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论文集

Abstract Book

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NaoMaiTong Mediates Akkermansia muciniphila Dependent Regulation of Tryptophan-Serotonin Metabolism in the Brain-Gut Axis on Ischemic Stroke

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Abstract: Naomaitong (NMT) incorporates a combination of *Panax ginseng* C.A.Mey., *Rheum palmatum* L., *Pueraria lobata* (Willd.) Ohwi, and *Ligusticum chuanxiong* Hort, has been successfully employed in clinical practice for numerous years and has been scientifically validated for treating stroke in China. However, its mechanism is still to be explored in the brain-gut axis. This study aims to investigate the mechanism of the action of NMT treatment of middle cerebral ischemia/reperfusion (MCAO/R) model rats based on brain-intestinal LT/5-HLTP/5-HT/5-HIAA (L-tryptophan/5-Hydroxy-L-tryptophan/5-HT/5-Hydroxy -indoleacetic acid) metabolic disorders. We conducted MCAO/R model rats to assess the efficacy of NMT treatment. The efficacy of NMT was evaluated by measuring the TTC infarct area and neurological score. Western blotting and histopathological experiments were utilized to determine the protective effect of NMT on brain tissue and intestinal barrier. Moreover, non-targeted and quantitative standard curve were performed to focus on the changes of metabolites in the cerebrospinal fluid (CSF), plasm, and colon. Bioinformatics identification of metabolites and key enzymes associated with tryptophan-5-HT metabolic pathway were excavated by R language combined with Metaboanalyst. The concentrations of the key metabolites were measured by ELISA kits. Western blotting was used to verify the expression of the Wnt7a, β-Catenin, MMP9, AQP4, and Occludin related metabolic pathways. The Pseudo-Germ-Free experiment was designed to explore whether NMT regulation of colonic 5-HT depended on microbiota remodelling. Next， to validate the LT/5-HLTP/5-HT/5-HIAA metabolic pathway, immunohistochemistry was used to

examine the expression of key enzymes in the brain and colon tissue. Finally, real-time fluorescence quantitative PCR was used to detect the number of *Akkermansia muciniphila*（AKK） in the cecal contents, and its pharmacological and metabolomic effects were studied after administration of AKK. NMT reduced the infarct area of MCAO/R model rats and restored neural function in MCAO/R model rats. NMT improved the regular arrangement of nerve cells in the cerebral cortex and hippocampus, reduced cell edema, decreased the expression of Bax and Caspase-9 proteins, and increased the expression of Bcl-2 protein. NMT also protected the damaged intestinal barrier. NMT significantly changed 60 different metabolites in the CSF, 54 different metabolites in the colon, and 30 different metabolites in the plasma when the NMT group compared with the model group. In addition, NMT altered the LT/5-HLTP/5-HT/5-HIAA metabolism in the CSF, plasm, and colon. After NMT treatment, 5-HT levels were significantly increased in the plasm and CSF but significantly decreased in the colon. Meanwhile, NMT treatment significantly up-regulated the expressions of colonic MAO-A and SERT, and significantly down-regulated the expressions of colonic TPH1. NMT treatment upregulated the expression of Wnt7a, β-Catenin, Occludin proteins, and decreased the expression of p-β-Catenin, MMP9 and AQP4 proteins in the brain. In addition, NMT regulation of intestinal 5-HT is associated with microbiota remodelling. Compared with the model group, the AKK group showed a decrease in cerebral infarction area and neurological deficit score, and regulated the tryptophan metabolic pathway and its six metabolites. NMT regulated the tryptophan-5-HT metabolic pathway by improving the intestinal barrier, flora and 5-HT-related proteins, thereby reducing damage to the brain and gut. At the same time, NMT activated the Wnt signaling pathway and restored the blood-brain barrier and nerve function in the brain, which is closely related to the tryptophan-5-HT metabolic pathway. Moreover, NMT may partially exert its therapeutic effect on ischemic stroke by enriching intestinal AKK. These findings lay a solid foundation for NMT in treating ischemic stroke.

