toxic diester diterpenoid alkaloids, the beneficial effects of aconite can still be retained and the frequency of toxicity occurrences can be greatly reduced. Knowledge of tissue-specific metabolite distribution can guide users and herbal drug manufacturers in prudent selection of relatively safer and therapeutically more effective parts of the root. The information provided from this study can contribute towards improved and effective management of therapeutically important, nonetheless, toxic drug such as Aconite.

82. Identification of Chinese herbal medicines by fluorescence microscopy: fluorescent characteristics of medicinal bark.

X.X. Huang, Z.T. Liang, H.B. Chen, Z.Z. Zhao, P. Li

Journal of Microscopy, 2014, 256, 6-22

Abstract

Medicinal bark refers to structures outside the vascular cambium of stems, branches and roots of gymnospermous and dicotyledonous plants that are used as medicinal materials; bark is an important type of Chinese herbal medicine. However, identification of the species from which the bark comes can be very difficult, especially when the bark is dried and sliced. In our previous studies, we have found that fluorescence microscopy is a powerful tool for the identification of easily confused Chinese herbal medicines, powdered Chinese herbal medicines and decoction dregs. To establish the fluorescent characteristics by which medicinal barks can be identified, for ensuring their safe and effective use, a systematic microscopic investigation by normal light and fluorescence microscope was carried out on transverse section samples of 11 medicinal barks commonly used in China. Specifically, the fluorescent characteristics of mechanical tissues, including stone cells and fibres as well as secretory tissues, have been observed. The microscopic features of medicinal bark are here systematically and comparatively described and illustrated. Under the fluorescence microscope, various tissues emitted fluorescence of different colours, and we found that both the colours and the intensity can be used to distinguish and identify these barks.

83. A comparative tissue-specific metabolite analysis and determination of protodioscin content in Asparagus species used in traditional Chinese medicine and Ayurveda by use of laser microdissection, UHPLC-QTOF/MS and LC-MS/MS.

Y. Jaiswal, Z.T. Liang, A. Ho, H.B. Chen, Z.Z. Zhao

Phytochemical Analysis, 2014, 25, 514-528

Abstract

INTRODUCTION:

Asparagus is esteemed in Traditional Chinese Medicine and Ayurveda, and it is commercially one of the most important drugs in the global herbal market. Comparative metabolite profiling of different species would help in determining the similarities and ascertain their validity for being used as substitutes for each other. Laser microdissection (LMD) facilitates identification of metabolites in specific tissues, and thus it can aid in exploration of metabolic pathways in target tissues.

OBJECTIVE:

To compare tissue-specific metabolites and protodioscin content of Asparagus cochinchinensis (Lour.) Merr. and Asparagus racemosus Willd. used in China and India.

METHODS:

Metabolite analysis of laser-dissected tissues was carried out using UHPLC-QTOF/MS and LC-MS/MS. The protodioscin contents were determined and the method was validated as per the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.

RESULTS:

Metabolite analysis reveals that the velamen tissue, among other tissues such as cortex, vascular bundles and pith, contained maximum components, specifically those belonging to the steroidal saponin class. Although the metabolite profiles were similar, the content of protodioscin was found to be higher in Chinese than Indian species.

CONCLUSION:

The study provided a suitable methodology for metabolite profiling and protodioscin content determination of *Asparagus* by use of LMD, UHPLC-QTOF/MS and LC-MS/MS. The similarities in metabolite profiles indicate that *Asparagus* species from India and China can serve as substitute for each other in various therapeutic and pharmaceutical applications.

84. Isolation and identification of antioxidant compounds in *Vaccinium bracteatum* Thunb. by UHPLC-Q-TOF LC/MS and their kidney damage protection.

J. Zhang, C.J. Chu, X.L. Li, S. Yao, B. Yan, H.L. Ren, N.Y. Xu, Z.T. Liang & Z.Z. Zhao.

Journal of Functional Foods, 2014, 11, 62-70

Abstract

Vaccinium bracteatum Thunb. is commonly used to stain and cook the rice, which then becomes black, named Wu Fan in Chinese. However, there are limited modern data on its pharmacological effects and active components relating to its traditional use. In the present study, the 50% EtOH eluates (C-b) of *V. bracteatum* exhibited a strong antioxidant effect assessed by two antioxidant assays (DPPH, ORAC) *in vitro* and a protective effect on KBrO₃-induced kidney damage. Seven compounds in the C-b were identified as isoorientin (1), orientin (2), vitexin (3), isovitexin (4), isoquercitrin (5), quercetin-3-O- α -L-rhamnoside (6), and chrysoeriol-7-O- β -D-glucopyranoside (7). These seven compounds accounted for 0.31 mg/mg of C-b by HPLC analysis, and C-b mainly contained flavonoid compounds by UHPLC-Q-TOF-MS analysis. Flavonoids, especially isoorientin and orientin, were found to be responsible for the antioxidant effect. The results demonstrate that flavonoids may contribute to the health benefits of *V. bracteatum* in Chinese traditional food.

85. Tissue-specific metabolites profiling and quantitative analyses of flavonoids in the rhizome of *Belamcanda chinensis* by combining laser-microdissection with UHPLC-Q/TOF-MS and UHPLC-QqQ-MS".

Y.J. Chen, Z.T. Liang, Y. Zhu, G.Y. Xie, M. Tian, Z.Z. Zhao & M.J. Qin.

Talanta, 2014, 130, 585-597

Abstract

The rhizome of *Belamcanda chinensis* (L.) DC. is a traditionally used medicinal material in China. Due to increasing demand, *B. chinensis* has been cultivated widely, and thus the study on its rational utilization of medicinal part and guidelines for the optimal cultivation and harvest is an important issue. Considering flavonoids were the main bioactive secondary metabolites of *B. chinensis*, fluorescence microscopy, laser microdissection (LMD), ultra-high performance liquid

chromatography-quadrupole/time-of-flight-mass spectrometry (UHPLC-Q/TOF-MS), and UHPLC coupled with triple quadrupole mass spectrometer (UHPLC-QqQ-MS) were applied to profile and determine flavonoids in various tissues in this study. Consequently, 43 peaks were detected by UHPLC-Q/TOF-MS, and 26 flavonoid compounds combined with seven triterpene compounds were identified or tentatively identified in the tissue extractions. The results indicated that the hydrophobic compounds, especially flavonoid or isoflavonoid aglycones and xanthone mainly accumulated in the cork, whereas the hydrophilic compounds, namely the flavonoid and isoflavonoid glycosides were usually found in the cortex or center (the part inside of endodermis). Samples of rhizomes from different growth ages and origins were simultaneously analyzed. It was shown that the bulb or lateral part of the rhizome generally possessed more total flavonoids than the vertical part or the primordium. The present study established a new practical method to evaluate the quality of the rhizome of *B. chinensis* and to explore the relationship between distribution patterns of secondary metabolites and growth years of plants, thus important information for cultivation and processing was provided

86. A novel and rapid HPGPC-based strategy for quality control of saccharide-dominant herbal materials: *Dendrobium officinale*, a case study

J. Xu, S.L. Li, R.Q. Yue, C.H. Ko, J.M. Hu, J. Liu, H.M. Ho, T. Yi, Z.Z. Zhao, J. Zhou, P. C. Leung, H.B. Chen, Q.B. Han.

Analytical and Bioanalytical Chemistry, 2014, 406, 6409-6417

Abstract

Qualitative and quantitative characterization of natural saccharides, especially polysaccharides, in herb materials remains a challenge due to their complicated structures and high macromolecular masses. Currently available methods involve time-consuming and complicated operations, and present poor specificity. Here, a novel and rapid highperformance gel permeation chromatography (HPGPC)-based approach is described for quality assessment of saccharidedominant herbal materials by simultaneous qualitative and quantitative analysis of saccharide components. *Dendrobium officinale*, one of the rarest tonic herbs worldwide, was employed as the model herb in this study. First, a HPGPC fingerprint based on the molecular weight distribution of its carbohydrate components was established for qualitative identification of *D. officinale*. Then, HPGPC-guided dominant holistic polysaccharide marker was separated using ultrafiltration followed by HPGPC determination for quantitative evaluation of *D. officinale*. The experimental results suggest that this method is more efficient, stable, and convenient compared with the currently available methods for authentication and quality evaluation of *D. officinale*, and we expect the method will have similar advantages when used for quality control of other saccharide-dominant herbal materials and products.

87. Comparative analysis of diosgenin in *Dioscorea* species and related medicinal plants by UPLC-DAD-MS

T. Yi, L.L. Fan, H.L. Chen, G.Y. Zhu, H.M. Suen, Y.N. Tang, L. Zhu, C. Chu, Z.Z. Zhao, H.B. Chen.

BMC Biochemistry, 2014, 15, 19

Abstract

Background: *Dioscorea* is a genus of flowering plants, and some *Dioscorea* species are known and used as a source for the steroidal sapogenin diosgenin. To screen potential resource from *Dioscorea* species and related medicinal plants for diosgenin extraction, a rapid method to compare the contents of diosgenin in various plants is crucial.

