The Hong Kong University of Science and Technology

1. **Asarones from Acori Tatarinowii Rhizoma stimulate expression and secretion of neurotrophic factors in cultured astrocytes**

K.Y.C. Lam, Q.Y. Wu, W.H. Hu, P. Yao, H.Y. Wang, T.T.X. Dong, K.W.K. Tsim

Neuroscience Letters, 2019, 77, 134308

Abstract

Acori Tatarinowii Rhizoma (ATR, the dried rhizome of Acorus tatarinowii Schott.) is a traditional Chinese medicine widely used to treat brain diseases, e.g. depression, forgetfulness, anxiety and epilepsy. Several lines of evidence support that ATR has neuronal beneficial functions in animal models, but its action mechanism in cellular level is unknown. Here, we identified α-asarone and β-asarone could be the major active ingredients of ATR, which, when applied onto cultured rat astrocytes, significantly stimulated the expression and secretion of neurotrophic factors, i.e. nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF), in dose-dependent manners. These results suggested that the neuronal action of ATR, triggered by asarone, might be mediated by an increase of expression of neurotrophic factors in astrocytes, which therefore could support the clinical usage of ATR. In addition, application of PKA inhibitor, H89, in cultured astrocytes partially blocked the asarone-induced neurotrophic factor expression, suggesting the involvement of PKA signaling. The results proposed that α-asarone and β-asarone from ATR could serve as potential candidates for drug development in neurodegenerative diseases

2. **Discrimination of three Siegesbeckiae Herba species using UPLC-QTOF/MS-based metabolomics approach**

H.X. Tao, W. Xiong, G.D. Zhao, T. Peng, Z.F. Zhong, L. Xu, R.Duan, K.W.K. Tsim, H. Yu, Y.T. Wang

Food and Chemical Toxicology, 2018, 119, 400-406

Abstract

The plant origin is one of the most important factors for the quality control of traditional Chinese medicines (TCMs) and highly affected on their safety and effectiveness in clinical applications. Multi-origin has been widely observed for many TCMs. Siegesbeckiae Herba (SH) is a traditional anti-rheumatic TCM which is originated from the plants of Siegesbeckia pubescens Makino (SP), S. orientalis L. (SO), and S. glabrescens Makino (SG). In the present study, an UPLC-QTOF/MS method were validated and successfully applied for the determination of the chemical profiles in the three SH species. The data were statistical analyzed with the OPLS-DA analysis and One-Way ANOVA F-test. Obvious differences in chemistry were observed in different SH species and 40 components were identified. Finally, 6 components were selected as potential chemical markers for the discrimination of

SP, SO and SG based on the characteristic distribution in individual SH species.

3. **Asarone from Acori Tatarinowii Rhizome prevents oxidative stress-induced cell injury in cultured astrocytes: A signaling triggered by Akt activation**

K.Y.C. Lam, P. Yao, H. Wang, R. Duan, T.T.X. Dong, K.W.K. Tsim

PLoS One, 2017, 12: e0179077

Abstract

Acori Tatarinowii Rhizome (ATR; the dried rhizome of Acori tatarinowii Schott) is a well-known herb being used for mental disorder in China and Asia. Volatile oil is considered as the active ingredient of ATR, and asarones account for more than 90% of total volatile oil. Here, the protective effects of ATR oil and asarones, both α-asarone and β-asarone, were probed in cultured rat astrocytes. The cyto-protective effect of ATR oil and asarones against tBHP-induced astrocyte injury was revealed, and additionally ATR oil and asarones reduced the tBHP-induced intracellular reactive oxygen species (ROS) accumulation. In parallel, the activity of anti-oxidant response element (ARE) promoter construct (pARE-Luc), being transfected in cultured astrocytes, was markedly induced by application of ATR oil and asarones. The mRNAs encoding anti-oxidant enzymes, e.g. glutathione S-transferase (GST), glutamate-cysteine ligase modulatory subunit (GCLM), glutamate-cysteine ligase catalytic subunit (GCLC) and NAD(P)H quinone oxidoreductase (NQO1) were induced by ATR oil and asarones in a dose-dependent manner. The ATR oil/asarone-induced gene expression could be mediated by Akt phosphorylation; because the applied LY294002, a phosphoinositide 3-kinase inhibitor, fully abolished the induction. These results demonstrated that α-asarone and β-asarone could account, at least partly, the function of ATR being a Chinese medicinal herb.

4. **Comparative Study of Different Acorus Species in Potentiating Neuronal Differentiation in Cultured PC12 Cells.**

K.Y.C. Lam, Y. Huang, P. Yao, H. Wang, T.T.X. Dong, Z. Zhou, K.W.K. Tsim

Phytotherapy Research, 2017, 31,1757-1764

Abstract

Acori Tatarinowii Rhizoma (ATR), the rhizome of Acorus tatarinowii Schott, is a common traditional Chinese medicine being used clinically for mental disorder. However, other Acorus species herbs are all having the same Chinese name 'Chang Pu', making the confusion in herbal market. Acori Graminei Rhizoma (AGR) and Acori Calami Rhizoma (ACR) are common adulterants of ATR. Here, we aim to provide a comparative analysis between ATR, AGR, and ACR in potentiating neuronal differentiation. Volatile oil, derived from Acorus species, was applied onto cultured PC12 cells, and various parameters were determined: (i) transcriptional activation of neurofilament promoters was determined by the promoter-driven luciferase activity assay; (ii) the neurite outgrowth of PC12 cells was captured and measured; and (iii) the neurofilament expression and its underlying mechanism

were analyzed by western blotting. The co-treatment of ATR, AGR, or ACR volatile oil with low concentration of nerve growth factor (NGF) could potentiate the NGF-induced neuronal differentiation in cultured PC12 cells. In addition, application of protein kinase A inhibitor H89 in cultures blocked the induction of neurofilament. Among these three Acorus species, ATR volatile oil showed the highest NGF-induced induction in neurite outgrowth and neurofilament expression, as compared with that of AGR and ACR

5. **Authentication of Acori Tatarinowii Rhizoma (Shi Chang Pu) and its adulterants by morphological distinction, chemical composition and ITS sequencing**

K.Y.C. Lam, C.F. Ku, H.Y. Wang, G.K.L. Chan, P. Yao, H.Q. Lin, T.T.X. Dong, H.J. Zhang, K.W.K. Tsim

Chinese Medicine, 2016, 11:41

Abstract

Acori Tatarinowii Rhizoma (ATR; rhizome of Acorus tatarinowii Schott) (Shi Chang Pu) is widely used in Chinese medicine (CM) to resuscitate, calm the mind, resolve shi (dampness) and harmonize the wei (stomach). Seven different species have been identified as belonging to the genus Acorus, all of which can be found in China. However, it can be difficult to distinguish the different species of Acorus because of their morphological similarities. The aim of this study was to authenticate Acorus species using macroscopic and microscopic techniques, chemical analysis and DNA authentication and to compare the resolution power and reliability of these different methods.

