

1. Prenylated Isoflavonoids-Rich Extract of Erythrinae Cortex Exerted Bone Protective Effects by Modulating Gut Microbial Compositions and Metabolites in Ovariectomized Rats

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Nutrients, 2021,13, 2943

Abstract

Flavonoids, found in a wide variety of foods and plants, are considered to play an important role in the prevention and treatment of osteoporosis. Our previous studies demonstrated that Erythrina cortex extract (EC) rich in prenylated isoflavonoids exerted bone protective effects in ovariectomized (OVX) rats. The present study aimed to investigate the interactions of gut microbiota with the EC extract to explore the underlying mechanisms involved in its beneficial effects on bone. Sprague-Dawley female rats of 3-months-old were ovariectomized and treated with EC extract for 12 weeks. EC extract reversed ovariectomy-induced deterioration of bone mineral density and bone microarchitecture as well as downregulated cathepsin K (Ctsk) and upregulated runt-related transcription factor 2 (Runx2) and alkaline phosphatase (ALP) in the tibia of OVX rats. Its protective effects on bone were correlated with changes in microbial richness and the restorations of several genera. EC increased the serum circulating levels of acetate and propionate in OVX rats. We conclude that the bone protective effects of EC extract were associated with the changes in microbial compositions and serum short chain fatty acids (SCFAs) in OVX rats.

2. The lignan-rich fraction from Sambucus Williamsii Hance ameliorates dyslipidemia and insulin resistance and modulates gut microbiota composition in ovariectomized rats

H. H. Xiao, L. Lu, C. C. W. Poon, C. O. Chan, L. J. Wang, Y. X. Zhua, L. P. Zhou, S.S. Cao, W. X. Yu, K. Y. Wong, D. K. W. Mok, S. Chen, M.S. Wong

Biomedicine and Pharmacotherapy, 2021,137,111372

Abstract

Menopausal women are susceptible to have high risk of cardiovascular diseases, type II diabetes and osteoporosis due to the metabolic disorder caused by estrogen deficiency. Accumulating evidence supports that gut microbiota is a key regulator of metabolic diseases. Our previous metabolomics study interestingly demonstrated that the anti-osteoporotic effects of lignan-rich fraction (SWCA) from Sambucus wialiamsii Hance were related to the restoration of a series of lipid and glucose metabolites. This study aims to investigate how SWCA modulates lipid and glucose metabolism and the underlying mechanism. Our results show that oral administration of SWCA (140

mg/kg and 280 mg/kg) for 10 weeks alleviated dyslipidemia, improved liver functions, prevented glucose tolerance and insulin actions, attenuated system inflammation and improved intestinal barrier in OVX rats. It also induced a high abundance of Actinobacteria, and restored microbial composition. We are the first to report the protective effects of the lignan-rich fraction from *S. williamsii* on dyslipidemia and insulin resistance. Our findings provide strong evidence for the application of this lignan-rich fraction to treat menopausal lipid disorder and insulin resistance-related diseases.

3. Altered metabolites in guinea pigs with allergic asthma after acupoint sticking therapy: New insights from a metabolomics approach

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Phytomedicine, 2019, 54, 182-194

Abstract

Background: Clinical evidence gathered in Chinese communities suggested that acupoint sticking therapy could be an alternative treatment for asthma-related diseases. However, its underlying mechanism is still poorly understood.

Aim/hypothesis: In this study, we aimed to investigate the mechanism of the anti-inflammatory effect of acupoint sticking application with 'Treatment of Winter Disease in Summer' (TWDS) prescription by using metabolomics.

Methods: Allergic asthma in guinea pig was sensitized and challenged by ovalbumin (OVA). Histopathological evaluation of the lung tissue was performed by hematoxylin and eosin (H&E) staining and Masson's trichrome staining. The levels of Th2 cytokine and IgE level in serum were measured using enzyme-linked immunoassay (ELISA). The mRNA expression levels of IL-4, IL-5, IL-13 and orosomucoid-like 3 (ORMDL3) were measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR). Proteins of NF- κ B signaling pathway were measured using western blot. The serum metabolomics profiles were obtained by using ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS).

Results: The overall results confirmed that AST with TWDS prescription had a significant protective effect against OVA-induced allergic asthma in guinea pig. This treatment not only attenuated airway inflammation and collagen deposition in the airway, but also decreased the levels of IL-4, IL-5, IL-13 and IgE in serum. In addition, metabolomics results indicated that metabolisms of phospholipid, sphingolipid, purine, amino acid and level of epinephrine were restored back to the normal control level. Moreover, results of the gene expression of ORMDL3 in lung tissues indicated that AST using TWDS could alter the sphingolipid metabolism. Further western blotting analysis also showed that its anti-inflammatory mechanism was by decreasing the phosphorylation of p65 and I κ B.