Results: An ultra-performance liquid chromatography (UPLC) coupled with diode array detection (DAD) and electrospray ionization mass spectrometry (ESI-MS) method was developed for identification and determination of diosgenin in various plants. A comprehensive validation of the developed method was conducted. Twenty-four batches of plant samples from four *Dioscorea* species, one *Smilax* species and two *Heterosmilax* species were analyzed by using the developed method. The present method presented good sensitivity, precision and accuracy. Diosgenin was found in three *Dioscorea* species and one *Heterosmilax* species, namely *D. zingiberensis*, *D. septemloba*, *D. collettii* and *H. yunnanensis*.

Conclusion: The method is suitable for the screening of diosgenin resources from plants. *D. zingiberensis* is an important resource for diosgenin harvesting.

88. A Systematic Review of the Botanical, Phytochemical and Pharmacological Profile of *Dracaena cochinchinensis*, a Plant Source of the Ethnomedicine “Dragon’s Blood”

J.Y. Fan, T. Yi, C.M. Sze-To, L. Zhu, W.L. Peng, Y.Z. Zhang, Z.Z. Zhao, H.B. Chen.

Molecules, 2014, 19, 10650-10669

Abstract

“Dragon’s blood” is the name given to a deep red resin obtained from a variety of plant sources. The resin extracted from stems of *Dracaena cochinchinensis* is one such source of “dragon’s blood”. It has a reputation for facilitating blood circulation and dispersing blood stasis. In traditional Chinese medicine, this resinous medicine is commonly prescribed to invigorate blood circulation for the treatment of traumatic injuries, blood stasis and pain. Modern pharmacological studies have found that this resinous medicine has anti-bacterial, anti-spasmodic, anti-inflammatory, analgesic, anti-diabetic, and anti-tumor activities, while it is also known to enhance immune function, promote skin repair, stop bleeding and enhance blood circulation. Various compounds have been isolated from the plant, including loureirin A, loureirin B, loureirin C, cochinchinenin, socotrin-4'-ol, 4',7'-dihydroxyflavan, 4-methylcholest-7-ene-3-ol, ethylparaben, resveratrol, and hydroxyphenol. The present review summarizes current knowledge concerning the botany, phytochemistry, pharmacological effects, toxicology studies and clinical applications of this resinous medicine as derived from *D. cochinchinensis*.

89. Comparative Authentication of Three “Snow Lotus” Herbs by Macroscopic and Microscopic Features

Q.L. Chen, T. Yi, Y.N. Tang, L.L. Wong, X.X. Huang, Z.Z. Zhao, H.B. Chen.

Microscopy Research and Technique, 2014, 77, 631–641

Abstract

“Snow lotus” is a famous Chinese Materia Medica derived from species of the genus *Saussurea* (Compositae). To differentiate three representative easily-confused snow lotus herbs, namely, *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip, *Saussurea laniceps* Hand.-Mazz., and *Saussurea medusa* Maxim., macroscopic features of the three herbs were systemically observed, and microscopic features were compared by using ordinary light microscopy, polarized light microscopy and scanning electron microscopy (SEM). The results indicate that, as for macroscopic identification, capitula situation and arrangement, and as for microscopic identification, pollen grains, nonglandular hairs, glandular hairs, and cells of inner surface of the microdiodange can be used to authenticate the three snow lotus herbs. Comprehensive table comparing the characteristics were presented in this study. SEM has been found to provide a number of unique characteristics of pollen grains. Based on the observation of pollen grains, evolution sequence of the three species was speculated. The present method was proven to be efficient, convenient, simple, and reliable, which was successfully applied to the authentication of three snow lotus herbs.

90. Evaluation of measurement uncertainty of sporoderm-broken rate of *Ganoderma* spores, method modification and optimization

K.C. Lo, A.K.M. Leung, W.F. Mok

Chinese Journal of Pharmaceutical Analysis, 2014, 19, 5650-5673

Abstract

Objective: To evaluate the measurement uncertainty of sporoderm-broken rate of *Ganoderma* spores according to the China Agriculture Professional Standard NY/T1677-2008 “Determination of sporoderm-broken rate of *Ganoderma* spore product”. By investigating the major sources of uncertainty, optimize the method this study provides the basis for the effective control of sporoderm-broken rate determination by standard NY/T1677-2008.

Methods: Measurement uncertainty in sporoderm-broken rate measurement was evaluated throughout the production process. Based on the results, the method for determination of sporoderm-broken rate was further modified and optimized.

Results: The relative expanded uncertainty for sporoderm-broken rate was found to be 29.8% ($k = 2$). The relative expanded uncertainty by modified and optimized method was lowered to 13.0% ($k = 2$).

Conclusion: After method optimization, the uncertainty level was decreased, and the accuracy of analysis results was increased.

91. Chemical profile analysis and comparison of two versions of the classic TCM formula Danggui Buxue Tang by HPLC-DAD-ESI-IT-TOF-MSⁿ

Y.Z. Zhang, F. Xu, T. Yi, J.Y. Zhang, J. Xu, Y.N. Tang, X.C. He, J. Liu, H.B. Chen

Molecules, 2014, 19, 5650-5673

Abstract

Danggui Buxue Tang (DBT) is a Traditional Chinese Medicine (TCM) formula primarily used to treat symptoms associated with menopause in women. Usually, DBT is composed of one portion of Radix Angelicae Sinensis (RAS) and five portions of Radix Astragali (RA). Clinically, Radix Hedysari (RH) is sometimes used by TCM physicians to replace RA in DBT. In order to verify whether the chemical constituents of the DBT1 (RA:RAS = 5:1, w/w) and DBT2 (RH:RAS = 5:1, w/w) share similarities the chemical profiles of the two DBTs crude extracts and urine samples were analyzed and compared with the aid of HPLC-DAD-ESI-IT-TOF-MSⁿ, which determines the total ion chromatogram (TIC) and multi-stage mass spectra (MSⁿ). Then, the DBT1 and DBT2 were identified and compared on the basis of the TIC and the MSⁿ. In the first experiment (with crude extracts), 69 compounds (C1-C69) were identified from the DBT1; 46 compounds (c1-c46) were identified from the DBT2. In the second experiment (with urine samples), 44 compounds (M1-M44) were identified from the urine samples of rats that had been administered DBT1, and 34 compounds (m1-m34) were identified from the urine samples of rats that had been administered DBT2. Identification and comparison of the chemical compositions were carried out between the DBT1 and DBT2 of the crude extracts and urine samples respectively. Our results showed that the two crude extracts of the DBTs have quite different chemical profiles. The reasons for their differences were that the special astragalosides in DBT1 and the isoflavonoid glycosides formed the malonic acid esters undergo single esterification and acetyl esters undergo acetylation in DBT1. In contrast, the urine from DBT1-treated rats strongly resembled that of DBT2-treated rats. These metabolites originate mainly from formononetin, calycosin and their related glycosides, and they were formed mainly by the metabolic process of reduction, deglycosylation, demethylation, hydrogenation and sulfation. The HPLC-DAD-ESI-IT-TOF-MSⁿ method was successfully applied for the rapid chemical profiles evaluation of two DBTs and their related urine samples.

92. An integrated strategy based on UPLC–DAD–QTOF-MS for metabolism and pharmacokinetic studies of herbal medicines: Tibetan ‘Snow Lotus’ herb (*Saussurea laniceps*), a case study

Y. Tao, L. Zhu, Y.N. Tang, J.Y. Zhang, Z.T. Liang, J. Xu, Z.Z. Zhao, Z.L. Yu, Z.X. Bian, Z.J. Yang, H.B. Chen

Journal of Ethnopharmacology, 2014, 153, 701-713

Abstract

ETHNOPHARMACOLOGICAL RELEVANCE: *Saussurea laniceps* Hand.-Mazz. (SL) has long been used under the herbal name Tibetan "Snow Lotus" for the treatment of rheumatoid arthritis, stomachache and dysmenorrhea in Tibetan folk medicine. Since herbal medicine (HM) is a synergistical system with multiple components, both of the metabolism and pharmacokinetic studies

of HM are interdependent. This study aimed to develop an integrated strategy based on the UPLC-DAD-QTOF-MS technique for metabolism and pharmacokinetic studies of HM.

MATERIAL AND METHODS: SL was used here as a test herb to verify the feasibility of the proposed strategy. SL was administered to rats, then, the blood plasma, urine and feces were analyzed to determine the metabolic profiles. Using our strategy, umbelliferone and scopoletin were evaluated to be the key bioactive components. Their pharmacokinetic parameters were measured and biotransformation pathways were elucidated.

RESULTS: After oral administration of SL to rats, 17 components in blood, 10 components in urine and 2 components in feces were identified and characterized using our UPLC-DAD-QTOF-MS method. Umbelliferone, scopoletin and their metabolites were found to be the major components involved in the metabolism process. Literature reports also suggest that umbelliferone and scopoletin are responsible for the therapeutic effects of SL, thus these two components were selected as the active markers for pharmacokinetic study. In the test of validity, the established method presented good linearity with $R(2) > 0.99$. The relative standard deviation value was below 13.9% for precision, and recovery studies for accuracy were found to be within the range 91.8-112.5%.

CONCLUSION: The present strategy offers, simultaneously, precision in quantitative analysis (metabolism study) and accuracy in quantitative analysis (pharmacokinetic study) with greater efficiency and less costs, which is therefore reliably used for integrated metabolism and pharmacokinetic studies of HM.