METHODS:

Four batches of ATR, Acori Graminei Rhizoma (AGR), Acori Calami Rhizoma (ACR) and Anemones Altaicae Rhizoma (AAR) (totaling 16 samples) were collected from Hong Kong and mainland China. The major characteristic features of these Acorus species were identified by macroscopic and microscopic examination. The identified samples were also analyzed by UHPLC analysis. Principal component analysis (PCA) and hierarchal clustering analysis (HCA) on UHPLC results were used to differentiate between the samples. An internal transcribed spacer (ITS) was selected as a molecular probe and a modified DNA extraction method was developed to obtain trace amounts of DNA from the different Acorus species. All extracted DNA sequences were edited by Bioedit and aligned with the ClustalW. And the sequence distances were calculated using the Maximum Parsimony method.

RESULTS:

Macroscopic and microscopic analyses allowed for AAR to be readily distinguished from ATR, AGR and ACR. However, it was difficult to distinguish between ATR, AGR and ACR because of their similar morphological features. Chemical profiling revealed that α- and β-asarone were only found in the ATR, AGR and ACR samples, but not in the AAR samples. Furthermore, PCA and HCA allowed for the differentiation of these three species based on their asarone contents. Morphological authentication and chemical profiling allowed for the partial differentiation of ATR, AGR ACR and AAR. DNA analysis was the only method capable of accurately differentiating between all four species.

CONCLUSION:

DNA authentication exhibited higher resolution power and reliability than conventional morphological identification and UHPLC in differentiating between different Acorus species.

6. **Danggui Buxue Tang, Chinese Herbal Decoction Containing Astragali Radix and Angelicae Sinensis Radix, Induces Production of Nitric Oxide in Endothelial Cells: Signaling Mediated by Phosphorylation of Endothelial Nitric Oxide Synthase**

A.G.W. Gong, K.M. Lau, L.M.L. Zhang, H.Q. Lin, T.T.X. Dong, K.W.K. Tsim

Planta Medica, 2016, 82, 418-523

Abstract

Danggui Buxue Tang, an ancient Chinese herbal decoction containing Astragali Radix and Angelicae Sinensis Radix at the weight ratio of 5 : 1, is used to mitigate menopausal syndromes in women. The pharmacological properties of Danggui Buxue Tang have been illustrated in bone development, blood enhancement, and immune stimulation. Here, we extended the possible pharmacological role of Danggui Buxue Tang in cardiovascular function. In cultured human umbilical vein endothelial cells, the application of Danggui Buxue Tang induced the release of nitric oxide and the phosphorylation of endothelial nitric oxide synthase and Akt kinase in time- and dose-dependent manners. The robust activation of nitric oxide signaling, however, required the boiling of Astragali Radix and Angelicae Sinensis Radix together, i.e., as Danggui Buxue Tang instead of other herbal extracts. The Danggui Buxue Tang-induced phosphorylation of endothelial nitric oxide synthase and Akt kinase in human umbilical vein endothelial cells were fully blocked by treatment with an endothelial nitric oxide synthase inhibitor (L-NAME), a PI3K/Akt inhibitor (LY294002), and a Ca2+ chelator (BAPTA-AM). In parallel, the blockage of endothelial nitric oxide synthase and Akt activation subsequently fully abolished the Danggui Buxue Tang-induced nitric oxide production.

7. **Asarone from Acori Tatarinowii Rhizoma Potentiates the Nerve Growth Factor-Induced Neuronal Differentiation in Cultured PC12 Cells: A Signaling Mediated by Protein Kinase A**

K.Y.C. Lam, J.P. Chen, C.T.W. Lam, Q.Y. Wu , P. Yao, T.T.X. Dong, H.Q. Lin, K.W.K. Tsim

PLoS One, 2016, 11, e0163337.

Abstract

Acori Tatarinowii Rhizoma (ATR), the rhizome of Acorus tatarinowii Schott, is being used clinically to treat neurological disorders. The volatile oil of ATR is being considered as an active ingredient. Here, α-asarone and β-asarone, accounting about 95% of ATR oil, were evaluated for its function in stimulating neurogenesis. In cultured PC12 cells, application of ATR volatile oil, α-asarone or

β-asarone, stimulated the expression of neurofilaments, a bio-marker for neurite outgrowth, in a concentration-dependent manner. The co-treatment of ATR volatile oil, α-asarone or β-asarone, with low concentration of nerve growth factor (NGF) potentiated the NGF-induced neuronal differentiation in cultured PC12 cells. In addition, application of protein kinase A inhibitors, H89 and KT5720, in cultures blocked the ATR-induced neurofilament expression, as well as the phosphorylation of cAMP-responsive element binding protein (CREB). In the potentiation of NGF-induced signaling in cultured PC12 cells, α-asarone and β-asarone showed synergistic effects. These results proposed the neurite-promoting asarone, or ATR volatile oil, could be useful in finding potential drugs for treating various neurodegenerative diseases, in which neurotrophin deficiency is normally involved.

8. **The Volatile Oil of Nardostachyos Radix et Rhizoma Induces Endothelial Nitric Oxide Synthase Activity in HUVEC Cells**

M. Maiwulanjiang, C.W.C. Bi, P.S.C. Lee, G. Xin , A. Miernisha, K.M. Lau , A. Xiong, N. Li, T.T.X. Dong, H A. Aisa , K.W. K. Tsim

PLoS One, 2015, 10, e0116761.