Conclusion: The study demonstrated that metabolomics provides a better understanding of the

4. Qualitative and quantitative analysis of sesquiterpene lactones in *Centipeda minima* by UPLC-Orbitrap-MS & UPLC-QQQ-MS

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Journal of Pharmaceutical and Biomedical Analysis, 2019, 174, 360-366

Abstract

An ultra-high performance liquid chromatography coupled with high-resolution Orbitrap mass spectrometry (UPLC-Orbitrap-MS) method has been used to identify sesquiterpene lactones in the methanolic extract of *Centipeda minima*. Fifteen sesquiterpene lactones were tentatively identified based on retention time and accurate mass of external standards or exact accurate mass searching (within 2 ppm) by comparison of some previous isolated sesquiterpene lactones. Meanwhile, a rapid, sensitive, precise, and reliable ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-QQQ-MS) method has been developed to evaluate the quality of *Centipeda minima* through a simultaneous determination of five sesquiterpene lactones, namely brevilin A, arnicolide C, arnicolide D, microhelenin C, minimolide F. Chromatographic separation was achieved on a Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) with a mobile phase consisting of a) 0.1% formic acid and b) a mixture of acetonitrile and methanol 50:50 v/v under an isocratic elution (42:58) manner. Positive electrospray ionization mode with multiple reaction monitoring was applied for the detection of the five sesquiterpene lactones. Method validation for linearity, accuracy and precision was also carried out. Finally, the method was successfully used for the analysis of 10 batches of *Centipeda minima* samples collected in China. Brevilin A and arnicolide D were the dominant sesquiterpene lactones in *Centipeda minima* and could be proposed as suitable markers for the quality control of *Centipeda minima*.

5. A Metabolomics Study on the Bone Protective Effects of a Lignan-Rich Fraction From *Sambucus Williamsii* Ramulus in Aged Rats

H. H. Xiao, T. T. Sham, C. O. Chan, M. H. Li, X. Chen, Q. C. Wu, D. K.W. Mok, X.S. Yao, M. S. Wong

Frontiers in Pharmacology, 2018, 9, 932

Abstract

The lignan-rich fraction (SWR) of *Sambucus Williamsii* Ramulus, a folk herbal medicine in China for treatment of bone diseases, has previously reported to exert protective effects on bone without exerting uterotrophic effects in ovariectomized (OVX) mice. The aim of the present study was to identify the potential metabolites and the associated metabolic pathways that contribute to the beneficial effects of SWR on bone in vivo. Aged female Sprague Dawley rats (9 months old) were either sham-operated or ovariectomized for 12 weeks, before receiving treatment for another 12

weeks with the following treatment groups ($n = 12$ each): vehicle (Sham), vehicle (OVX), Premarin (130 $\mu\text{g}/\text{kg}$) or low (57 mg/kg), medium (114 mg/kg), and high (228 mg/kg) doses of SWR. The results showed that SWRH significantly suppressed bone loss, improved bone micro-architecture and increased bone strength on tibia without stimulating uterus weight gain in OVX rats. Premarin exerted similar bone protective effects as SWRH but elicited uterotrophic effects in OVX rats. The metabolic profiles of serum samples were analyzed by using ultra-performance liquid chromatography quadrupole time-of flight mass spectrometry and gas chromatography time-of flight mass spectrometry, and the metabolites that were significantly altered were identified by multivariate statistical analysis. Our study indicated that SWRH effectively restored the changes of 26 metabolites induced by estrogen-deficiency in OVX rats, which related to lipids, amino acids, tryptophan metabolisms, and anti-oxidative system. A subsequent validation showed that the serum level of superoxide dismutase and catalase were indeed up-regulated, while the serotonin level in a tryptophan hydroxylase 1 (TPH1) high expressing cells (rats RBL-2H3 cells) was down regulated after treatment with SWR. The results also suggested that the gut-microbiota may play an important role on the bone protective effects of SWR. The current study provides insight for understanding the unique mechanism of actions of SWR that might be involved in achieving bone protective effects in vivo.

6. Chemical Analysis of Astragali Complanati Semen and Its Hypocholesterolemic Effect Using Serum Metabolomics Based on Gas Chromatography-Mass Spectrometry