TdT combined with Cas14a for the electrochemical biosensing of NPC-derived exosomes

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Abstract: In this work, the electrochemical biosensor based on the subtly combination of terminal deoxynucleotidyl transferase (TdT), CRISPR/Cas14a and magnetic nanoparticles (MNPs) was exploited for the detection of nasopharyngeal carcinoma (NPC)-derived exosomes. Ascribed to the synergistic effect of the followed factors: the powerful elongation capacity of TdT for single-stranded DNA (ssDNA) with 3-hydroxy terminus, the outstanding trans-cleavage ability of CRISPR/Cas14a specifcally activated by the crRNA binding to target DNA and the excellent separation ability of MNPs, the developed electrochemical biosensor exhibited high sensitivity for the detection of NPC-derived exosome, with the linear range from 6.0×10^2 ~1.0×10^5 particles/mL and the limit of detection as lown down to 80 particles/mL. In addition, this electrochemical biosensor successfully distinguish the exosome from the NPC patients and the healthy people. This electrochemical biosensor opened up a new way for the early diagnosis of NPC.

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Machine Learning Coupled with Critical Components Elucidates *Peucedanum praeruptorum* Dunn Quality under Multi-Factors

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Abstract: *Peucedanum praeruptorum* Dunn as a traditional Chinese medicine (TCM) with many clinical applications. Praeruptorin A (PA) and Praeruptorin B (PB) are quality markers. Recently, PB has attracted attention for its difficulty in satisfying Chinese Pharmacopoeia in *Peucedanum praeruptorum* Dunn. However, the quality science connotation behind this is not clear. Hence, we conducted a study on PA and PB of 538 batches of samples from the main production areas of Chinese provinces. A negative correlation between PA and PB was observed for the first time, with a wide range of fluctuation coefficients and poor quality stability. This relates closely to the main production areas and growth patterns. Particularly, the sum of PA and PB reveals the quality stability and maintains the satisfactory rate to avoid quality evaluation bias. Six machine learning algorithms were used to build the model after optimisation and evaluation. We found that the prediction accuracies for the DaoDi producing regions Anhui and Zhejiang reached 93.3% and 87% with Stacking and SVM, respectively.

The kNN predicts wild and domestic species patterns with an accuracy of 93.3%. Compared with traditional chemometrics, machine learning has absolute advantages. This study comprehensively revealed the quality formation factors of *Peucedanum praeruptorum* Dunn. It provides a scientific basis for improving the quality standard, grade evaluation and scientific supervision of *Peucedanum praeruptorum* Dunn.

Analysis of lipid components in five kinds of Cordyceps based on lipidomics

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Abstract Submission

Objective:In recent years, the of Cordyceps sinensis（CS）, which has been listed as a national second level endangered species，the production of CS is low, the demand is high, and it is easy to be adulterated. Since the 2000 edition of the Chinese Pharmacopoeia, the quality standards for CS have been established based on the content of adenosine as an indicator. However, the adenosine is also commonly present in other fungi, lacking specificity. Therefore, it is incomplete to take adenosine as a quality indicator for CS. Lipids are a diverse group of compounds with many key roles enabling them to serve as forms of energy storge, cellular membrane and signaling molecules.Guo et al. have demonstrated that fatty acid composition might be sensitive makers for determinantion of CS geographical origins. We attempt to identificate and achieve a preliminary evaluation of the quality of CS through the research of lipidomics in this study. Method：Were analyzed using an LC-ESI-MS/MS system ,the lipids of wild Cordyceps sinensis（YS）, cultivated Cordyceps sinensis(RG), Cordyceps taii（DS）, Liangshan Cordyceps sinensis（LS）, and Cordyceps hawkesii（YXB） were analyzed from the perspective of lipidomics.Result：A total of 671 lipid components, were identified in all samples, among which the highest number of lipid components of TG was detected as 341. It was found that the detection of lipid components of LDGTS and DGGA classes is expected to be the key

lipid type components for the identification of CS.The number of double bonds of unsaturated lipid components in the five species of Cordyceps were all between 2-5, and the difference in the number of double bonds was not obvious, but there was a significant difference in the content of the unsaturated lipid components, especially the difference was significant in the number of carbon chains of 2 and 5. When the unsaturated carbon chain element was 2, the unsaturated lipid content was the highest among the TG-like lipids of the five samples; when the number of unsaturated carbon bonds was 5, the lipid content of YS and RG, DS, LS, and YXB was decreasing. Six lipid components unique to ys were found, Cer(t18:0/25:1(2OH)) (C43H85NO5), HexCer(d16:2/22:0(2OH))(C44H83NO9), PI(18:1_22:3)(C49H87O13P), PMeOH(16:0_18:2)(C38H71O8P), TG(24:0_18:3_18:3)(C63H110O6), and TG(18:2_20:0_20:1) (C61H112O6). Conclusion:It is found that the detection of ldgts and dgga lipid components is expected to become the key lipid type components for the identification of CS. Six lipid components unique to YS were screened; can be used as the relevant marker lipid identity for distinguishing YS from other CS, especially for distinguishing RG from YS. The main metabolic pathway that differed among different groups was the metabolic pathways, suggesting that this pathway can be used as one of the main metabolic pathways to explore the quality differences and identify the differences between different kinds of CS.