93. Microscopic research on a multi-source traditional Chinese medicine: Astragali Radix

K.Z. Yu, J. Liu, B.L. Guo, Z.Z. Zhao, H. Hong, H.B. Chen, S.Q. Cai

Journal of Natural Medicines, 2014, 68, 340-350

Abstract

Astragali Radix is a widely and commonly used Chinese herbal medicine, which is derived from roots of Astragalus membranaceus var. mongholicus and Astragalus membranaceus. To find a quick and reliable method of distinguishing these two species of Astragali Radix and of determining the age of a sample, microscopic characteristics of the two species were compared using light microscopy. The results showed that the microscopic characteristics, such as number of layers of phellem, continuing lignified xylem bundles within spring wood and lignified parenchyma cells in the central part of the xylem could be used for the differentiation of the root of A. membranaceus from the root of A. membranaceus var. mongholicus. Growth rings (annual rings) were found for the first time in the roots of both species, and could determine the age of a sample. For the first time, radial fibers in both species of Astragali Radix and pipette-shaped fibers in A. membranaceus var. mongholicus were found. The structure of "rotten heart" cork tissue (decayed central xylem) and tubular cork tissue was carefully studied, and the arranged order of tissues in both "rotten heart" and tubular cork tissues is phelloderm and phellem from outside to inside, which is contrary to that in the periderm.

94. A mixed microscopic method for differentiating seven species of 'Bixie'-related Chinese Materia Medica

Y.N. Tang, X.C. He, Q.L. Chen, L.L. Fan, J.Y. Zhang, Z.Z. Zhao, L.S. Dong, Z.T. Liang, T. Yi, H.B.

Chen

Microscopy Research and Technique, 2014, 77, 57-70

Abstract

Confusion in the species associated with the name of "Bixie" in Chinese Materia Medica began centuries ago. In recent decades, diverse medicinal plants from the genera Dioscorea and Smilax, and even minor species from the genus Heterosmilax, have been documented under the name Bixie or a very similar name as folk medicines in different areas of China. However, the traditional efficacies and chemical profiles of these herbs are not exactly the same and even vary wildly. Comprehensive authentication of multiple Bixie herbs has not yet been attempted. To differentiate and ensure the correct use of these Bixie-related herbs, in this study, seven sorts of representative Bixie herbs (Dioscorea colleti, D. zingiberensis, D. nipponica, D. septemloba, Smilax china, S. glabra, and Heterosmilax japonica) were characterized based on the microscopic examination of their powders and cryotomed transverse sections. This is not only the first attempt to distinguish Bixie herbs by a comprehensive microscopic techniques, including common light microscopy, fluorescence microscopy, and polarized light microscopy, but also it is the first research to observe characteristics of transections of crude drugs under polarized lighting for the purpose of authentication. Polarized light has been found to provide a number of unique characteristics. The results indicate that starch granules, stone cells, vascular bundles, and other significant tissue features can be used to authenticate "Bixie" herbs. The method was proven to be quick, handy, specific, and simple. It should be widely applicable to other herbal materials.

95. Combined therapeutic effects of vinblastine and Astragalus saponins (AST) in human colon cancer cells and tumor xenograft via inhibition of tumor growth and proangiogenic factors

K.K.W. Auyeung, P.C. Law, J. Ko

Nutrition and Cancer, 2014, 66, 662-674

Abstract

Our previous study had demonstrated that Astragalus saponins (AST) could reduce the side effects of orthodox chemotherapeutic drugs, while concurrently promote antitumor activity. In the present study, we attempted to investigate the potential synergistic anticarcinogenic effects of AST and a vinca alkaloid vinblastine (VBL). Reduced expression of key proangiogenic and metastatic factors including VEGF, bFGF, metalloproteinase (MMP)-2, and MMP-9 was detected in VBL-treated colon cancer cells, with further downregulation by combined VBL/AST treatment. Subsequently, VBL or AST decreased LoVo cell invasiveness, with further reduction when the drugs were cotreated. Significant growth inhibition and cell cycle arrest at G2/M phase were achieved by either drug treatment with apparent synergistic effects. VBL-induced apoptosis was confirmed but found to be unrelated to induction of the novel apoptotic protein NSAID-activated gene 1. In vivo study in tumor xenograft indicates that combined VBL/AST treatment resulted in sustained regression of tumor

growth, with attenuation of the neutropenic and anemic effects of VBL. In addition, downregulation of proangiogenic and proliferative factors was also visualized, with boosting effect by combined drug treatment. These findings have provided evidence that AST combined with adjuvant chemotherapeutics like VBL could alleviate cancer development through diversified modes of action, including the regulation of angiogenesis.

96. Cell type-specific qualitative and quantitative analysis of saikosaponins in three Bupleurum species using laser microdissection and liquid chromatography-quadrupole/time of flight-mass spectrometry

Z.T. Liang, K.Y. Oh, Y.Q. Wang, T. Yi, H.B. Chen, Z.Z. Zhao

Journal of Pharmaceutical and Biomedical Analysis, 2014, 97, 157-165

Abstract

Cell type-specific metabolite analysis is a promising method for understanding plant metabolite production, function, transport and storage. In the present study, laser microdissection (LMD) and ultra-high performance liquid chromatography quadrupole/time of flight-mass spectrometry are combined to determine where secondary metabolites are accumulated in the roots of *Bupleurum scorzonrifolium* Willd, *Bupleurum chinense* DC. and *Bupleurum falcatum* L. Four tissues, namely cork, cortex, phloem and xylem, were microdissected by laser microdissection, and their chemical profiles were analyzed. The main metabolites are saikosaponins. Different tissues contained different saikosaponins. Generally, the cork and cortex from all three species contained more types of saikosaponins and higher contents of saikosaponins a, c and d than did the phloem and xylem. Interestingly, in the roots of *Bupleurum scorzonrifolium* and *B. falcatum*, the cork contained much higher contents of saikosaponins a, c and d than did the cortex; while in the root of *B. chinense*, the cortex contained higher contents of saikosaponins a, c and d than the cork. Explanation and application of the results are discussed. The present findings yield valuable insights into the quality evaluation of *Bupleuri Radix* by morphological features.

97. UHPLC UHD Q-TOF MS/MS analysis of the impact of sulfur fumigation on the chemical profile of Codonopsis Radix (Dangshen)

X.Q. Ma, A.K.M. Leung, C.L. Chan, T. Su, W.D. Li, S.M. Li, D.W.F. Fong, Z.L. Yu

Analyst, 2014, 139, 505-516

Abstract

Over recent decades sulfur fumigation has been becoming abused in processing some freshly harvested Chinese medicinal herbs, although it is questioned whether sulfur fumigation can result in changes in efficacy and safety of the herbs. One of the herbs commonly processed by sulfur fumigation is *Codonopsis Radix* (Dangshen). A report showed that lobetyolin content in sulfur-fumigated Dangshen was lower than in air-dried Dangshen. Whereas there is no investigation designed to compare the chemical profiles of the sulfur-fumigated Dangshen and the air-dried Dangshen. In the present study, a rapid and versatile ultra-high-performance liquid chromatography coupled with ultra-high resolution quadrupole time-of-flight mass spectrometry (UHPLC UHD Q-TOF MS/MS) method was developed for comprehensive analysis of the chemical profiles of sulfur-

fumigated and air-dried Dangshen samples. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) demonstrated that there were significant chemical differences between sulfur-fumigated and air-dried Dangshen samples. Among the changed components, 57 compounds were identified, in which 15 sulfur-containing compounds were detected only in sulfur-fumigated samples. The established methods were successfully applied to discriminate sulfur-fumigated Dangshen among commercial samples. Whether the chemical changes caused by sulfur fumigation affect the clinical efficacy and safety of Dangshen needs to be further investigated.

98. Characterization of Flavonoids in the Ethnomedicine *Fordia Cauliflorae Radix* and Its Adulterant *Millettiae Pulchrae Radix* by HPLC-DAD-ESI-IT-TOF-MSⁿ

L.L. Fan, T. Yi, F. Xu, Y.Z. Zhang, J.Y. Zhang, D.P. Li, Y.J. Xie, S.D. Qin, H.B. Chen

Molecules, 2013, 18, 15134-15152

Abstract

Fordia Cauliflorae Radix (FC, the root of *Fordia cauliflora* Hemsl) and *Millettiae Pulchrae Radix* [MP, the root of *Millettia pulchra* (Benth.) Kurz var. *laxior* (Dunn) Z. Wei], which go under the same local name of “Daluosan”, have long been used in Southern China for the treatment of stroke, paralysis, dementia in children, Alzheimer’s disease and other diseases. The same local name and similar functions always confuse users. To further utilize these two ethnodrugs and identify them unambiguously, an HPLC-DAD-ESI-IT-TOF-MSⁿ method was developed to separate and characterize the flavonoids in FC and MP. A total of 41 flavonoids were detected, of which six compounds were identified by comparing their retention time and MS data with those of the reference standards, and the others were tentatively identified based on their tandem mass spectrometry data obtained in the positive ion detection mode. Nineteen of these characterized compounds are reported from these two plants for the first time.