T.T. Sham, H. Zhang, D.K.W. Mok, S.W. Chan, J. Wu, S. Tang, C.O. Chan

Antioxidants, 2017, 6, 57

Abstract

The hypocholesterolemic protective effect of the dried seed of *Astragalus complanatus*(ACS) was investigated in rats fed with normal diet, high cholesterol diet (HCD), and HCD plus 70% ethanol extract of ACS (600 $\text{mg}/\text{kg}/\text{day}$) by oral gavage for four weeks. ACS extract was tested to be rich in antioxidants, which may be contributed to its high content of phenolic compounds. Consumption of ACS remarkably suppressed the elevated total cholesterol ($p < 0.01$) and LDL-C ($p < 0.001$) induced by HCD. Chemical constituents of ACS extract were analyzed by ultra-performance liquid chromatography coupled with electrospray ionization orbitrap mass spectrometry and the results showed that the ACS extract mainly consisted of phenolic compounds including flavonoids and flavonoid glycosides. In addition, based on the serum fatty acid profiles, elucidated using gas chromatography-mass spectrometry, free and esterified fatty acids including docosapentaenoic acid, adrenic acid, dihomo--linolenic acid and arachidonic acid were regulated in ACS treatment group. Western blot results further indicated the protein expression of peroxisome proliferator-activated receptor alpha (PPAR) ($p < 0.05$) in liver was upregulated in ACS treatment group. To conclude, our results clearly demonstrated that ACS provides beneficial effect on lowering HCD associated detrimental change.

7. Novel cycloartane triterpenoid from *Cimicifuga foetida* (Sheng ma) induces mitochondrial apoptosis via inhibiting Raf/MEK/ERK pathway and Akt phosphorylation in human breast carcinoma MCF-7 cells

H.Y. Sun, B.B. Liu, J.Y. Hu, L.J. Xu, S.W. Chan, C.O. Chan, D.K.W. Mok, D.M. Zhang, W.C. Ye, S.B. Chen

Chinese Medicine, 2016, 11:1

Abstract

Background: Cycloartane triterpenoids exhibited anticancer effects. This study aims to identify any potential novel anticancer cycloartane triterpenoids from *Cimicifuga foetida* L. rhizome (Sheng ma) and the mode of actions.

Methods: Cycloartane triterpenoids were isolated from the *C. foetida* rhizome by a series of column chromatography and identified by IR, MS and NMR. Their anticancer effects on several human cancer cell lines, MCF-7, HepG2, HepG2/ADM, HeLa, and PC3, and normal human mammary epithelial cells MCF10A were investigated by colony formation and MTT assays. Morphological analysis of apoptosis induction was performed by acridine orange/ethidium bromide dual staining and Hoechst 33258 nuclear staining. The cell cycle profile and annexin V staining were evaluated by flow cytometry. Apoptosis were investigated by measuring changes in mitochondrial membrane potential and analyzing expression of cell cycle- and apoptosis related proteins in MCF-7 cells by Western blotting.

Results: A novel cycloartane triterpenoid, 25-O-acetyl-7,8-didehydrocimigenol-3-O-β-D-(2-acetyl)xylopyranoside (ADHC-AXpn), together with the known 7,8-didehydrocimigenol-3-O-β-D-xylopyranoside (DHC-Xpn) were isolated. MCF-7 growth was significantly inhibited by ADHC-AXpn in a dose- and time dependent manner (IC₅₀: 27.81 μM at 48 h; P = 0.004 vs. control at 25 μM for 48 h treatment), and ADHC-AXpn was selectively cytotoxic for cancerous cells (MCF-7, HepG2/ADM, HepG2 and HELA cells) based on its higher IC₅₀ values for normal cells MCF10A (IC₅₀: 78.63 μM at 48 h) than for tumor cells. In MCF-7 cells, ADHC-AXpn induced G₂/M cell cycle arrest by mediating cyclin B1, and CDK1 and its phosphorylation; and induced apoptosis through the mitochondrial-mediated apoptotic pathway, with inhibition of Akt activation. As ADHC-AXpn suppressed phosphorylation of ERK1/2, Raf and Akt proteins in MCF-7 cells, its apoptotic effect might be associated with Raf/MEK/ERK signaling and Akt activation.

Conclusions: ADHC-AXpn significantly suppressed the growth of MCF-7 cells, induced mitochondrial apoptosis and cell cycle arrest, and inhibited Raf/MEK/ERK signaling pathway and Akt phosphorylation.

8. *Centipeda minima* (Ebushicao) extract inhibits PI3K-Akt-mTOR signaling in nasopharyngeal carcinoma CNE-1 cells

Y.Q. Guo, H.Y. Sun, C.O. Chan, B.B. Liu, J.H. Wu, S.W. Chan, D.K.W. Mok, A.K.WE.Tse, Z.L. Yu, S.B. Chen

Chinese Medicine, 2015, 10:26

Abstract

BACKGROUND:

Centipeda minima (Ebushicao) has been used for the treatment of various diseases, such as nasal allergies, rhinitis and sinusitis, nasopharyngeal carcinoma, cough, and headache. This study aims to investigate the anticancer activities of *Centipeda minima* ethanol extracts (CME) against nasopharyngeal carcinoma cell CNE-1 and their underlying mechanism.

METHODS:

CNE-1 cells were treated with different concentrations (15-50 $\mu\text{g}/\text{mL}$) of CME for different time intervals (24, 48, and 72 h). Cytotoxicity of CME was determined by MTT assay. Cell morphological changes were observed by fluorescence microscopy after HO 33258 staining. Cell cycle status was evaluated by flow cytometry following propidium iodide staining. Apoptosis was detected by flow cytometry following annexin V-FITC/PI staining. The levels of apoptosis-associated and PI3K-Akt-mTOR signaling related proteins were measured by western blotting analysis.