Adulteration Identification of *Angelica Sinensis* based on "Digital Identity" of Mass Spectrometry

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Abstract：Background and objectives: *Angelica Sinensis* (AS) is the dried root of *Angelica sinensis(Oliv.) Diels* of Umbelliferae. *Levisticum officinale* (LO) is the dried root of *Levisticum Officinale Koch* of Umbelliferae Angelica. *Angelica acutiloba* (AA) is the dried root of *A.acutiloba Kitagava* and *A.acutiloba Kitagava var.Sugiyama Hikino* of Umbelliferae. In addition, *Angelica gigas* (AG) is the dried root of *Angelica gigas Nakai* of Umbelliferae. In the Chinese Pharmacopoeia, only AS can be used as a medicinal herb. However, due to their similar macroscopic and microscopic characteristics, LO, AA, and AG are common umbelliferous adulterants of AS. In the Chinese medicine market, LO, AA, and AG are often regarded as AS or mixed into AS, which affects the market supervision, quality control and clinical application of AS. Therefore, it is necessary to analyze and distinguish AS, AA, AG, and LO. In this paper, in order to strengthen the quality control of AS and develop digital identification methods to enrich AS's identification methods, based on the high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS^{E}), the "digital identities" of AS, AG, AA, and LO were put forward and established based on mass spectrometry analysis for matching test samples to realize the digital identification of AS adulteration. **Methods:** Firstly, the AS, AA, AG and LO were analyzed by UHPLC-QTOF-MS^{E} to obtain their mass spectrometry information. Further, the quantized data information of AS, AA, AG and LO were obtained by Progenesis QI. The shared ions (SI) were extracted from AS, AA, AG and LO from different batches and origins, respectively, as their "data representation of ions". Then, the data matrices of unique ions of AS, AA, AG and LO relative to each other were screened out, and the Top-N ions were outputted as the "digital identities" of AS, AA, AG, and LO, respectively, sorted by ionic strength from smallest to largest. Finally, the above "digital identities" were used as benchmarks for matching single herb, mixed positive samples, and market blind samples to feedback on matching credibility (MC). **Results:** Based on the "digital identity" of AS, AA, AG, and LO, the digital identification of single herb can be realized at the individual level

of Chinese medicine with the MC not less than 78.0 %. In digital identification of mixed positive samples, even if 1 % adulterant in mixed positive samples can still be identified efficiently and accurately. Based on identification results of mixed positive samples and taking into account that 3.0 % impurity is allowed, the MC detection limits of AA, AG and LO were initially set to 15.0 %, 65.0 %, and 20.0 %, respectively. In addition, through digital identification based on "digital identity" and the set detection limit, 2 batches of market blind samples were adulterated with AG, and their MC results compared to AG's "digital identity" are 94.0 %, 83.0 %, which are greater than the MC detection limits of AG (65.0 %). It is sufficient to show that the 2 batch of market blind samples are indeed adulterated with AG. On the other hand, the MCs of other market blind samples did not exceed the MC detection limits, which suggesting that these samples were not adulterated with AG, AA and LO. **Conclusion**: The digital identification and adulteration analysis of AS, AA, AG, and LO can be realized efficiently and accurately through the "digital identities" of AS, AA, AG, and LO based on mass spectrometry analysis. The digital identification methods based on "digital identity" can enrich current AS's identification methods. In the digital era of Chinese medicines, it has important reference significance for developing non-targeted digital identification of herbal medicines, which is beneficial to the construction of digital quality control of Chinese medicines.