99. A bioactivity-guided study on the anti-diarrheal activity of *Polygonum chinense* Linn.

H.T. Xiao, S.W. Tsang, H.Y. Qin, F.F. Choi, Z.J. Yang, Q.B. Han, H.B. Chen, H.X. Xu, H. Shen, A.P.

Lu, Z.X. Bian

Journal of Ethnopharmacology, 2013, 149, 499-505

Abstract

Ethnopharmacological Relevance: *Polygonum chinense* Linn., a folk medicine, has long been used for the treatment of diarrhea and enteritis in southwestern China. However, the components responsible for its anti-diarrheal activity are still poorly understood.

AIM OF THE STUDY: To determine anti-diarrheal activities of *Polygonum chinense* L. and to identify its active components through bioactivity-guided isolation technique.

Materials and Methods: Animals were orally administered with the extract of *Polygonum chinense* L. The anti-diarrheal effects of 75% ethanol extract, four fractions with different polarities from 75% ethanol extract, different eluates collected from Diaion HP-20 macroporous resin chromatography,

ellagic acid and corilagin, were examined based on mouse models of castor oil- and magnesium sulfate-induced diarrhea.

Results: The results showed that the 75% ethanol extract of *Polygonum chinense* L. exhibited significant anti-diarrheal activities in a dose-dependent manner in two mouse models. Through in vivo bioactivity-guided fractionation processes, *n*-butanol and aqueous fractions were found to exhibit prominent anti-diarrheal activities, and two major compounds, ellagic acid and corilagin, from these active fractions were found to possess anti-diarrheal effects.

Conclusion: Present study provides evidence of the utilization of *Polygonum chinense* L. for diarrhea, and ellagic acid and corilagin are two components contributing to the anti-diarrheal effect.

100. Determination of five flavonoids in different parts of *Fordia cauliflora* by ultra performance liquid chromatography / triple-quadrupole mass spectrometry and chemical comparison with the root of *Millettia pulchra* var. *laxior*

L.L. Fan, Y.Z. Zhang, R.B. Huang, S.D. Qin, T. Yi, F. Xu, Y.N. Tang, X.S. Qu, H.B. Chen, J.H. Miao

Chemistry Central Journal, 2013, 7, 126

Abstract

Background: The root of *Fordia cauliflora* Hemsl (FC) has long been used in southern China for the treatment of rheumatism, bruises, dementia in children, and valetudinarianism. However, sometimes it is mixed with other parts. And it has always been confused with the root of *Millettia pulchra* (Benth.) Kurz var. *laxior* (Dunn) Z. Wei (MP) by the local people. The chemical differences between the two ethnic drugs are not clear until now. The aim of this study is to develop a precise and accurate method to characterize and quantify multiple chemical components of FC, which is helpful for the quality evaluation and identification of FC.

Results: A method coupling ultra performance liquid chromatography (UPLC) with triple-quadrupole mass spectrometry (QQ-MS) was first developed for simultaneous determination of five flavonoids in different parts of FC and the root of MP, based on a UPLC-diode array detection (DAD) fingerprint method. All calibration curves showed good linearity ($R^2 > 0.99$) within test ranges. The overall LOD and LOQ were lower than 2.5 ng/mL and 5.0 ng/mL, respectively. The RSDs for intra- and inter-day of five analytes were less than 2.83% and 3.04%, respectively. Recovery studies for the quantified compounds were found to be within the range 93.6-99.8% with RSD less than 5.73%. The results suggest that the root, traditionally used medicinal part, yields the highest flavanoid content in FC. Pachycarin A, 3',4'-dimethoxy(2'',3'':7,8) furanoflavone, karanjachromene and isoderricin A can be used to differentiate between FC and MP samples.

Conclusions: The present method is specific, precise and reliable, and is suitable for characterizing and quantifying multiple chemical components of FC.

101. Why are Angelicae Sinensis radix and Chuanxiong Rhizoma different? An explanation from a chemical perspective

J. Xu, H.B. Chen, J. Liu, K.Y. Kwok, R.Q. Yue, T. Yi, H.M. Ho, Z.Z. Zhao, Q.B. Han

Food Research International, 2013, 54, 439-447

Abstract

Angelicae Sinensis radix (ASR) and Chuanxiong Rhizoma (CR) are two commonly used herbs with different blood-related bioactivities for tonic and medicinal purpose, respectively. However, current available scientific evidences indicate that they share similar chemical profiles in which volatile components are the majority. In order to figure out the decisive chemical differences and then establish their individual quality control methods, comprehensive comparison on chemical ingredients between ASR and CR was carried out in this study. First, volatile components between ASR and CR were determined and compared by GC–MS (gas chromatography–mass spectrometry). Then, carbohydrates and polysaccharides in ASR and CR were qualitatively and quantitatively characterized using HPGPC (high performance gel permeation chromatography) and HPLC (high performance liquid chromatography). Furthermore, these components in different botanical parts and processed products of ASR were also investigated. The results not only confirmed that ASR and CR contain similar profiles of volatile components, but also suggested that the volatile components might not be the nutrient ingredients of ASR and that the polysaccharides make the real differences between ASR and CR.

102. A comparative study on the traditional Indian Shodhana and Chinese processing methods for aconite roots by characterization and determination of the major components

Y. Jaiswal, Z.T. Liang, P. Yong, H.B. Chen, Z.Z. Zhao

Chemistry Central Journal, 2013, 7, 169

Abstract

Background: *Aconitum is an indispensable entity of the traditional medicine therapy in Ayurveda and Traditional Chinese medicine (TCM), in spite of its known fatal toxicity characteristics. The prolonged use of this drug, irrespective of its known lethal effects, is governed by the practice of effective detoxification processes that have been used for decades. However, the processing methods of Ayurveda and TCM are different, and no comparative study has been carried out to evaluate their differences. The objective of the present study was to carry out comparative chemical profiling of the roots of Aconitum heterophyllum Wall, A. carmichaelii Debx., and A. kusnezoffii Reichb. after application of two detoxification methods used in Ayurveda and one method used in TCM.*

Results: *Analysis of the processed samples was carried out by ultra-high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF/MS). The results obtained in the study demonstrate that all three processing methods used in Ayurveda and TCM effectively extract the diester diterpenoid alkaloids and led to their conversion into monoester diterpenoid alkaloids. The efficiency of the processes in reduction of toxic alkaloid contents can be stated as: Processing with water > Shodhana with cow milk > Shodhana with cow urine. The analysis method was validated as per ICH-Q2R1 guidelines and all the parameters were*

found to comply with the recommendations stated in the guidelines.

Conclusions: There have been no reports till date, to compare the processing methods used in Ayurveda with the methods used in TCM for detoxification of aconite roots. Our study demonstrates that, these methods used in both the traditional systems of medicine, efficiently detoxify the aconite roots. Amongst the three selected procedures, the TCM method of decoction with water is the most efficient. Through experimental evidences, we prove the conversion of toxic diester diterpenoid alkaloids to relatively safer monoester diterpenoid alkaloids. Thus, this study demonstrates that comparative study on the traditional experiences accumulated in different medical systems is useful for expanding their respective applications.

103. Quantitative comparison of multiple components in *Dioscorea nipponica* and *D. panthaica* by ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

Y.N. Tang, T. Yi, H.M. Chen, Z.Z. Zhao, Z.T. Liang, H.B. Chen

Phytochemical Analyst, 2013, 24, 413-422

Abstract

Introduction: *Dioscorea nipponica* (DN) and *D. panthaica* (DP) have been uniquely prepared as herbal medicinal products for treating coronary heart disease (CHD) in China. However, so far there has been little discussion and no work comparing the qualitative and quantitative differences between the two herbs nor assessing whether they have similarities in chemical composition that would support their common application for treating CHD.

Objective: To develop an efficient and reliable method based on UPLC–qTOF–MS for quantitative comparison of saponins in both DN and DP.

Methods: Using electrospray ionisation and atmospheric-pressure chemical ionisation respectively, six steroidal glycosides and one aglycone were determined in 13 DN samples and 13 DP samples. The comparative analysis of chemical components in DN and DP was carried out by chromatographic fingerprint similarity evaluation, test of significance (t-test) and principle component analysis (PCA).

Results: The UPLC–qTOF–MS method showed limit of detection and quantitation within the range 0.02–0.2 ng and 0.08–0.5 ng, respectively, for the seven saponins studied. The intra- and interday precision (RSD) were below 5%. The recoveries for the quantified compounds were within the range of 72.79–118.31%.

Conclusion: This UPLC–qTOF–MS assay provides a suitable method for the identification and determination of major bioactive constituents both in DN and DP. The chemical composition of all DN and DP samples studied exhibited a high level of global similarity. This chemical similarity validates their common application in the pharmaceutical industry as anti-CHD herbal drugs.

104. Chemical quantification and antioxidant assay of four active components in *Ficus hirta* root using UPLC-PAD-MS fingerprinting combined with cluster analysis

T. Yi, Q.L. Chen, X.C. He, S.W. So, Y.L. Lo, L.L. Fan, J. Xu, Y.N. Tang, J.Y. Zhang, Z.Z. Zhao, H.B.

Chen

Chemistry Central Journal, 2013, 7, 115

Abstract

Background: Root of *Ficus hirta* (RFH) is widely consumed in China as a plant-derived popular food. However, contents of the active constituents of RFH are unknown, and the chemical as well as bioactive properties of RFH may be affected by growing area. In order to ensure the standard efficacy of health products made with RFH, its active constituents should firstly be determined and, secondly, a means of assessing samples for their contents of these constituents is needed.