RESULTS:

CME (15-50 $\mu\text{g}/\text{mL}$) significantly inhibited the proliferation of CNE-1 in a dose- and time-dependent manner ($P = 0.026$ for 15 $\mu\text{g}/\text{mL}$, $P < 0.001$ for 25, 30, 40, and 50 $\mu\text{g}/\text{mL}$, respectively); the IC50 values ($\mu\text{g}/\text{mL}$) were 41.57 ± 0.17 , 30.34 ± 0.06 and 24.98 ± 0.08 for 24, 48 and 72 h treatments, respectively. Significant morphological changes of CNE-1 cells displaying apoptosis were observed after CME treatment. CME showed low cytotoxicity toward normal LO2 cells. CNE-1 cells were arrested in the G2/M phase while treated with 15, 25, 40 $\mu\text{g}/\text{mL}$ of CME, respectively ($P = 0.032$, $P = 0.0053$, $P < 0.001$). CME (15, 25, 40 $\mu\text{g}/\text{mL}$) down-regulated Bcl-2 expression ($P = 0.032$, $P = 0.0074$, $P < 0.001$), and up-regulated Bax ($P = 0.026$, $P = 0.0056$, $P < 0.001$) with activation of caspase-3, caspase-8, caspase-9, and PARP observed in CNE-1 cells ($P = 0.015$, $P = 0.0067$, $P < 0.001$ for caspase 3; $P = 0.210$, 0.028 , < 0.001 for caspase 8; $P = 0.152$, 0.082 , 0.0080 for caspase 9; $P = 0.265$, 0.0072 , < 0.001 for PARP). CME suppressed the activation of the PI3K-AKT-mTOR pathway ($P = 0.03$, 0.0007 , 0.004 , 0.006 , 0.022 for p-PI3K, p-Akt-Ser(473), p-Akt-Thr(308), p-mTOR-Ser(2448), p-mTOR-Ser(2481), respectively after 40 $\mu\text{g}/\text{mL}$ of CME treated for 24 h).

CONCLUSION:

CME inhibited the proliferation of CNE-1 cells and activation of the PI3K-AKT-mTOR signaling pathway.

9. Identifying bioactive components in natural products through chromatographic fingerprint

J. Xu, Q.S. Xu, C.O. Chan, D.K.W. Mok, L.Z. Yi, F.T. Chau

Analytica Chimica Acta, 2015, 870, 45-55

Abstract

Bioactive component identification is a crucial issue in search for new drug leads. We provide a new strategy to search for bioactive components based on Sure Independence Screening (SIS) and interval PLS (iPLS). The method, which is termed as SIS-iPLS, is not only able to find out the chief bioactive components, but also able to judge how many components should be there responsible for the total bioactivity. The method is totally "data-driven" with no need for prior knowledge about the unknown mixture analyzed, therefore especially suitable for effect-directed work like bioassay-guided fractionation. Two data sets, a synthetic mixture system of twelve components and a suite of Radix Puerariae Lobatae extracts samples, are used to test the identification ability of the SIS-iPLS method.

10. Discrimination between Leave of Apocynum venetum and Its Adulterant, A. pictum Based on Antioxidant Assay and Chemical Profiles Combined with Multivariate Statistical Analysis

C.O. Chan, C.C. Lau, Y.F. Ng, L.J. Xu, S.B. Chen, S. W. Chan and D. K.W.M

Antioxidants, 2015, 4, 359-372

Abstract

An integrated approach including chemical and biological assessments was developed to investigate the differences between Apocynum venetum L. (AV) and its adulterant, Apocynum pictum Schrenk (AP). Ten flavonoids were tentatively identified by ultra-visible and mass spectra data. The chemical component, hyperoside, was identified as a critical parameter for discrimination of two species from the results of principal component analysis (PCA) and quantitative analysis. The anti-oxidative power of the herbal extracts were determined using 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay and H₂O₂-induced cell damage on LO₂ cells. The results of the biological assays suggested that the chemical differences between AV and AP do lead to difference in activity and AV is demonstrated to have higher anti-oxidant activity.