Abstract

Nardostahyos Radix et Rhizoma (NRR; the root and rhizome of Nardostachys jatamansi DC.) is a widely used medicinal herb. Historically, NRR is being used for the treatment of cardiovascular and neurological diseases. To search for active ingredients of NRR, we investigated the vascular benefit of NRR volatile oil in (i) the vasodilation in rat aorta ring, and (ii) the release of nitric oxide (NO) and the phosphorylation of endothelial NO synthase (eNOS) in cultured human umbilical vein endothelial cells (HUVECs). By measuring the fluorescence signal in cultures, application of NRR volatile oil resulted in a rapid activation of NO release as well as the phosphorylation of eNOS: both inductions were markedly reduced by L-NAME. In parallel, the phosphorylation level of Akt kinase was markedly increased by the oil treatment, which was partially attenuated by PI3K/Akt inhibitor LY294002. This inhibitor also blocked the NRR-induced NO production and eNOS phosphorylation. In HUVECs, application of NRR volatile oil elevated the intracellular Ca2+ level, and BAPTA-AM, a Ca2+ chelator, reduced the Ca2+ surge: the blockage were also applied to NRR-induced eNOS phosphorylation and NO production. These findings suggested the volatile oil of NRR was the major ingredient in triggering the vascular dilatation, and which was mediated via the NO production.

9. **The water extract of Angelica Sinensis Radix protects cultured PC12 cells against oxidative stress: Suppression of reactive oxygen species and activation of antioxidant response elements**

P.S.C. Lee, A.L.Yan, A.G.W. Gong, R.C.Y. Choi, H.Q. Lin, K.W.K. Tsim

Oxidants and Antioxidants in Medical Science, 2015, 4, 39-48

Abstract

Angelica Sinensis Radix (ASR; Dang Gui; the root of Angelica sinensis) is an herbal supplement that

has been used in invigorating blood circulation. Here, we provided different lines of evidence to support the beneficial role of ASR against oxidative stress in cultured PC12 cell, a rat pheochromocytoma cell line. Materials and Methods: The water extract of ASR inhibited the activity of xanthine oxidase in vitro. In cultures, the pre-treatment of ASR water extract reduced the cytotoxic effect of tert-butyl hydroperoxide (tBHP), an oxidative stress inducer. Results: The protecting mechanisms of ASR were shown to be mediated by: (i) suppression of tBHP-induced reactive oxygen species (ROS) formation; (ii) induction of caspase-3 and PARP activities; and (iii) stimulation of mRNAs encoding antioxidative genes, glutathione S-transferase A2 and NAD(P)H dehydrogenase quinone oxidoreductase 1 (NQO1), via the transcriptional activation of antioxidant response element (ARE). The outcome was the prevention of tBHP-induced cell apoptosis. Conclusion: Interestingly, the protective effect of ASR extract in PC12 cells was insignificant when challenging by insults of β-amlyoid (for Alzheimer's disease) and 1-methyl-4-phenylpyridinium (for Parkinson's disease). Taken together, we revealed a neurobeneficial role of ASR in protecting neuronal cells against oxidative damage, which might be useful in developing health food supplements for disease prevention in the future.

10. Authentication of Cordyceps sinensis by DNA Analyses: Comparison of ITS Sequence Analysis and RAPD-Derived Molecular Markers

K.Y.C. Lam, G.K.L. Chan, G.Z. Xin, H. Xu, C.F. Ku, J.P. Chen, P. Yao, H.Q. Lin, T.T.X. Dong, K.W.K. Tsim

Molecules, 2015, 20, 22454-22462

Abstract

Cordyceps sinensis is an endoparasitic fungus widely used as a tonic and medicinal food in the practice of traditional Chinese medicine (TCM). In historical usage, Cordyceps specifically is referring to the species of C. sinensis. However, a number of closely related species are named themselves as Cordyceps, and they are sold commonly as C. sinensis. The substitutes and adulterants of C. sinensis are often introduced either intentionally or accidentally in the herbal market, which seriously affects the therapeutic effects or even leads to life-threatening poisoning. Here, we aim to identify Cordyceps by DNA sequencing technology. Two different DNA-based approaches were compared. The internal transcribed spacer (ITS) sequences and the random amplified polymorphic DNA (RAPD)-sequence characterized amplified region (SCAR) were developed here to authenticate different species of Cordyceps. Both approaches generally enabled discrimination of C. sinensis from others. The application of the two methods, supporting each other, increases the security of identification. For better reproducibility and faster analysis, the SCAR markers derived from the RAPD results provide a new method for quick authentication of Cordyceps.

11. **Calycosin orchestrates the functions of Danggui Buxue Tang, a Chinese herbal decoction composing of Astragali Radix and Angelica Sinensis Radix: An evaluation by using calycosin-knock out herbal extract**

A.G.W. Gong, N. Li, K.M. Lau, P.S.C. Lee, L. Yan, M.L. Xu, C.T.W. Lam, A.Y.Y. Kong, H.Q. Lin, T.T.X. Dong, K.W.K. Tsim

Journal of Ethnopharmacology, 2015, 168: 150–157

Abstract

Ethnopharmacological relevance: Danggui Buxue Tang (DBT) is a classical Chinese herbal decoction containing two herbs, Astragali Radix (AR) and Angelicae Sinensis Radix (ASR), which serves as dietary supplement for treating women menopausal syndromes. Pharmacological studies indicate that DBT has estrogenic, erythropoietic and osteogenic properties; however, the action mechanism for this complex herbal decoction is not known. Calycosin, a major flavonoid in AR, shares similar structure with βestradiol, and thus which is hypothesized to be the critical compound of DBT. Here, we aim to investigate the role of calycosin in DBT in terms of its biological functions by using a calycosin-depleted DBT decoction (DBTΔcal). The biological functions of DBTΔcal and parental DBT were systematically compared. Materials and methods: In order to standardize DBT decoction, four chemical markers were determined and quantified by HPLC. A semi-preparative HPLC method was utilized to prepare DBTΔcal. The authenticity of DBTΔcal was evaluated by LC-QQQ-MS/MS. To reveal the effect of calycosin on DBT functions, several cell assays related to the known properties of DBT were revealed, including estrogenic, erythropoietic and osteogenic functions. Results: As compared to parental DBT, the estrogenic, erythropoietic and osteogenic abilities were markedly reduced in DBTΔcal. However, calycosin alone did not show significant responses. Conclusions: Our results suggest that calycosin is a bioactive chemical in DBT decoction, and which could play a key linker in orchestrating multi-components of DBT as to achieve maximal functions. These discoveries should be invaluable in drug development and in investigating the modernization of traditional Chinese medicine from a new perspective.