Results: Four active components, including two coumarins, namely psoralen and bergapten, and two flavonoids, namely luteolin and apigenin, in twenty RFH samples were quantified using a new ultra performance liquid chromatography coupled with photodiode array detector and mass spectrometry (UPLC-PAD-MS) method, and the content level in descending order was psoralen > bergapten > luteolin > apigenin. Chromatographic fingerprint similarity evaluation and cluster analysis were used to assess geographical origin of RFH, and the results revealed a high level of similarity for the tested RFH samples obtained from Hainan, Guangdong, Guangxi provinces and Hong Kong. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted to evaluate the antioxidant potencies of the four components, and the results clearly demonstrated that luteolin was most effective; apigenin exhibited a moderate potency, whereas psoralen and bergapten possessed little effect against free radical reactions. Structure-activity relationship of the components was elucidated, and the 3'-hydroxyl group of luteolin was found to be directly responsible for its antioxidant activity.

Conclusion: The present UPLC-PAD-MS method and DPPH radical scavenging assay performed well for the purpose of constituent quantification and antioxidant assay. Global profiles were highly similar for RFH samples from different origins. Both the coumarins and flavonoids were involved in the health benefit of RFH.

105. Development of high-performance liquid chromatographic fingerprint for quality analysis of *Hedysari Radix*

Y. Liu, Y.Y. Zhao, H.B. Chen, H. Liang, Q.Y. Zhang

Natural Product Research, 2013, 27, 1398-1403

Abstract

In this study, a simple, sensitive and reliable fingerprint analysis method by high-performance liquid chromatography coupled with diode array detection was developed for raw materials of *Hedysari Radix*, which is a famous traditional Chinese medicine and widely used as a restorative food. Then the developed method was subsequently applied to analyse 48 samples collected or purchased from different origins. Twenty-six common fingerprint peaks, including six definitely identified peaks, were found in total, and were involved for further characterisation and quality evaluation of *Hedysari*

Radix. The chemical quality of commercial samples and samples collected in spring varied obviously, while those of the cultivated samples collected in autumn were relatively stable, indicating that it is better to harvest *Hedysari Radix* in autumn and that more attention should be paid to the standardisation of the process of harvesting, manufacturing and storage of *Hedysari Radix*.

106. Characterization of secondary metabolites from the raphides of calcium oxalate contained in three Araceae family plants using laser microdissection and ultra-high performance liquid chromatography- quadrupole/time of flight-mass spectrometry

Z.T. Liang, J. Zhang, L.L. Wong, T. Yi, H.B. Chen, Z.Z. Zhao

European Journal of Mass Spectrometry, 2013, 19, 195-210

Abstract

The dried stem tubers of *Pinellia ternata* (Thunb.) Breit, *P. pedatisecta* Schott and *Typhonium flagelliforme* (Lodd.) Blume from the Araceae family are used as Chinese medicines, namely Banxia, Zhangye Banxia and Shui Banxia, respectively. They have been reported to have a strong irritative effect on mucosa. Previous studies have indicated that the pure raphides of calcium oxalate contained in the stem tubers of three plants have attributed to this strong irritation. However, the processed products of the stem tubers of *P. ternata*, including Fabanxia, Qingbanxia and Jiangbanxia, have been found to have no irritative effects on mucosa. Currently, the secondary metabolites from the raphides of calcium oxalate contained in the stem tubers of *P. ternata* with its processed products, *P. pedatisecta* and *T. flagelliforme* were analyzed by means of laser microdissection and ultra-high performance liquid chromatography-quadrupole/time of flight-mass spectrometry. The results indicate that the stem tubers of *P. ternata*, *P. pedatisecta* and *T. flagelliforme* mainly contain amino acids, fatty acids, cyclic dipeptides and alkaloids. The secondary metabolite profile of the stem tuber of *P. ternata* was different from those of its processed products, Fabanxia, Qingbanxia and Jiangbanxia. However, the secondary metabolites from their raphides of calcium oxalate all contained beta-sitosterol, sitosterol palmitate, trigonelline, octadecenoic acid, pedatisectine A and thymidine. The raphides of calcium oxalate from *P. ternata*, *P. pedatisecta* and *T. flagelliforme* do not include any specific irritants. The results indicated that the irritation induced by raphides of calcium oxalate of three Araceae family plants is not relevant to the secondary metabolites but relates to the special needle shape.

107. Identification of Daqingye and Banlangen including crude drugs and decoction dregs from three plant species by normal light and fluorescence microscopy

X.J. Wan, Z.T. Liang, H.B. Chen, Z.Z. Zhao, P. Li

Microscopy Research Technique, 2013, 76, 774-782

Abstract

Daqingye and Banlangen are commonly used Chinese medicinal materials derived from the leaves and roots of *Isatis indigotica* Fort., respectively, which clinical effects have been confirmed by many studies in recent years. However, many problems have arisen concerning the quality and identity

of materials sold in the market under these two names. Thus, the identification of Daqingye and Banlangen has drawn public attention. In this work, transverse sections of Daqingye and Banlangen from *I. indigotica* Fort. and two easily confused species, namely *Baphicacanthus cusia* (Nees) Bremek. and *Clerodendrum cyrtophyllum* Turcz., were investigated with normal light and fluorescence microscopy. The distinguishing features were 7–9 vascular bundles, cystoliths and nonglandular hairs in the leaves of *I. indigotica*, *B. cusia*, and *C. cyrtophyllum*, respectively. The Banlangen could be distinguished according to the characteristics of parenchymous cells, cystoliths, and stone cells. Moreover, the fluorescence features of Daqingye and Banlangen investigated in this study can provide direct points for differentiating those samples. Importantly, whether the crude drugs were decocted could be easily identified by their different fluorescence features, which can ensure their quality in clinical application. This is the first report to distinguish the three species that are commonly found in the market sold as Daqingye and Banlangen by normal light and fluorescence microscopy. This work indicates that the combination of normal light and fluorescence microscopy could be powerful, convenient, and economical for authenticating Daqingye and Banlangen from the three species, including crude drugs and decoction dregs.

108. Chemical profiling and histochemical analysis of *Bupleurum marginatum* roots from different growing areas of Hubei province

Z.T. Liang, J. Zhang, G.Y. Yang, H.B. Chen, Z.Z. Zhao

Acta Pharmaceutica Sinica B, 2013, 3, 193-204

Abstract

Bupleuri Radix has been widely used in traditional Chinese medicine. In the current herbal market, the species *Bupleurum marginatum* Wall. ex DC. is the main source of *Bupleuri Radix*. Although *Bupleuri Radix* from the roots of *B. marginatum* grown wild in the North West of Hubei province has higher quality compared with those from other regions according to the previous investigations, the exhaustive exploitation driven by increasing demand has drastically reduced the wild resource. As a result, germplasm evaluation and quality resource exploration are important for the sustainable utilization and cultivation of *B. marginatum*. A preliminary study indicated differences in the tissue structure of *B. marginatum* grown in different areas of North Western Hubei province. In the current study, various tissues of the roots of *B. marginatum* grown in different areas of North Western Hubei were subjected to laser microdissection and analyzed by microscopy and ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC–Q-TOF-MS). The results show that wild plants from Maqiao Town, Baokang County contain the most saikosaponins distributed mainly in cork, cortex and phloem. This study provides key chemical information for evaluating the quality of *B. marginatum* roots.

109. Profiling of secondary metabolites in tissues from *Rheum palmatum* L. using laser microdissection and liquid chromatography mass spectrometry

Z.T. Liang, T.T. Sham, G.Y. Yang, L. Yi, H.B. Chen, Z.Z. Zhao

Analytical and Bioanalytical Chemistry, 2013, 405, 4199-4212

Abstract

Evaluating the quality of herbal medicines by morphological features is a convenient, quick, and practical method compared with other methods that mostly depend on modern instruments. Here, laser microdissection and ultra-performance liquid chromatography are combined with mass spectrometry to map the distribution of secondary metabolites in cells or tissues of a herb itself for correlating its bioactive components and morphological features. The root and rhizome of *Rheum palmatum* L. were taken as research target, which is the Chinese medicine, *Radix et Rhizoma Rhei*. According to fluorescent microscopic characteristics, 12 herbal cells or tissues of *Radix et Rhizoma Rhei* were separated by laser microdissection. Thirty-eight compounds were identified or tentatively characterized in the microdissected tissues. (+)-Catechin, 1-O-galloyl-2-O-cinnamoyl- β -D-glucose, and emodin were found to be the major components in most of the tissues. The brown ergastic substances found in rays of normal and anomalous vascular bundles as well as the parenchymatous cells of rhizome pith and the parenchymatous cells of root xylem contained higher than average amounts of these three components and more kinds of secondary metabolites. Overall, results suggest that *Radix et Rhizoma Rhei* of larger size and with conspicuous “brocaded patterns” and star spots are of higher quality as they tend to have greater contents of bioactive components. The study provides quantitative and specific criteria by which the quality of *Radix et Rhizoma Rhei* can be judged. This research also established a new, reliable, and practical method for direct profiling and imaging of secondary metabolites in any herbal tissue.