11. Semen Astragali Complanati: An Ethnopharmacological, Phytochemical and Pharmacological Review

Y.F. Ng, P.C.T. Tang, T.T. Sham, W.S. Lam, D.K.W. Mok, and S.W. Chan

Journal of Ethnopharmacology, 2014, 155, 39-53

Abstract

Ethnopharmacological relevance: Semen Astragali Complanati (SAC), the dried ripe seed of Flatstem Milkvetch (Astragalus complanatus Bunge) (Leguminosae), is commonly used in traditional Chinese medicine (TCM) for treating muscle, liver, kidney, blood, skin and reproductive system diseases. Materials and methods: Relevant information about SAC was gathered via "Google Scholar", "ISI Web of Knowledge", "PubMed", "ScienceDirect", "Medline Plus", "ACS", "CNKI" and "Wiley Online Library" and from books in local libraries. Results: The major contents of SAC include fatty acids, amino acids, polysaccharides, flavonoids, triterpene glycosides and trace elements. Previous scientific studies have reported that SAC exhibits a number of therapeutic effects on chronic diseases such as cardiovascular diseases, diabetes mellitus and cancers. It has been found that flavonoids are the main bioactive component in SAC. However most of the previous studies have shown the effects brought by the total flavonoid fraction extracted from SAC only; further studies are warranted for the biological effects produced by individual components. There are only a few studies on the toxicity of SAC and the overall results show that its toxicity is quite low or even non-existent. Conclusions: SAC is a valuable TCM herb with multiple pharmacological effects for treating some chronic diseases. More studies on SAC will help us to have a better understanding of its pharmacological mechanisms so as to provide more scientific evidence to explain its traditional uses, identify its therapeutic potential on other diseases and understand its possible harmful effects. Based on previous studies, it is easy to identify that antioxidant effect of SAC might play an important role on its pharmacological effects. Studying the effects of SAC on handling intracellular reactive oxygen species may be a potential direction to help understanding the molecular mechanisms of SAC on preventing and/or treating chronic diseases.

12. Petroleum ether extractive of the hips of Rosa multiflora ameliorates collagen-induced arthritis in rats

J.H. Wu, X.X. Liu, C.O. Chan, D.K.W. Mok, S.W. Chan, Z.L. Yu, S.B. Chen

Journal of Ethnopharmacology, 2014, 157, 45-54

Abstract

Ethnopharmacological relevance: The hip of Rosa multiflora Thunb. (HRM) has been traditionally used as a dietary supplement and a herbal remedy for the treatment of various diseases, including inflammation, osteoarthritis, rheumatoid arthritis and chronic pain, in China. The current study was to evaluate the therapeutic efficacy of the petroleum ether extractive of HRM (PEE) on type II collagen-induced rheumatoid arthritis (CIA) in male Wistar rats. In addition, the anti-inflammatory mechanism(s) of PEE on type II CIA was explored. Materials and methods: Rheumatoid arthritis (RA)

was induced by intradermal injection of bovine type II collagen on Day 1 and Day 8. Starting from Day 13, normal rats were treated with vehicle (serving as the control group); the CIA rats were treated with vehicle (CIA group), dexamethasone (0.25 mg/kg bw per day, p.o.) (a positive control), lei-gong-teng (LGT: 10 mg/kg bw per day, p.o.) (a clinically used Chinese patent medicine in RA therapy) or PEE (12, 36 or 120 mg/kg bw per day, p.o.) for 28 days. Results and conclusions: PEE (120 mg/kg bw per day) efficiently attenuated the severity of arthritis in the CIA rats by reducing the mean arthritis severity scores and the fore/hind paw swelling as well as reduced histological changes by decreasing the cartilage surface erosion and cartilage proteoglycan depletion. PEE's therapeutic effect in RA may involve the inhibition of pro-inflammatory cytokines, such as TNF-alpha, IL-1 beta and IL-6, in serum and/or the elevation of the activities of hepatic anti-oxidative enzymes including SOD, CAT and GSH-Px. However, the detailed anti-inflammatory mechanism, the main effective components and the interaction between different ingredients in PEE are still not clear and require more studies.

13.A Review on the Traditional Chinese Medicinal Herbs and Formulae with Hypolipidemic Effect

T.T. Sham, C.O. Chan, Y.H. Wang, J.M. Yang, D.K.W. Mok, S.W. Chan

Biomed Research International, Volume 2014 (2014), Article ID 925302, 21 pages

Abstract

Hyperlipidemia, characterized by the abnormal blood lipid profiles, is one of the dominant factors of many chronic diseases such as diabetes, obesity, and cardiovascular diseases (CVD). For the low cost, effectiveness, and fewer side effects, the popularity of using traditional Chinese medicine (TCM) to handle hyperlipidemia is increasing and its role in health care has been recognized by the public at large. Despite the importance of TCM herbs and formulations, there is no comprehensive review summarizing their scientific findings on handling hyperlipidemia. This review summarizes the recent experimental and clinical results of nine representative single Chinese herbs and seven classic TCM formulae that could improve lipid profiles so as to help understand and compare their underlying mechanisms. Most of single herbs and formulae demonstrated the improvement of hyperlipidemic conditions with multiple and diverse mechanisms of actions similar to conventional Western drugs in spite of their mild side effects. Due to increasing popularity of TCM, more extensive, well-designed preclinical and clinical trials on the potential synergistic and adverse side effects of herb-drug interactions as well as their mechanisms are warranted. Hyperlipidemic patients should be warned about the potential risks of herb-drug interactions, particularly those taking anticoagulants and antiplatelet drugs.