12. **The sulfur-fumigation reduces chemical composition and biological properties of Angelicae sinensis radix**

J.Y.X. Zhana, P. Yao, C.W.C. Bi, K.Y.Z. Zheng, W.L. Zhang, J.P. Chen, T.T.X. Dong, Z.R. Su, K.W.K. Tsim

Phytomedicine, 2014, 21, 1318–1324

Abstract

Angelica Sinensis Radix (roots of Angelica sinensis; ASR) is a popular herbal supplement in China for promoting blood circulation. Today, sulfur-fumigation is commonly used to treat ASR as a means of

pest control; however,the studies of sulfur-fumigation on the safety and efficacy of ASR are very limited. Here, we elucidated the destructive roles of sulfur-fumigation on ASR by chemical and biological assessments. After sulfur-fumigation, the chemicals in ASR were significantly lost. The biological activities of antiplatelet aggregation, induction of NO production and estrogenic properties were compared between the water extracts ofnon-fumigatedandsulfur-fumigatedASR.Inall cases,the sulfur-fumigationsignificantly reduced the biological properties of ASR. In addition, application of water extract deriving from sulfurfumigated ASR showed toxicity to cultured MCF-7 cells. In order to ensure the safety and to achieve the best therapeutic effect, it is recommended that sulfur-fumigation is an unacceptable approach for processing herbal materials.

13. **Identification of Angelica oil as a suppressor for the biological properties of Danggui Buxue tang: a Chinese herbal decoction composes of Astragali Radix and Angelica Sinensis Radix**

J.Y.X. Zhan, K.Y.Z. Zheng, W.L. Zhang, J.P. Chen, P.Yao, C.W.C. Bi, T.T.X. Dong, K.W.K.Tsim

Journal of Ethnopharmacology, 2014, 154, 825-831

Abstract

Ethnopharmacological relevance: Danggui Buxue Tang (DBT), a Chinese herbal decoction commonly used in treating women's ailments, contains two herbs: Angelica Sinensis Radix (ASR) and Astragali Radix (AR). Traditionally, ASR had to be pre-treated with yellow wine before the herbal preparation, which reduced the amount of volatile oil in water extract of ASR and DBT, and meanwhile the volatile oilreduced DBT processed better bioactivities in cell cultures. The present study aimed to investigate the effect of volatile oil from ASR (Angelica oil) on the solubility of AR-derived ingredients and the biological properties of DBT. Materials and methods: To standardize Angelica oil, four marker chemicals in ASR were determined by GC-QQQ-MS/MS. Subsequently, fifteen gram of AR was boiled with different amounts of Angelica oil. The amounts of astragaloside IV, calycosin, formononetin, total polysaccharides, total saponins and total flavonoids, all derived from AR, were extracted and determined by HPLC-UV/ELSD. To reveal the effect of Angelica oil on DBT functions, several cell assays related to the traditional functions of DBT were selected, including anti-platelet aggregation, induction of NO production, hematopoetic, estrogenic and osteogenic properties. Results: The inclusion of Angelica oil in AR during preparation significantly decreased the amount of ARderived astragaloside IV, calycosin, formononetin, total saponins and total flavonoids in the final water extract. In parallel, an inclusion of Angelica oil caused a decrease of DBT's estrogenic and hematopoetic activities in cultured cells. Moreover, the Angelica oil decreased DBT-induced cell proliferation of cultured MG-63 and endothelial cells. Conclusions: The results indicated that Angelica oil was a negative regulator for DBT chemically and biologically, which supported the traditional practice of preparing DBT by using the wine-treated ASR.

14. **The volatile oil of Nardostachyos Radix et Rhizoma inhibits the oxidative stress-induced cell injury via reactive oxygen species scavenging and Akt activation in H9c2 cardiomyocyte**

M. Maiwulanjiang, J.P. Chen, G.Z. Xin, A.G.W. Gong, A. Miernisha, C.Y.Q. Du, K.M. Lau, P.S.C. Lee, J. Chen, T.T.X. Dong, H.A. Aisa, K.W.K. Tsim

Journal of Ethnopharmacology, 2014, 153, 491–498

Abstract

Ethnopharmacological relevance: Nardostachyos Radix et Rhizoma (NRR; the root and rhizome of Nardostachys jatamansi DC.) is a well-known medicinal herb widely used in Chinese, Uyghur and Ayurvedic medicines for the treatment of cardiovascular disorders. The oxidative stress-induced cardiomyocyte loss is the major pathogenesis of heart disorders. Here, the total volatile oil of NRR was isolated, and its function in preventing the cell death of cardiomyocyte was demonstrated. Materials and methods: The cyto-protective effect of volatile oil of NRR against tBHP-induced H9c2 cardiomyocyte injury was measured by MTT assay. A promoter-report construct (pARE-Luc) containing four repeats of antioxidant response element (ARE) was applied to study the transcriptional activation of ARE. The amounts of phase II antioxidant enzymes were analyzed by quantitative real-time polymer chain reaction (qPCR) upon the volatile oil treatment at 30 μg/mL for 24 h. The activation of Akt pathway was analyzed by western blot. Results: In cultured H9c2 cardiomyocytes, application of NRR volatile oil exhibited strong potency in preventing tBHP-induced cell death and accumulation of intracellular reactive oxygen species (ROS) in a concentration-dependent manner. In addition, the application of NRR volatile oil in cultures stimulated the gene expressions of self-defense antioxidant enzymes, which was mediated by the transcriptional activation of antioxidant response element (ARE). The induced genes were glutathione S-transferase, NAD(P)H quinone oxidoreductase, glutamate-cysteine ligase catalytic and modulatory subunits. In addition, the volatile oil of NRR activated the phosphorylation of Akt in cultured H9c2 cells. The treatment of LY294002, an Akt inhibitor, significantly inhibited the volatile oil-mediated ARE transcriptional activity, as well as the cell protective effect of NRR oil. Conclusion: These results demonstrated that NRR volatile oil prevented the oxidative stress-induced cell death in H9c2 cells by (i) reducing intracellular ROS production, (ii) inducing antioxidant enzymes and (iii) activating Akt phosphorylation.