110. Chemical differentiation of two taste variants of *Gynostemma pentaphyllum* by using UPLC-Q-TOF-MS and HPLC-ELSD

J.G. Lu, L. Zhu, K.Y.W. Lo, A.K.M. Leung, A.H.M. Ho, H.Y. Zhang, Z.Z. Zhao, D.W.F. Fong, Z.H.

Jiang

Journal of Agricultural and Food Chemistry, 2013, 61, 90-97

Abstract

To differentiate the sweet and bitter taste variants of a Chinese medicinal tea *Gynostemma pentaphyllum* (GP), a method for the quantitative analysis of ginsenosides Rb1, Rb3, Rd, and F2 in GP by using UPLC-Q-TOF-MS was developed. According to the different contents of the four ginsenosides, chemical differentiation of the two taste variants of GP was achieved by principal component analysis (PCA). A supplementary quantitative analysis method of using HPLC-ELSD for determination of 20(S)-panaxadiol in the hydrolysates of GP was also developed. Similarly, chemical differentiation based on different amounts of 20(S)-panaxadiol was established and the result was well consistent with that based on the analysis of the four ginsenosides. It was found that the amounts of the four ginsenosides and 20(S)-panaxadiol in the sweet taste variant were significantly higher than those in the bitter one. The significant difference between the sweet and bitter taste variants of GP was easily visualized in 3D-PCA score plots. The PCA loading plot also indicated the contributions among the four ginsenosides ($Rd > Rb3 > F2 > Rb1$) for distinguishing the two taste variants. This is the first report to describe the use of these two quantitative methods (UPLC-Q-TOF-MS and HPLC-ELSD) for the accurate authentication and quality control of GP.

111. Determination of the content of rosmarinic acid by HPLC and analytical comparison of volatile constituents by GC-MS in different parts of *Perilla frutescens* (L.) Britt

J. Liu, Y.L. Wan, Z.Z. Zhao, H.B. Chen

Chemistry Central Journal, 2013, 7, 61

Abstract

Background *Perilla frutescens* (L.) Britt. is not only an edible plant but also a traditional medicinal plant commonly used for treating common cold. It is widely cultivated in southern China. The anatomical parts of *P. frutescens* that are recorded as medicines in the Chinese material medica are: *Perillae Caulis*, *Perillae Folium* and *Perillae Fructus*, which are the dried stems, the dried leaves and the dried ripe fruits, respectively. Rosmarinic acid is one of major polyphenolic ingredients found in all three *Perillae Caulis*, *Perillae Folium* and *Perillae Fructus*. The characteristic volatile oil of *P. frutescens* is believed to be another essential composition of the herb, giving its wide range of use.

Results A simple, rapid and accurate HPLC-DAD method was set up, suitable for the assay of rosmarinic acid in *Perillae Fructus*, *Perillae Folium* and *Perillae Caulis*. 12 batches of *Perillae Caulis*, 12 batches of *Perillae Folium* and 13 batches of *Perillae Fructus* from 8 different regions of mainland China and Hong Kong herbal markets were collected for evaluating the quality of *P. frutescens*. Results showed that *Perillae Folium* typically had the highest content of rosmarinic acid. Certain macroscopic characteristics were related to the concentration of rosmarinic acid. The volatile components were identified and compared in *Perillae Fructus*, *Perillae Folium* and *Perillae Caulis* by gas chromatography–mass spectrometry (GC-MS). Extracts were prepared by steam distillation. Twelve, seventeen and nine compounds were identified and accounted for 69.71%, 50.54% and 81.73% of all identified peak areas in *Perillae Caulis*, *Perillae Folium* and *Perillae Fructus* respectively. The identified components were analyzed for comparison of *Perillae Caulis*, *Perillae Folium* and *Perillae Fructus* more effectively.

Conclusions This work provides a means by which samples of various parts of *P. frutescens* can be evaluated in terms of their pharmacologically active components. It should be of value in the efficient exploitation of *P. frutescens* plant material in clinical applications and drug development.

112. Characterization and determination of six flavonoids in the ethnomedicine “Dragon’s blood” by UPLC-PAD-MS

T. Yi, Y. Tang, J.Y. Zhang, Z.Z. Zhao, Z.J. Yang and H.B. Chen

Chemistry Central Journal, 2012, 6, 116

Abstract

Background “Dragon’s Blood” (DB) has long been used as an ethnomedicine in China to invigorate blood circulation for the treatment of traumatic injuries, blood stasis and pain. To comprehensively assess the quality of DB medicine, a precise and accurate method that can rapidly separate, characterize and quantify multiple active components of DB is crucial.

Results An ultra performance liquid chromatography (UPLC) coupled with photodiode array detection (PAD) and electrospray ionization mass spectrometry (ESI-MS) method was developed for characterization and determination of six flavonoids in DB. A comprehensive validation of the developed method was conducted, and confirmed that the method presented good sensitivity,

precision and accuracy. All linear regressions were acquired with $R^2 > 0.99$, and the limits of detection ranged from 0.06 to 0.83 ng. The relative standard deviation (RSD) values were found to be within the range 1.4–3.8% for the method repeatability test. Recovery studies for the quantified compounds were found to be within the range 94.2–102.8% with RSD less than 4.9%. DB samples collected from different geographical regions were analyzed by the present method, and the results demonstrated that the contents of the six flavonoids in DB samples varied significantly. Three major active components among the six flavonoids, namely dracorhodin, (2S)-5-methoxyflavan-7-ol and (2S)-5-methoxy-6-methylflavan-7-ol, are suggested as the index for DB quality evaluation.

Conclusions Overall, the present hyphenation method is highly efficient and reliable, and hence suitable for the characterization and determination of the flavonoids of DB ethnomedicine.

113. Quality evaluation of various commercial specifications of *Polygoni Multiflori Radix* and its dregs by determination of active compounds

Z.T. Liang, N.N. Leung, H.B. Chen, Z.Z. Zhao

Chemistry Central Journal, 2012, 6, 53

Abstract

Background According to market investigation, two kinds of *Polygoni Multiflori Radix* decoction pieces with different specifications are commercially available: irregular thick slices (0.7-1.3 cm) and length-wise into thin slices (0.11-0.2 cm). The objective of this study was to evaluate the quality of various samples of *Polygoni Multiflori Radix* decoction pieces and its dregs.

Results A simple and reliable high performance liquid chromatographic method was developed for determination the contents of 2,3,5,4'-tetrahydroxystilbene-2-O- β -D- glucopyranoside (THSG), emodin and physcion, which were considered to be potent active ingredients. The results showed that the contents of THSG, emodin and physcion varied in samples of different diameters and thicknesses. The results also indicated the dregs of *Polygoni Multiflori Radix* still contained a considerable amount of THSG, emodin and physcion.

Conclusion The various commercial specifications of *Polygoni Multiflori Radix* sold in the markets did not correlate with their prices, and the dregs of *Polygoni Multiflori Radix* can be further utilized.

114. Tissue-specific metabolite profiling of alkaloids in *Sinomenii Caulis* using laser microdissection and liquid chromatography-quadrupole/time of flight-mass spectrometry

L. Yi, Z.T. Liang, Y. Peng, X. Yao, H.B. Chen, Z.Z. Zhao

Journal of Chromatography A, 2012, 1248, 93-103

Abstract

Secondary metabolites accumulated in different tissues and cells of herbs are usually bioactive components of herbal medicines. Thus, tissue- and cell-specific phytochemical profiling should be useful for indicating relationship between herbal tissues and chemicals, and evaluating the quality of a medicinal herb. Here, a method that combining laser microdissection and ultra-performance liquid chromatography–quadrupole/time-of-flight mass spectrometry (LMD with UPLC–Q/TOF-MS) was established to achieve simultaneous localization and determination of bioactive components in herbal medicines. *Sinomenii Caulis*, sourced from the stems of *Sinomenium acutum* (Thunb.)

Rehd. et Wils., was set as an illustrative case, and its phytochemicals were profiled by the present method through analyses of different microdissected tissues and cells, involving epidermis, cortex, stone cells, pericycle, vascular bundles and pith. Results revealed that different tissues and cells contained varied alkaloids, among which six alkaloids, i.e. 6-Me-ether-12-O- β -d-glucopyranoside-laudanosoline (peak 4), sinomenine (peak 6), N-norsinoacutine (peak 7), magnoflorine (peak 11), laurifoline (peak 16) and menisperine (peak 17) were detected in all microdissected parts, and sinomenine and magnoflorine were the two most abundant components. By further quantitative determination, alkaloids were generally demonstrated to distribute in the outer part of the cortex, phloem and xylem. According to the relationship between alkaloids and tissues revealed in our study, *Sinomenii Caulis* of larger diameter has proportionately more bioactive components, and is therefore of higher quality for medicinal use. The method of LMD with UPLC–Q/TOF-MS developed in this study was initially applied to the research of medicinal herbs, and proved to be high sensitive, low cost, convenient and practical.