Application of Lipidomics in the Study of Traditional Chinese Medicine

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Abstract Submission

Lipidomics is an emerging discipline that systematically studies the various types, functions, and metabolic pathways of lipids within living organisms. This field compares changes in diseases or drug impact, identifying biomarkers and molecular mechanisms present in lipid metabolic networks across different physiological or pathological states. Through employing analytical chemistry within the realm of lipidomics, researchers analyze traditional Chinese medicine (TCM). This analysis aids in uncovering potential mechanisms for treating diverse physiopathological conditions, assessing drug efficacy, understanding mechanisms of action and toxicity, and generating innovative ideas for disease prevention and treatment. This manuscript assesses recent literature, summarizing existing lipidomics technologies and their applications in TCM research. It delineates the efficacy, mechanisms, and toxicity research related to lipidomics in Chinese medicine. Additionally, it explores the utilization of lipidomics in quality control research for Chinese medicine, aiming to expand the application of lipidomics within this field. Ultimately, this initiative seeks to foster the integration of traditional medicine theory with modern science and technology, promoting an organic fusion between the two domains.

PBOX-sRNA-seq uncovers species-specific miRNAs in *Artemisia annua*

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Abstract: Artemisinin is the most effective anti-malarial drug, and the application of metabolic engineering to increase its content is of great significance for ensuring the supply of medicinal materials. Studies on the regulation of artemisinin synthesis

mainly focus on transcriptional level. Species-specifically expressed miRNAs are particularly critical for the determination and maintenance of medicinal plants-specific traits. Relevant studies on the regulation of artemisinin biosynthesis by A. annua-specifically expressed miRNAs are largely unknown. We developed PBOX-sRNA-seq to improve the sensitivity and accuracy of low-abundance and species-specific miRNAs identification. Compared with conventional sRNA sequencing, the proportion of the 2'OMe sRNAs was elevated from 40% to 90%, and the replicability was also significantly increased. We conducted sequencing using RNA with variable degrees of degradation and the results showed that conventional approach contained predominantly transfer RNA (tRNA) contamination. By contrast, PBOX-sRNA-seq tolerated severe RNA degradation. Besides, the sensitivity of PBOX-sRNA-seq has been significantly improved. A. annua treated with plant hormone MeJA (which can promote artemisinin synthesis) were sequenced by PBOX-sRNA-seq and three candidate miRNAs were obtained. We hope PBOX-sRNA-seq will provide a new perspective for future miRNA research in medicinal plants.

Ursodesoxycholic acid nanoparticles with rapid hepatic excretion for near-infrared imaging and cholestasis liver injury detection

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Abstract:

Ursodesoxycholic acid (UA), a hydrophilic bile acid widely used to treat cholestasis, is known to enhance the hepatic excretion of bilirubin and bile salts. However, most near-infrared II (NIR-II) fluorophores suffer poor water solubility and prolonged liver retention, leading to potential hepatic toxicity and other side effects. In this study, we present the synthesis of a novel UA derivative with excellent water solubility and enhanced hepatic excretion properties. First, we designed four UA derivatives by conjugating UA with different types of polyethylene glycol (PEG) and introducing the acetyl groups. These derivatives are capable of loading a donor-acceptor-donor NIR-II fluorophore (DYE) to form nanoparticles with diameters under 100 nm. Following intravenous administration in mice, fluorescence monitoring revealed that acetylated UA conjugated with PEG-containing hydroxy groups (Aco-UA-PEG-OH) significantly accelerated hepatobiliary excretion of DYE. Further *in vivo* studies confirmed that Aco-UA-PEG-OH could release UA and its metabolites effectively. Additionally, in a cholestasis model, DYE-loaded Aco-UDCA-PEG-OH nanoparticle exhibited reduced hepatic clearance due to impaired hepatobiliary metabolism, resulting in uneven hepatic fluorescence distribution. These findings suggest that Aco-UDCA-PEG-OH is a promising nanomaterial for improving the water solubility and hepatic excretion of NIR-II fluorophores. This advancement could have significant implications for the detection and monitoring of cholestasis, contributing to disease progression understanding and drug efficacy evaluation.