15. **Rapid identification of plant materials by wooden-tip electrospray ionization mass spectrometry and a strategy to differentiate the bulbs of Fritillaria**

G.Z. Xin, B. Hub, Z.Q. Shi, Y.C. Lam, T.T.X Dong, P. Li, Z.P. Yao, K.W.K. Tsim

Analytica Chimica Acta, 2014, 820, 84–91

Abstract

The counterfeit plant products, especially by using incorrect plant materials in pharmaceutical industry, have become a global problem. The plant materials belonging to closely related species but differing in medicinal properties are difficult to be identified. Here, a novel and generally applicable approach to identify the sources of plant materials was developed, which was based on the use of wooden-tip electrospray ionization mass spectrometry (wooden-tip ESI-MS) and multivariate statistical analysis of unidentified MS features (non-targeted). Using this approach, six officinal species of Fritillariae Cirrhosae Bulbus had been successfully differentiated. In addition, Fritillariae Pallidiflorae Bulbus, a common adulterant of Fritillariae Cirrhosae Bulbus, was also identified by using the strategy reported here. Compared with DNA phylogenetic trees, our approach provided finer resolution in distinguishing the closely related Fritillaria species. By combining wooden-tip ESI-MS and multivariate statistical analysis, a useful method was developed here for rapid identification of the sources of herbs, which showed promising perspectives in tracking the supply chain of pharmaceutical suppliers

16. **Authentication of Bulbus Fritillariae Cirrhosae by RAPD-Derived DNA Markers**

G.Z. Xin, Y.C. Lam, M. Maiwulanjang, G.K.L. Chan, K.Y. Zhu, W.L. Tang, T.T.X Dong, Z.Q. Shi, P. Li, K.W.K. Tsim

Molecules, 2014*,* 19, 3450-3459

Abstract

Bulbus Fritillariae is the most commonly used antitussive herb in China. Eleven species of Fritillaria are recorded as Bulbus Fritillariae in the Chinese Pharmacopoeia. Bulbus Fritillariae Cirrhosae is a group of six Fritillaria species with higher efficiency and lower toxicity derived mainly from wild sources. Because of their higher market price, five other Fritillaria species are often sold deceptively as Bulbus Fritillariae Cirrhosae in the herbal market. To ensure the efficacy and safety of medicinal herbs, the authentication of botanical resources is the first step in quality control. Here, a DNA based identification method was developed to authenticate the commercial sources of Bulbus Fritillariae Cirrhosae. A putative DNA marker (0.65 kb) specific for Bulbus Fritillariae Cirrhosae was identified using the Random Amplified Polymorphic DNA (RAPD) technique. A DNA marker representing a Sequence Characterized Amplified Region (SCAR) was developed from a RAPD amplicon. The SCAR marker was successfully applied to differentiate Bulbus Fritillariae Cirrhosae from different

species of Fritillaria. Additionally, the SCAR marker was also useful in identifying the commercial samples of Bulbus Fritillariae Cirrhosae. Our results indicated that the RAPD-SCAR method was rapid, accurate and applicable in identifying Bulbus Fritillariae Cirrhosae at the DNA level.

17. **Alkaloids of Linderae Radix suppressed the lipopolysaccharide-induced expression of cytokines in cultured macrophage RAW 264.7 cells**

D.J.Y. Chou, K.Y.C. Lam, J.P. Chen, P. Yao, T.T.X Dong, A.Z. Xiong, G.X. Chou, Z.T. Wang, K.W.K. Tsim

TANG [humanitas medicine], 2014, 4, e28

Abstract

Linderae Radix, the dry roots of Lindera aggregata (Sims) Kosterm, has long been used as traditional Chinese medicine for treatment of inflammatory diseases. The total alkaloids are believed to be the active components responsible for anti-inflammation of Linderae Radix. Here, the total alkaloids of Linderae Radix were extracted and isolated, including 12 isoquinoline alkaloids and 1 furan sesquiterpene. Within the alkaloids, norisoboldine, boldine, linderaline, isoboldine, reticuline, N-methyllaurotetanine, norjuziphine were found to be the major ingredients. In lipopolysaccharide-treated macrophage RAW 264.7 cells, application of Linderae Radix extract, or total alkaloids, suppressed the transcription of pro-inflammatory cytokines, interleukin-1β and interleukin-6. Out of the 12 alkaloids, norisoboldine, boldine, and isoboldine were tested in lipopolysaccharide-treated macrophages, and norisoboldine was the strongest alkaloid in suppressing the cytokine expressions. The current studies suggested that the identification of alkaloids from Linderae Radix could provide a plausible explanation for herbal therapeutic functions.

18. **Chromatographic fingerprint analysis and simultaneous determination of β–acetoxyisovaleryalkannin in Arnebiae Radix**

A. Miernisha, T.X. Dong, J.Y. Guo, H.A. Aisa, K.W.K. Tsim

China Pharmacy, 2014, 3

Abstract

OBJECTIVE：To establish fingerprints to assess the quality of Arnebiae Radix and to determine the contents of β-acetoxyisovaleryalkannin derived from Arnebia euchroma (Royle) Johnst. which is in order to provide the evidence for the quality control of Arnebiae Radix in new version of the Chinese Pharmacopoeia . METHODS: HPLC fingerprinting and content determination methods were applied to evaluate the quality of Arnebiae Radix · Ten batched of samples were detected by an ACE C18 column (4.6 mm x 250 mm, 5 μm) using acetonitrile-0.1 % formic acid with water Isocratic system as mobile phase. The wavelength of detection is 516 nm for fingerprinting and content determination of

β-acetoxyisovaleryalkannin in Arnebiae Radix. RESULTS: HPLC fingerprint of Arnebiae Radix was established and could be used for quality assessment of Arnebiae Radix. The results showed the characteristic HPLC fingerprints peaks of these ten batches in Arnebiae Radix. The contents of β-acetoxyisovaleryalkannin showed the differences from differenr sources of Arnebiae Radix. CONCLUSION：β-acetoxyisovaleryalkannin can be good chemical marker for the quality control of Arnebiae Radix. It has been used in Hong Kong Chinese Materia Medica Standards.