115. Comparative chemical analysis of Radix Astragali and Radix Hedysari by HPLC

Y. Liu, X.J. Zhang, Y.Y. Zhao, H.B. Chen

Natural Product Research, 2012, 26, 1935-1938

Abstract

Radix Astragali and Radix Hedysari, two famous traditional Chinese medicines (TCM), were considered to possess the same efficacy in TCM traditionally. However, modern pharmacological and phytochemical investigations showed that they were different in efficacy and chemical constituents to some degree. In this study, the principal constituents of the two drugs, isoflavonoids and saponins, were comparatively analysed using HPLC–UV and HPLC–ELSD methods. The results showed that there were some common compounds, especially flavonoids, in both drugs, while the differences in the types and contents of their chemical constituents, especially in saponins, were also obvious. The similarities and differences of the chemicals may be responsible, at least partially, for the similarities and differences in their efficacies or bioactivities.

116. An ethnobotanical survey of medicinal spices used in Chinese hotpot

M.H. Wu, P. Guo, S.W. Tsui, H.B. Chen, Z.Z. Zhao

Food Research International, 2012, 48, 226-232

Abstract

Chinese cuisine is one of the most famous cuisines of the world. The use of spices and condiments is an indispensable procedure in Chinese culinary culture, especially the hotpot culture. However, there has been no systematic investigation on spices and condiments used in Chinese hotpot so far. An ethnobotanical survey was conducted to collect information on spices and condiments used in Chinese hotpot. The results showed that a total of 67 spices were commonly used for the preparation of Chinese hotpot, involving 82 plant species of 50 genera in 26 families. All of these spices are also used medicinally in China yet half of them were not native to China. Plants of Zingiberaceae and Apicaceae are important botanical resource, and fruit parts of a plant are the main source of the production of spices used in Chinese hotpot.

117. Authentication of Chinese Materia Medica decoction dregs, Part II: Comparison before and after decoction of four Chinese Materia Medica that mainly comprise storage tissue

L.L. Wong, Z.T. Liang, H.B. Chen, Z.Z. Zhao

Microscopy Research and Technique, 2012, 75, 164-175

Abstract

Authentication of Chinese Materia Medica (CMM) decoction dregs is important for ensuring the efficacy and safety of CMM when they are used in decoction. If someone got worse or poisoned after taking a decoction while the formula is appropriate, the authentication of CMM dregs is the effective method to explore the reasons. Therefore, a systematic study on the authentication of CMM dregs was carried out. In this study, two pairs of easily confused CMM dregs, Fenge (*Puerariae Thomsonii Radix*) and Shanyao (*Dioscoreae Rhizoma*), Dihuang (*Rehmanniae Radix*) and Huangjing (*Polygonati Rhizoma*), which mostly comprise storage tissues, were investigated by comparing the morphological and microscopic characteristics. Fenge and Shanyao contain abundant starch granules. After decoction their dregs were hard, nonstarchy and horn-like. Fully gelatinized starch granules were found in the powder of Fenge dregs while incompletely gelatinized starch granules were occasionally found in the Shanyao dregs. In contrast, Dihuang and Huangjing contain water-soluble sugars. After decoction, their dregs were hard and brittle. Their parenchymatous cells were shrunk and unknown crystals were found under microscope. Hence, the morphological and microscopic characteristics of CMM before and after decoction were different, and different changes in the CMM dregs can be illustrated by the different nature of their ergastic substance. These differences could be used to authenticate CMM dregs.

118. Comparison of the chemical composition and pharmacological effects of the aqueous and ethanolic extracts of a Tibetan 'Snow Lotus' (*Saussurea laniceps*) herb

T. Yi, H.W. Lo, Z.Z. Zhao, Z.L. Yu, Z.J. Yang, H.B. Chen

Molecules, 2012, 17, 7183-7194

Abstract

To understand the impacts of different processing methods on the composition and effects of the herb *Saussurea laniceps* (SL), the present study report the first comparison of the chemical constituents of aqueous and ethanolic SL extracts using chromatographic analysis, and to compare their pharmacological effects in a mouse anti-inflammatory, anti-nociceptive model and an *in vitro* anti-oxidant test. Chemical comparison demonstrated that the types of chemicals in the two extracts were identical, but the contents of the main constituents in the aqueous extract were lower than those of the ethanolic extract. A transesterification of dicaffeoylquinic acids took place in the aqueous extract during boiling. As for pharmacological effects, oral administration of aqueous and ethanolic SL extracts significantly inhibited croton oil-induced mice ear edema, and significantly inhibited acetic acid-induced mice writhings, respectively. In the DPPH anti-oxidant activity test, the IC50 values were calculated as 409.6 mg/L and 523.4 mg/L for the ethanolic and aqueous extracts,

respectively. The inhibitory effects of the ethanolic extract were more potent than those of the aqueous extract in all pharmacological tests, although there was no significant difference. This study suggests that the two preparations should be distinguished when used.

119. Authentication of Chinese Materia Medica decoction dregs, Part 1: Comparison of morphological and microscopic features of four Chinese Materia Medica before and after decoction

L.L. Wong, Z.T. Liang, H.B. Chen, Z.Z. Zhao

Microscopy Research and Technique, 2011, 74, 320-328

Abstract

Chinese Materia Medica (CMM) decoction dregs are the residues of medicinal materials after decoction. Accurate identification of CMM in decoction dregs will be helpful for exploring the causes of poisoning or other medical incidents arising after the ingestion of CMM decoctions. To determine how decoction affects the characteristics used to authenticate specific CMM, a systematic study was carried out. In this study, two pairs of Materia Medica that are commonly confused-namely, Baizhu (*Atractylodes Macrocephalae Rhizoma*) and Cangzhu (*Atractylodes Rhizoma*), Baishao (*Paeoniae Alba Radix*) and Chishao (*Paeoniae Rubra Radix*)-were chosen for investigation. Each pair of Materia Medica has similar morphology in appearance, but they have different functions in Chinese clinic. After decoction, with regard to gross morphological characters, the results showed that bark and wood could be easily distinguished. The striation of vessels and fibers became more prominent because of the contraction of parenchymatous cells, but the lignified cells did not. As for the microscopic characteristics, the cells with thickened walls, such as stone cells and fibers, were basically stable. Most of the parenchymatous cells were broken. Crystals of calcium oxalate showed no changes as they were insoluble in water. Starch granules were gelatinized and aggregated in parenchymatous cells. Inulins were substantially reduced in number as they dissolved in water during decoction. According to these changes in morphological and microscopic characteristics after decoction, the dregs of two pairs of Materia Medica could be distinguished.

120. Ethanol Extract of Fructus Schisandrae Decreases Hepatic Triglyceride Level in Mice Fed with a High Fat/Cholesterol Diet, with Attention to Acute Toxicity

S.Y. Pan, Z.L. Yu, H. Dong, C.J. Xiang, W.F. Fong, K.M. Ko

Evidence-based Complementary and Alternative Medicine, 2011, 729412

Abstract

Effects of the ethanol extract of Fructus Schisandrae (EtFSC) on serum and liver lipid contents were investigated in mice fed with high fat/cholesterol (HFC) diet for 8 or 15 days. The induction of hypercholesterolemia by HFC diet caused significant increases in serum and hepatic total cholesterol (TC) levels (up to 62% and 165%, resp.) and hepatic triglyceride (TG) levels (up to 528%) in mice. EtFSC treatment (1 or 5 g/kg/day for 7 days; from Day 1 to 7 or from Day 8 to 14, i.g.) significantly decreased the hepatic TG level (down to 35%) and slightly increased the hepatic index (by 8%) in hypercholesterolemic mice. Whereas fenofibrate treatment (0.1 g/kg/day for 7 days, i.g.) significantly lowered the hepatic TG level (by 61%), it elevated the hepatic index (by 77%) in

hypercholesterolemic mice. Acute toxicity test showed that EtFSC was relatively non-toxic, with an LD50 value of 35.63 ± 6.46 g/kg in mice. The results indicate that EtFSC treatment can invariably decrease hepatic TG in hypercholesterolemic mice, as assessed by both preventive and therapeutic protocols, suggesting its potential use for fatty liver treatment.

121. Macroscopic identification of Chinese medicinal materials: Traditional experiences and modern understanding

Z.Z. Zhao, Z.T. Liang, P. Guo

Journal of Ethnopharmacology, 2011, 134, 556-564

Abstract

Authentication is fundamental for the standardization of Chinese Materia Medica. Macroscopic identification, an important method of authentication, has a long history rooted in practice and experience over generations. In ancient times, macroscopic features were used not only to identify the genuineness, but also to evaluate the quality of Chinese medicinal materials (CMMs). In many ancient documents, picturesque terms were used to describe the quality of certain specific CMMs. These traditional macroscopic descriptions used for identification are filled with characteristics, quality descriptions, and specifications of CMM that need to be confirmed by modern scientific analysis. In this paper, the essential and fundamental components of macroscopic identification are introduced. As the quality and macroscopic characteristics of CMM are traditionally thought to be closely related, modern scientific approaches to confirm the validity of these associations are discussed. This review clarifies the role of macroscopic features in the quality assessment of CMM.