14. Gui-ling-gao (turtle jelly), a traditional Chinese functional food, exerts anti-inflammatory effects by inhibiting iNOS and pro-inflammatory cytokine expressions in splenocytes isolated from BALB/c mice

H. Zhang, M.Y. Wu, D.J. Guo, C.W. Wan, C.C. Lau, C.O. Chan, D.K.W. Mok, S.W. Chan

Journal of Functional Foods, 2013, 5, 625-632

Abstract

Gui-ling-gao (GLG), also known as turtle jelly, is a popular medicinal Chinese health/functional food prepd. from several traditional Chinese medicinal herbs. The present study aimed at examg. and explaining the anti-inflammatory properties of GLG by using lipopolysaccharide (LPS)-induced inflammation in splenocytes isolated from BALB/c mice. Addnl., the effects of GLG on mRNA and protein expressions of inducible nitric oxide synthase (iNOS) and pro-inflammatory cytokines in LPS-stimulated splenocyte proliferation were evaluated by real-time RT-PCR and Western blot assays. We demonstrated that GLG significantly inhibited LPS-induced splenocyte proliferation in a concn.-dependent manner. LPS-mediated up-regulations in the gene and protein expressions of iNOS, interleukin-1 β (IL-1 β) and tumor necrosis factor-alpha (TNF- α) were suppressed by GLG. These findings suggest for the first time that GLG exerts anti-inflammatory effects by inhibiting the expression of iNOS and pro-inflammatory cytokines such as IL-1 β and TNF- α . The current study provides strong scientific evidence for the health-beneficial claim that consumption of GLG could help the body to modulate the immune system

15. A Review of the Phytochemistry and Pharmacological Activities of Raphani Semen

T.T. Sham, A.C.Y. Yuen, Y.F. Ng, C.O. Chan, D.K.W. Mok*, S.W. Chan*

Evidence-Based Complementary and Alternative Medicine, Volume 2013 (2013), Article ID 636194, 16 pages

Abstract

*The dried ripe seed of *Raphanus sativus* L., commonly known as radish seed (or Raphani Semen), is used as traditional Chinese medicine (TCM) to treat constipation, chronic tracheitis, and hypertension. The major active compounds in Raphani Semen are alkaloids, glucosinolates, brassinosteroids, and flavonoids. Fatty acids are its main nutritional contents. Raphani Semen has been demonstrated to have beneficial effects on hypertension, obesity, diabetes mellitus, constipation, and cough. So far, there is no report about the adverse/toxic effects of this herb on humans. However, Raphani Semen processed by roasting was reported to exhibit some adverse effects on mice. Additionally, erucic acid, the main fatty acid in Raphani Semen, was shown to enhance the toxicity of doxorubicin. Thus, Raphani Semen has a potential risk of causing toxicity and drug interaction. In summary, Raphani Semen is a valuable TCM herb with multiple pharmacological effects. More studies on Raphani Semen could help better understand its pharmacological mechanisms so as to provide clear scientific evidence to explain its traditional uses, to identify its*

therapeutic potential on other diseases, and to understand its possible harmful effects.

16. Cholesterol lowering and vascular protective effects of ethanolic extract of dried fruit of *Crataegus pinnatifida*, hawthorn (Shan Zha), in diet-induced hypercholesterolaemic rat model

C.Y. Kwok, C. Li, H.L. Cheng, Y.F. Ng, T.Y. Chan, Y.W. Kwan, G.P.H. Leung, S.M.Y. Lee, D.K.W. Mok, P.H.F. Yu, S.W. Chan

Journal of Functional Foods, 2013, 5, 1326-1335

Abstract

Consumption of functional foods for managing plasma cholesterol level has gained acceptance globally. The hypocholesterolemic and vascular protective effects of the dried fruit of *Crataegus pinnatifida*, hawthorn (Shan Zha), were investigated in rats fed with normal diet, high cholesterol diet (HCD) or HCD plus Shan Zha 80% ethanolic ext. treatment (30 or 100 mg/kg/day, p.o.) for 4 wk. Shan Zha ext. markedly reversed the increased plasma total cholesterol and high d. lipoprotein cholesterol induced by HCD with a dose-dependent improvement on the atherogenic index. It also demonstrated good hepatoprotective function by reducing lipid content in the liver. The blunted endothelium-mediated aortic relaxation in HCD-fed rats was restored by high dosage of Shan Zha ext. treatment. The current results showed that Shan Zha ext. could provide its cholesterol lowering effect by up-regulating hepatic CYP7A1 mRNA expression which leads to enhanced bile acid biosynthesis. It is postulated that the hypocholesterolemic effect is the primary beneficial effect given by Shan Zha ext.; it then leads to other secondary beneficial effects such as vascular protective and hepatoprotective functions. Thus, Shan Zha ext. could provide an overall improvement on the hepatic and vascular systems that may be important in relieving hypercholesterolemia-related complications.