19. **Study on identification of Cordyceps- a Chinese traditional medicine**

H. Xu, T.X. Dong, K.J. Zhao, G.K.L. Chan, Y.J. Lin, K.W.K. Tsim

Chinese traditional medicine China Pharmacy Journal, 2014, 49, 120

Abstract

OBJECTIVE: To study on the identification of Cordyceps and establish its traditional pharmacognostic identification and modern identification method based on DNA molecular marker technology. METHODS: The methods of macroscopic, microscopic examination and RAPD-SCAR were employed here to authenticate Cordyceps. RESULTS: Based on the macroscopic identification, the features of transverse section and powder of Cordyceps were described in detail. The digital photographs were presented here in revealing the main macroscopic and microscopic characteristic of Cordyceps. The specific RAPD fragment of Cordyceps was conversed into SCAR marker, and Cordyceps could be identified by the optimized PCR conditions. CONCLUSIONS: The authenticated method of microscopic and macroscopic identification is intuitive. However, the identification method based on DNA molecular marker is sample, which provides the new scientific evidences for the identification of authenticity of Cordyceps.

20. **Kai-Xin-San, a Chinese herbal decoction containing Ginseng Radix et Rhizoma, Polygalae Radix, Acori Tatarinowii Rhizoma and Poria, stimulates the expression and secretion of neurotrophic factors in cultured astrocytes**

K.Y. Zhu, S.L. Xu, R.C.Y. Choi, A.L. Yan, T.T.X. Dong, K.W.K. Tsim

Evidence-Based Complementary and Alternative Medicine, 2013: 731385.

Abstract

Kai-xin-san (KXS), a Chinese herbal decoction prescribed by Sun Simiao in Beiji Qianjin Yaofang about 1400 years ago, contains Ginseng Radix et Rhizoma, Polygalae Radix, Acori Tatarinowii Rhizoma, and Poria. In China, KXS has been used to treat stress-related psychiatric diseases with the symptoms of depression and forgetfulness. Although animal study has supported the antidepression function of KXS, the mechanism in cellular level is still unknown. Here, a chemically standardized water extract of KXS was applied onto cultured astrocytes in exploring the action mechanisms of KXS treatment, which significantly stimulated the expression and secretion of

neurotrophic factors, including NGF, BDNF, and GDNF, in a dose-dependent manner: the stimulation was both in mRNA and protein levels. In addition, the water extracts of four individual herbs did not significantly stimulate the expression of neurotrophic factors, which could explain the optimized effect of KXS in a herbal decoction. The KXS-induced expression of neurotrophic factors did not depend on signaling mediated by estrogen receptor or protein kinase. The results suggested that the antidepressant-like action of KXS might be mediated by an increase of expression of neurotrophic factors in astrocytes, which fully supported the clinical usage of this decoction.

21. **Chemical fingerprinting and quantitative analysis of two common Gleditsia sinensis fruits using HPLC-DAD**

J.P. Chen,Z.G. Li, K.Y.Z. Zheng, A.J.Y. Guo, K.Y. Zhu, W.L. Zhang, J.Y.X. Zhan, T.T.X. Dong, Z.R. Su, K.W.K. Tsim

Acta Pharm, 2013, 63, 505–515.

Abstract

Gleditsiae Fructus Abnormalis and Gleditsiae Sinensis Fructus are obtained from different developmental stages of fruits from Gleditsia sinensis Lam. (Leguminosae). The possible interchangeable usage of the two fruits, however, has long been very controversial. Here, high performance liquid chromatography coupled with diode array detection was developed to explore their chemical fingerprinting profiles. Besides, the amounts of aglycones of saponin compounds, echinocystic acid and oleanolic acid in both fruits were quantified. The results indicated that there was no significant difference in the content of aglycones from the two types of fruits. However, their chromatographic fingerprints showed distinct characteristics. Therefore, the interchangeable application of these fruits has to be taken with a specific precaution.

22. **Analysis of HPLC fingerprints and determination of isofraxidin and rosmarinic acid of Sarcandrae Herba from Sarcandra glabra (Thunb.) Nakai**

R.W.L. Tang, Y.Y. Ng, D. Bi, R. Duan, K.W.K. Tsim, T.X. Dong

China Pharmacy, 2013, 47

Abstract

OBJECTIVE: To determine the contents of isofraxidin and rosmarinic acid, and establish fingerprints to assess the quality of Sarcandrae Herba from Sarcandra glabra (Thunb.) Nakai, which is in order to establish the quality evaluation of Sarcandrae Herba. Methods: HPLC fingerprint and content determination were applied to evaluate ten batches of Sarcandrae Herba from Sarcandra glabra (Thunb.) Nakai · For the determination of isofraxidin and rosmarinic acid, the samples were separated by Inertsil ODS-4 column (250 mm × 4.6 mm id. 5 μm) using acetonitrile and 0.1% phosphoric acid gradient system as mobile phase · The flow rate was 1.0 ml•min-1, and the detection

wavelength was at 342 nm. Results: HPLC fingerprint of Sarcandrae Herba was established with good separation and repeatability, which could be used for quality assessment of Sarcandrae Herba. The results showed that the HPLC fingerprints are similar among the ten batches. The contents of rosmarinic acid from ten batches were obviously different from each other. Conclusion：The method is sensitive, repeatable and accurate, it can be used as quality control for Sarcandrae Herba ·

23. Study on Quality Standard of Andrographis Herba

L.J. Liang, K.J. Zhao, T.X. Dong, K.W.K. Tsim

Chinese Journal of Information on Traditional Chinese Medicine, 2013, 9, 63-65

Abstract

Objective: To improve the quality standard of Andrographis Herba through determination of effective components,moisture,total ashes,acid insoluble ashes,extracts and heavy metals.Methods TLC and HPLC were used for qualitative and quantitative identification of andrographolide and dehydroandrograpolide in Andrographis Herba.Routine examinations were based on the procedures recorded in the Appendix Ⅸ A,Ⅸ H,Ⅸ K,ⅩA and Ⅸ E of Chinese Pharmacopoeia(2010),for foreign matter,moisture,ashes,extracts determination and heavy metal test respectively.Results Total content of andrographolide and dehydroandrographolide,extractives(70% ethanol) all complied with Chinese pharmacopoeia.Conclusion The established method was simple,accurate and can be used as the quality standard for the quality control of Andrographis Herba.