122. Authentication of the 31 species of toxic and potent Chinese Materia Medica by microscopic technique assisted by ICP-MS analysis, part 4: four kinds of toxic and potent mineral arsenical CMMs

Q. Li, C. Chu, Y.Q. Wang, H.B. Chen, P. Li, Z.Z. Zhao

Microscopy Research and Technique, 2011, 74, 1-8

Abstract

Toxic and Potent Chinese Materia Medica (T/PCMM) is a special and very important category of Chinese medicines. They have long been used in traditional medical practice and are being used more and more widely throughout the world in recent years. As there may be many fatal toxic effects caused by misusing or confusion of T/PCMM, their quality and safety control arouse increasing attention internationally. Researches on the accurate identification to ensure the safe use of T/PCMM are acquired; however, there are few reports on authentication. We are carrying out a series of studies on 31 T/PCMM originating from plants, animals, minerals, and secreta. In our previous studies, we proved that modern microscopic authentication is a simple, fast, effective, low cost, and less toxic method for identifying animal, seed, and flower T/PCMM. In the present study, we focused on the authentication of four kinds of mineral arsenicals, including orpiment (mainly containing As_2S_3), realgar (mainly containing As_4S_4), arsenolite, and arsenic trioxide (mainly containing As_2O_3). We examined the macroscopic and microscopic characteristics of the above minerals and found that they all can be easily identified and authenticated by using light microscopy coupled with polarized microscopy. Moreover, the authentication results for arsenolite and arsenic

trioxide are confirmed by ICP-MS analysis. We are sure that the morphological and microscopic characteristics indicated here are indispensable to establishing standards for these four mineral T/PCMMs.

123. A unique issue in the standardization of Chinese Materia Medica: processing

Z.Z. Zhao, Z.T. Liang, K. Chan, G.H. Lu, E.L.M. Lee, H.B. Chen, L. Li

Planta Medica, 2010, 76, 1975-1986

Abstract

Processing of Chinese Materia Medica (CMM) is a pharmaceutical technique to fulfill the different requirements of therapy, dispensing and making preparations according to traditional Chinese medicine theory. The aims of processing are to enhance the efficacy and/or reduce the toxicity of crude drugs. Those processed products are named as decoction pieces, which are used in clinics. Therefore, there is a close relationship between processing, safety, and efficacy of Chinese medicines. Some toxicity or side effects are caused by improper processing methods and some are due to improper combination of herbal mixtures. Standardization of processing methods for Chinese herbs is as important as authentication to maintain their quality and ensure their safe use. The objective of this paper is to review the literature covering the current situation and problems of CMM processing as well as recent progress in research in this area. A summary of the most urgent work needed is proposed.

124. An experimental study on four kinds of Chinese herbal medicines containing alkaloids using fluorescence microscope and microspectrometer

Z.T. Liang, H.B. Chen, Z.Z. Zhao

Journal of Microscopy, 2009, 233, 24-34

Abstract

In order to find a useful method for uniting the identification and quality evaluation of Chinese herbal medicine, the techniques of fluorescence microscopy and microspectrometer were firstly applied to authenticate four kinds of herbal medicines, Caulis Sinomenii, Rhizoma Coptidis, Radix Sophorae Tonkinensis and Rhizoma Menispermii, as well as to measure the distribution of alkaloids in their cross-sections. The results showed that the fluorescence microscopic characteristics and the fluorescence emission spectra of the same tissues from the four kinds of Chinese herbal medicine were different, for example, the cortex of Radix Sophorae Tonkinensis emitted blue fluorescence instead of the yellow of Rhizoma Coptidis observed with emission filter of long-pass 397 nm, which could be effective to identify them. Furthermore, alkaloids, as active components of the above herbal medicines, were distributed in each part of herbal tissue but their fluorescence intensities in different parts of tissue were different. The results indicated that fluorescence microscope combined with microspectrometer could be a helpful method for identification and quality evaluation of Chinese herbal medicine.

125. Studies on microscopic identification of animal drugs' remnant hair (3): identification of several species of Cauda Cervi

X.X. Cheng, T.G. Kang, Z.Z. Zhao

Journal of Natural Medicines, 2007, 61, 51-55

Abstract

Cauda Cervi has been used as a nourishing invigorant in China for a long time. As recorded, the origins of *Cauda Cervi* are the dried tails of *Cervus elaphus* Linnaeus and *Cervus nippon* Temminck. In addition, *Cervus albirostris* Przewalski and *Cervus unicolor* Kerr are also used in some regions. Hair grows periodically and has high biological stability, which allows it to be used as specific markers for certain species. In order to differentiate four species of *Cauda Cervi*, microscopic characteristics of remnant hair were observed. Digital parameters such as the diameter of the hair shaft, medulla index and vein distance of the hair cuticle were compiled. It was shown that the method is convenient and feasible.

126. Quantification of Two Polyacetylenes in Radix Ginseng and Related Panax Herbs Using a Gas Chromatography-Mass Spectrometric Method

J. Liu, C. S. Lee, K.M. Leung, Z. Yan, B. Shen, Z. Zhao, Z.H. Jiang

Journal of Agricultural and Food Chemistry, 2007, 55, 8830-8835

Abstract

A sensitive method for quantitating the pharmacologically active polyacetylenes panaxynol and panaxydol in *Radix Ginseng* was developed using a capillary gas chromatography-mass spectrometric (GC-MS) method. The detection mode of selected ion monitoring (SIM) allowed sensitive and selective quantitation of the two compounds in ginseng. Method validation showed that the GC-MS method has much lower detection and quantitation limits than the high-performance liquid chromatography (HPLC)-UV method. This indicates that GC-MS is particularly useful for the analysis of polyacetylene compounds, which have relatively low abundances compared with ginsenosides in ginseng. Based on the quantitative results of different types of ginseng herbs, it was found that the panaxydol and panaxynol contents were higher in forest ginseng than in cultivated ginseng. This method was further applied to the quantitative analyses of panaxynol and panaxydol in *Radix Notoginseng* and American ginseng. The ratio of panaxydol to panaxynol can be utilized as a marker for differentiating ginseng, notoginseng, and American ginseng. This study introduces the first GC-MS method for the quantitative analysis of polyacetylenes in herbs of the *Panax* genus.

127. Quality Assessment of Rhizoma et Radix Notopterygii by HPTLC, HPLC Fingerprinting and HPLC Quantitative Analysis

G.S. Qian, Q. Wang, K.S.Y. Leung, Y. Qin, Z. Zhao, Z.H. Jiang

Journal of Pharmaceutical and Biomedical Analysis, 2007, 44, 812-817

Abstract

This paper describes an improved quality assessment method for Rhizoma et Radix Notopterygii (the rhizome and root of Notopterygium incisum Ting ex H.T. Chang or Notopterygium forbesii Boiss). The method was established by using fingerprinting and quantitation of marker compounds (isoimperatorin, notopterol and bergapten) in this herbal medicine. The authentication of Rhizoma et Radix Notopterygii using high performance thin-layer chromatography (HPTLC) fingerprinting was achieved by comparing the colors and R_f values of the bands in TLC fingerprints with those of the marker compounds. The HPLC fingerprints of 16 batches of herbal samples from different regions of China showed similar chromatographic patterns. Five peaks were selected as characteristic peaks, and three of these were identified by using LC–MS–MS techniques. The relative retention times of these characteristic peaks in the HPLC fingerprint were established as an important parameter for identification of Rhizoma et Radix Notopterygii. Finally, the pharmacologically active marker compounds isoimperatorin, notopterol and bergapten in this herb were quantitatively determined using a validated reverse-phase HPLC method.

128. Authentication is fundamental for standardization of Chinese medicines

Z.Z. Zhao, Y.N. Hu, Z.T. Liang, J.P.S. Yuen, Z.H. Jiang, K.S.Y. Leung

Planta Medica, 2006, 72, 865-874

Abstract

Chinese medicines (CMs) are being used more and more widely throughout the world. Since there are many poisoning incidents caused by misuse or confusion of CMs, their safe use has become a critical issue internationally. In this paper, based on the investigation of the current market of CMs, reasons for various confusions of Chinese Materia Medica (CMM) are analyzed and clarified, such as herbs with multiple sources, regional custom-herbs, confusion in nomenclature, similarity in appearance, and complexity of processed products. Authentication of plant material is critical to the safe and effective use of CMM. In this paper, several authentication methods, such as taxonomy, morphology, microscopy, physical and chemical authentication, DNA molecular biology and their advanced applications in this area, are introduced. Furthermore, it is proposed that an authority on the authentication of CMM be established, as a physical institution and/or as an electronic database.

129. Application of microscopy in authentication of Chinese patent medicine- -Bo Ying compound

Z.Z. Zhao, Y.N. Hu, Y.W. Wong, Gigi W.C. Wong, K. Wu, Z.H. Jiang, T. Kang

Microscopy Research and Technique, 2005, 67, 305-311

Abstract

To establish a quality control method for the Chinese Patent Medicine (CPM)-Bo Ying Compound (BYC), a comparison study was carried out on it with microscopy. The micro-morphological characteristics of its 22 components in the CPM and in the crude constituents have been documented and compared with each other. Their corresponding features were described and documented with color digital micrographs, so as to authenticate the presence of genuine crude constituents in BYC. The results showed that almost all constituents of BYC are found within their representative fragments in the CPM except one (Borax) that could dissolve or merge with the other components. Also the study indicated that light microscopy, an easy and economical method, could be used for the identification of this kind of CPM that contains plant and animal materials without the specific characteristic chemical marker compounds.