17. Fingerprint Analysis and Simultaneous Determination of Phenolic Compounds in Extracts of *Curculiginis Rhizoma* by HPLC-Diode Array Detector

Q.Y. Bian, H. Yang, C.O. Chan, D.P. Jin, D.K.W. Mok, S.B. Chen

Chemical and Pharmaceutical Bulletin, 2013, 61, 802-808

Abstract

Curculiginis Rhizoma (*Curculigo orchoides* GAERTN.) is a well-known Chinese herbal medicine, as well as an important Rasayana drug in India. Current criteria of quality control on this herb are to quant. analyze single compd. curculigoside, which fail to comprehensively evaluate quality of this herb. In this paper, a simple and reliable HPLC coupled with diode array detector (DAD) method was developed to evaluate the quality of *Curculiginis Rhizoma* through establishing chromatog. fingerprint and simultaneously quantitating 4 phenolic compds., orcinol glucoside, orcinol, 2,6-dimethoxybenzoic acid and curculigoside. The fingerprint displayed eleven common peaks, and

the similarity index of different samples was in a range of 0.890-0.977. Validation of the method was acceptable, with 96.03-102.82% accuracy in recovery test and inter and intra-day precisions were less than 2%. This developed method by having a combination of chromatog. fingerprint and quantitation anal. could be applied to the quality control of *Curculiginis Rhizoma*

18. Simultaneous Determination of Eight Anthraquinones in Semen Cassiae by HPLC-DAD

L.J. Xu, C.O. Chan, C.C. Lau, Z.L. Yu, D.K.W. Mok, S.B. Chen

Phytochemical Analysis, 2012, 23, 110-116

Abstract

Introduction - Semen Cassiae (SC), a traditional Chinese herbal medicine for the treatment of various diseases, is known to contain active anthraquinone ingredients. However, since the content of some anthraquinones is too low, previous analytical methods only allow the quantitation of a few anthraquinones or a hydrolysis step has to be included in the sample preparation. A rapid and accurate method to examine the content of as many anthraquinones as possible in SC would be desirable.

Acknowledge to the support of Department of Health

19. Development of an in-line HPLC fingerprint ion-trap mass spectrometric method for identification and quality control of Radix Scrophulariae

J. Jing, C.O. Chan, L.J. Xu, D.P. Jin, X.W. Cao, D.K.W. Mok, H.S. Parekh, S.B. Chen

Journal of Pharmaceutical and Biomedical Analysis, 2011, 56, 830-835

Abstract

Chromatographic fingerprinting has been widely accepted as a crucial method for qualitative and quantitative analyses of bioactives within traditional Chinese medicine. A fingerprint provides detailed information, specific for any given herb, thus facilitating the quality control measures of a given traditional Chinese medicine. In this article, quality assessment of Radix Scrophulariae was achieved by using high performance liquid chromatography combining diode-array detection and electrospray ionization mass spectrometry (HPLC-DAD-ESI/MS). Eight batches of sample obtained from different origins in China were used to establish the fingerprint and quantitative analyses. By comparing the retention times, UV and MS spectral data with reference standards, four characteristic peaks in the chromatograms were confirmed as corresponding to acetoside, angoroside C, cinnamic acid, and harpagoside. In addition, other two characteristic peaks were tentatively identified, following the literature interpretation of HPLC-ESI-MS and LC-MS/MS (affording structural information) to be sibirioside A and scrophuloside B(4), respectively. The results indicated that the

newly developed HPLC-DAD-MS fingerprint method would be suitable for quality control of *Radix Scrophulariae*.

20. Proteomic identification of differentially expressed proteins in curcumin-treated MCF-7 cells

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Phytomedicine, 2011, 18, 697-703

Abstract

Curcumin (CM), a well-known dietary pigment derived from *Curcuma longa* L., possess anticancer activities against a variety of tumors including human breast carcinoma. In combination with docetaxel, CM has been used in breast cancer management in the clinic. In order to explore the possible mechanism of anticancer activity of CM, in the present study, we aimed to identify proteins involved in the anticancer activity of CM in human breast cancer cell line MCF-7 using the two-dimensional electrophoresis (2-DE)-based proteomic analysis. MCF-7 cells were cultured at 37°C in an atmosphere of 5.0% CO₂. All the following experiments were repeated three times. Cell viability assay showed that after a 48-h incubation CM dose-dependently inhibited cell growth with an IC₅₀ value of 47.42μM. Treatment of CM at 47.42μM for 48h induced apoptosis as determined by nuclear morphologic changes of Hoechst stained cells and flow cytometric analysis of Annexin V-FITC/PI stained cells. Proteomic analysis identified 12 differentially expressed proteins which contributed to multiple functional activities such as DNA transcription, mRNA splicing and translation, amino acid synthesis, protein synthesis, folding and degradation, lipid metabolism, glycolysis, and cell motility. Among them 7 proteins were up-regulated and 5 down-regulated. The up-regulated ones were verified by quantitative real-time PCR. The down-regulated proteins, TDP-43, SF2/ASF and eIF3i, as well as up-regulated ones, 3-PGDH, ERP29, and platelet-activating factor acetylhydrolase IB subunit beta positively contribute to the anticancer activity of CM in MCF-7 cells. These molecules are implicated in the bioactivities of CM for the first time. The findings of this study would shed new insights for systematically understanding the mechanisms of CM in breast cancer intervention.