24. HPLC determination of 5-heneicosylresorcinol and linoleic acid in Fructus Tritici Levis

M.J. Gao, D. Bi, W.L. Tang, C. Xu, T.X. Dong, K.W.K. Tsim

China Pharmacy, 2013, 3

Abstract

To develop an HPLC method for determination of 5-heneicosyresorcinol and linoleic acid in Fructus Tritici Levis

Methods: The separation was carried out on Phenomenex Luna C18（250 mm x4.6, 5 μm）column. The moblile phase was acetonitrile and phosphoric acid in aqueous solution (0.1%) with gradient elution. The flow rate of mobile phase was maintained at 1.0 mL·min-1. The detection wavelength was set at 210 nm.

Results: There were good linear relationships between the concentrations and peak areas of 5-heneicosyresorcinol and linoleic acid in the ranges of 2.83 – 113.2 μg/mL (r=0.9998)，17.55– 702.0 μg/mL (r=0.9997), respectively. The average recoveries (n=6) were 97.0% and 98.2%, and RSDs were 1.8% and 2.5%, respectively.

Conclusion: This method is simple, precise, reliable and can be used for the quality control of Fructus Tritici Levis.

25. **Pharmacognostical identification of Allii Tuberosi Semen**

H. Xu, T.X. Dong, Y.C. Lam, W.L. Tang, K.W.K. Tsim, Z.T. Wang

China Pharmacy, 2013, 3

Abstract

To establish the pharmacognostic identification and authentication method of Allii Tuberosi Semen. METHODS: The methods of macroscopic, microscopic examination and thin layer chromatography (TLC) were employed here to authenticate Allii Tuberosi Semen.

RESULTS: Based on the macroscopic identification, the features of transverse section and powder of Allii Tuberosi Semen were described in detail. The digital photographs were presented here in revealing the main macroscopic and microscopic characteristic of Allii Tuberosi Semen. Thin layer chromatography method was established to identify the crude drugs of Allii Tuberosi Semen. CONCLUSIONS: The authenticated method is sample and intuitive, and which provides the basis for further establishment of quality control of Allii Tuberosi Semen.

26. **Determination of Galactitol in Cistanche deserticola and Cistanche tubulosa by HPLC-ELSD**

K.J. Zhao, L.J. Liang, D. Bi, T.X. Dong, K.W.K. Tsim

Chinese Journal of Information on Traditional Chinese Medicine; 2012, 8, 52-54

Abstract

Objective: To establish a method for the determination of galactitol in Cistanche deserticola and Cistanche tubulosa,and to provide evidence for the quality control of Cistanches Herba. Methods: The contents of galactitol in 20 batches samples of Cistanche deserticola and Cistanche tubulosa were determined by HPLC-ELSD.The samples were separated by Alltech Pevail Carbohydrate ES column(4.6 mm×250 mm,5 μm) with acetonitrile-water(80:20) as mobile phase.The flow rate was 1.0 mL/min and the injection volume was 10 μL.Shift tube temperature of ELSD was set at 80 ℃ and the gas flow rate was 3.0 L/min. Results: The linear range of galactitol was 89.6-1 120 mg/L(r=0.999 0),method detection limit was 15.998 mg/L,limit of quantitation was 79.903 mg/L.The average recovery rate of galactitol in Cistanche deserticola and Cistanche tubulosa was 100.34% and 101.11% respectively with RSD of 1.72% and 1.33 %(n=5). Conclusion: The method is sensitive,r epeatable and accurate,which can be used for the quality control of Cistanches Herba.

27. **Analysis of HPLC fingerprints of Fructus Aurantii from different habitats and contents of naringin, neohesperidin and synephrine**

K.J. Zhao, Y.Z. Zheng, T.T.X. Dong, K.W.K. Tsim

Chinese Pharmaceutical Journal, 2011, 46, 955-959

Abstract

OBJECTIVE To establish the HPLC fingerprints for assessing the quality of Fructus Aurantii from different habitats, and to determine the contents of naringin, neohesperidin and synephrine.

METHODS HPLC fingerprinting and content determination methods were applied to evaluate 10 batches of Fructus Aurantii from different habitats. The samples were separated by Alltima C18 column (4.6 mm x 250 mm, 5 μm) using acetonitrile 0.1% phosphoric acid and 0.1% SDS water solution as mobile phase with gradient elution. The flow rate was 1.0 mL · min- 1. The detection wavelengths were 224 nm for fingerprinting, 283 nm for content determination of naringin and neohesperidin, and 224 nm for content determination of synephrine, respectively.

RESULTS HPLC fingerprint of Fructus Aurantii was established with good separation and repeatability, which could be used for quality assessment of Fructus Aurantii. Six common peaks were defined in characteristic fingerprints, and similarity evaluation system was applied to evaluate the fingerprints of the 10 batches of Fructus Aurantii. The contents of naringin, neohesperidin and synephrine in Fructus Aurantii from different habitats were obviously different from each other.

CONCLUSION The method is sensitive, repeatable and accurate, which can be used for the quality control of Fructus Aurantii. The contents of naringin, neohesperidin and synephrine vary significantly in Fructus Auranti from different habitats, which suggests that more attention should be paid to the clinical use of Fructus Aurantii.

Acknowledge to the support of Department of Health

28. ## Study on Determination of Indigo and Indirubin Contents and HPLC Fingerprints of Isatidis Folium from Various Sources

Y.X. Zhan, Q. Fu, D. Ran, D.T.W. Lau, Y.Z. Zheng, Y. Zhu, W.C. Bi, T.X. Dong, K.W.K. Tsim

Modern Chinese Medicine, 2011, 13, 15-18

Abstract

Objective: To determine the contents of indigo and indirubin and establish fingerprints of Isatidis Folium from various sources in order to control their qualities. Methods: We selected 17 batches of Isatidis Folium from various sources. The contents of indigo and indirubin were determined and the fingerprints were established by high performance liquid chromatography (HPLC) method. The fingerprints were compared using similarity evaluation software published by the Committee of China Codex. Results: The indigo content in Polygonum Folium was higher than that in Isatis Folium while its indirubin content was lower than that in Folium Isatis. Indigo and indirubin were not detected in the leaves of Baphicacanthus cusia and Clerodendron cyrtophyllum. The contents of indigo and indirubin in Isatidis Folium from different sources are varying largely. Conclusion: The above methods have desirable precision, reproducibility, stability, providing an experimental basis for quality control of Folium Isatidis.