21. Rapid analysis of Radix puerariae by near-infrared spectroscopy

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Journal of Chromatography A, 2009, 1216, 2130-2135

Abstract

A new, rapid analytical method using near-infrared spectroscopy (NIRS) was developed to differentiate two species of *Radix puerariae* (GG), *Pueraria lobata* (YG) and *Pueraria thomsonii* (FG), and to determine the contents of puerarin, daidzin and total isoflavonoid in the samples. Five isoflavonoids, puerarin, daidzin, daidzein, genistin and genistein were analyzed simultaneously by high-performance liquid chromatography-diode array detection (HPLC-DAD). The total isoflavonoid content was exploited as critical parameter for successful discrimination of the two species. Scattering effect and baseline shift in the NIR spectra were corrected and the spectral features were enhanced by several pre-processing methods. By using linear discriminant analysis (LDA) and soft independent modeling class analogy (SIMCA), samples were separated successfully into two different clusters corresponding to the two GG species. Furthermore, sensitivity and specificity of the classification models were determined to evaluate the performance. Finally, partial least squares (PLS) regression was used to build the correlation models. The results showed that the correlation coefficients of the prediction models are $R = 0.970$ for the puerarin, $R = 0.939$ for daidzin and $R = 0.969$ for total isoflavonoid. The outcome showed that NIRS can serve as routine screening in the quality control of Chinese herbal medicine (CHM).

22. Seasonal Variations in the Isoflavonoids of Radix Puerariae

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Phytochemical Analysis, 2007, 18, 245-250

Abstract

The variations in isoflavonoid content in *Radix Puerariae*, the root of *Pueraria lobata* (Wild) Ohwi, have been examined in order to determine the optimum time to harvest the herb. Total isoflavonoid contents have been analysed by a UV spectrophotometric method whilst HPLC with photodiode array detection has been used to monitor the contents of seven major individual isoflavonoids, namely, 3'-hydroxypuerarin, puerarin, 3'-methoxypuerarin, daidzin, genistin, formononetin-7-glucoside and daidzein. Ninety-six samples of *Radix Puerariae* of different ages and harvested in different months were collected and analysed. The results clearly showed that 3-year-old roots harvested in January have the highest yields of isoflavonoid compounds. Moreover, the results obtained by both methods have a strong correlation. This suggested that the simple and fast UV spectrophotometric method could be used for monitoring the phytochemical quality of *Radix Puerariae*.

23. High-performance thin-layer chromatographic fingerprints of isoflavonoids for distinguishing between *Radix Puerariae Lobatae* and *Radix Puerariae Thomsonii*

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Journal of Chromatography A, 2006, 1121, 114–119

Abstract

The roots of Pueraria lobata (Wild.) Ohwi and Pueraria thomsonii Benth have been officially recorded in all editions of Chinese Pharmacopoeia under the same monograph 'Gegen' (Radix Puerariae, RP). However, in its 2005 edition, the two species were separated into both individual monographs, namely 'Gegen' (Radix Puerariae Lobatae, RPL) and 'Fenge' (Radix Puerariae Thomsonii, RPT), respectively, due to their obvious content discrepancy of puerarin, the major active constituent. In present paper, the fingerprint of high-performance thin-layer chromatography (HPTLC) combining digital scanning profiling was developed to identify and distinguish the both species in detail. The unique properties of the HPTLC fingerprints were validated by analyzing ten batches of Pueraria lobata and P. thomsonii samples, respectively. The common pattern of the HPTLC images of the roots of Pueraria spp. and the respective different ratios of the chemical distribution can directly discern the two species. The corresponding digital scanning profiles provided an easy way for quantifiable comparison among the samples. Obvious difference in ingredient content and HPTLC patterns of the two species questioned their bio-equivalence and explained that recording both species separately in the current edition of Chinese Pharmacopoeia (2005 edition) is reasonable due to not only the content of major constituent, puerarin, but also the peak-to-peak distribution in the fingerprint and integration value of the total components. Furthermore, the HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the subtle difference among samples with identical plant resource but different geographic locations