Acknowledge to the support of Department of Health

29. **Quality evaluation of Radix Glehniae (Glehnia littoralis) by HPLC-DAD chromatographic fingerprinting and quantitative analysis of the herbs from different regions of China**

J. Li, A.W.H. Cheung, C.W.C. Bi, R. Duan, K.Y.Z. Zheng, W. Huang, J.J. Chen, T.T.X. Dong, K.W.K. Tsim

Asian Journal of Traditional Medicines, 2010, 5, 40-48

Abstract

High performance liquid chromatography coupled with diode array detection (HPLC-DAD) was developed to evaluate the qualityof Radix Glehniae. Chromatographic fingerprints and the amounts of coumarins and polyacetylenes were determined in Radix Glehniae collected from different regions of China. The analysis was carried out on a Prevail C18 analytical column (250 mm ×4.6 mm id. 5 μm) using linear gradient elution of acetonitrile-water. The correlation coefficients of similarity were determined and twelve common peaks were defined from the HPLC fingerprints: five of the peaks were identified as psoralen, imperatorin, isoimperatorin, falcarindiol and falcarinol. The contents of these five identified compounds were subsequently determined by the validated HPLC-DAD method with high sensitivity, precision, repeatability and accuracy. This newly developed method involving a combination of chromatographic fingerprinting and quantitative analysis is suitable for the quality of Radix Glehniae.

Acknowledge to the support of Department of Health

30. **Quality evaluation of Rhizoma Belamcandae (Belamcanda chinensis (L.) DC.) by using high-performance liquid chromatography coupled with diode array detector and mass spectrometry**

J. Li, W.Z.M. Li, W. Huang, A.W.H. Cheung, C.W.C. Bi, R. Duan, A.J.Y. Guo, T.T.X. Dong, K.W.K. Tsim

Journal of Chromatography A, 2009, 1216, 2071-2078

Abstract

A high-performance liquid chromatography coupled with diode array detector and mass spectrometry (HPLC-DAD-MS) method was developed to evaluate the quality of Rhizoma Belamcandae (Belamcanda chinensis (L.) DC.) through establishing chromatographic fingerprint and simultaneous determination of seven phenolic compounds. The analysis was achieved on an Alltima C(18) analytical column (250 mm x 4.6 mm i.d. 5 microm) using linear gradient elution of acetonitrile-0.1% trifluoroacetic acid. The correlation coefficients of similarity were determined from the HPLC fingerprints, and they shared a close similarity. By using an online APCI-MS/MS, twenty phenols were identified. In addition, seven of these phenols including mangiferin, 7-O-methylmangiferin, tectoridin, resveratrol, tectorigenin, irigenin and irisflorentin were quantified

by the validated HPLC-DAD method. These phenols are considered to be major constituents in Rhizoma Belamcandae, and are generally regarded as the index for quality assessment of this herb. This developed method by having a combination of chromatographic fingerprint and quantification analysis could be applied to the quality control of Rhizoma Belamcandae.

Acknowledge to the support of Department of Health

31. **Simultaneous determination of phenols in Radix Polygalae by high performance liquid chromatography: quality assurance of herbs from different regions and seasons.**

J. Li, X.B. Dong, Y. Jiang, Q.T. Gao, Z.Y. Jiang, A.W.H. Cheung, R. Duan, T.T.X. Dong, P.F. Tu, K.W.K. Tsim

Journal of separation science, 2007, 30, 2583-2589

Abstract

Radix Polygalae, roots of Polygala tenuifolia or of Polygala sibirica, is a Chinese herbal medicine commonly used to prevent dementia. Reliable chemical markers for quality assurance of this herb are missing. Here, a high performance liquid chromatography method coupled with diode array detection was developed to simultaneously determine nine different phenols in Radix Polygalae, including sibiricose A(5), sibiricose A(6), glomeratose A, tenuifoliside A, glomeratose D, 3',6-di-O-sinapoyl sucrose ester, mangiferin, polygalaxanthone III, and polygalaxanthone XI. By using two different detection wavelengths in the HPLC analysis, the developed method was able to determine the phenols with excellent resolution, precision, and recovery. This established method was therefore applied to determine the amounts of phenols in thirty-two samples from different cultivation regions and harvest seasons in China, and significant variations were revealed. The amounts of phenols in the roots of P. tenuifolia collected in Shanxi and Shannxi Provinces were markedly higher than in roots collected from other Provinces. Moreover, the samples harvested in the spring contained higher contents of phenols than those collected in other seasons.

Acknowledge to the support of Department of Health

32. **Molecular genetic and chemical assessment of Rhizoma Curcumae in China.**

Q. Xia, K.J. Zhao, Z.G. Huang, P. Zhang, T.T.X. Dong, S.P. Li, K.W.K. Tsim

Journal of agricultural and food chemistry, 2005, 53, 6019-6026

Abstract

Rhizoma Curcumae (Ezhu) is a traditional Chinese medicine that has been used in removing blood stasis and alleviating pain for over a thousand years. Three species of Curcuma rhizomes are being used, which include Curcuma wenyujin, Curcuma phaeocaulis, and Curcuma kwangsiensis. In

China, the production of Rhizoma Curcumae largely depends on agricultural farming. The essential oils are considered as active constituents in Rhizoma Curcumae, which include curdione, curcumol, and germacrone. On the basis of the yield of curdione, curcumol, and germacrone in an orthogonal array design, the optimized extraction condition was developed. The amounts of these compounds within essential oils in Rhizoma Curcumae varied according to different species and their regions of cultivation. Chemical fingerprints were generated from different species of Curcuma, which therefore could serve as identification markers. In molecular genetic identification of Rhizoma Curcumae, the 5S-rRNA spacer domains of 5 Curcuma species, including the common adulterants of this herb, were amplified, and their nucleotide sequences were determined. Diversity in DNA sequences among various species was found in their 5S-rRNA spacer domains. Thus, the chemical fingerprint together with the genetic distinction could serve as markers for quality control of Curcuma species.

Acknowledge to the support of Department of Health