Functional evolution and diversification of the CYP82D subfamily governs flavonoid diversification in the genus *Scutellaria*

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Abstract

Flavonoids, the largest class of polyphenols, exhibit substantial structural and functional diversity, yet their evolutionary diversification and specialized functions remain largely unexplored. The genus *Scutellaria* is notable for its rich flavonoid diversity, particularly the 6/8-hydroxylated variants biosynthesized by the cytochrome P450 subfamily CYP82D. Our study analyzes metabolic differences between *Scutellaria baicalensis* and *Scutellaria barbata*, suggesting that CYP82Ds have acquired a broad range of catalytic functions over their evolution. By integrating analyses of metabolic networks and gene evolution across 22 *Scutellaria* species, we rapid identified 261 flavonoids and delineated five clades associated with various catalytic functions of CYP82Ds. This approach uncovered a unique catalytic mode for 6/8-hydroxylated function under flavanone substrates and the first instance of 7-*O*-demethylation of flavonoid substrates catalyzed by cytochrome P450. Ancestral sequence reconstruction and functional validation demonstrated that gradual neofunctionalization of CYP82Ds has driven the chemical diversity of flavonoids in *Scutellaria* throughout its evolutionary history. Our study enhances the understanding of flavonoid diversity, elucidates the intricate roles of CYP82Ds in *Scutellaria* plants,

and underscores the extensive catalytic versatility of cytochrome P450 members within plant taxa.

Keywords: *Scutellaria*, CYP82D subfamily, flavonoids, demethylation, evolutionary diversity

Simultaneous extraction and *in situ* separation of flavonoids and alkaloids from *Morus alba* leaves by a pH-responsive DES/H_2O system

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Abstract: Flavonoids and alkaloids are the major bioactive components of *Morus alba* leaves with significant pharmacological activity. We here developed a novel method for simultaneous extraction and *in situ* separation of flavonoids and alkaloids from *Morus alba* leaves by using a pH-responsive deep eutectic solvent (DES)/H_2O system. Five novel DESs were synthesized using long-chain fatty acids as hydrogen bond donors and 2-methyl-2,4-pentanediol (MPD) as hydrogen bond acceptor. The DES was mixed with water to form a single-phase system for extraction at a high pH, and the pH of the extract was then lowered to induce *in situ* phase separation, further separating the flavonoids and alkaloids. Isoquercitrin and deoxynojirimycin, representing the major flavonoids and alkaloids, respectively, were selected as model compounds to evaluate the extraction and separation efficiency. The optimal DES, [HexA (hexanoic acid)][MPD], was identified, and its extraction conditions ([HexA][MPD] concentration, extraction time, solid/liquid ratio and pH) and *in situ* separation condition (pH) were optimized. Under optimal conditions, flavonoids were mainly enriched in the DES phase, while alkaloids were primarily concentrated in the H_2O phase. Moreover, [HexA][MPD] exhibited excellent reusability. This study

demonstrates the application of a pH-responsive DES/H_2O system for simultaneous extraction and separation of different types of natural products for the first time.

UPLC-MS-based serum metabolomics reveals the anti-aging activity of polysaccharides from *Polygonum multiflorum* Thunb. in aging mice

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Abstract: Prolonging life is one of the main effects of *Polygonum multiflorum* Thunb (PM). Although polysaccharides, the primary constituents of PM, remain largely unexplored in terms of their anti-aging properties and underlying mechanisms, this study endeavors to unravel them. Therefore, we investigated the anti-aging effects of purified polysaccharide fractions from PM, namely RPMP-N and RPMP-A, utilizing a D-galactose-induced aging mouse model. Antioxidant enzymes, essential mechanisms for scavenging free radicals and an integral part of the body's antioxidant defense system, including SOD, CAT, and GSH-PX, were measured in aging mice; revealing significant improvements following treatment with RPMP-N and RPMP-A. Moreover, RPMP-N and RPMP-A demonstrated the ability to repair and protect against liver and brain injuries. P16, P21, and P53 proteins, which orchestrate the process of cellular senescence through distinct mechanisms, exhibited downregulated expression in liver and brain tissues post-treatment. Non-targeted metabolomics techniques facilitated the observation of metabolic alterations in endogenous small

molecules, illuminating their mechanisms of action within the organism. Notably, RPMP-N and RPMP-A exhibited significant anti-aging potency in the D-Gal aging mice model, primarily impacting lysine, sphingolipid, cysteine, and methionine metabolism. This study not only demonstrates that polysaccharides from PM possess anti-aging activity and uncovers their mechanisms but also provides a scientific foundation for the clinical application of PM, the development of novel pharmaceutical preparations, and regulatory science.

Establishment of in vivo imaging technique for inflammatory bowel disease and its application in rapid efficacy evaluation of natural products

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Abstract: Inflammatory bowel disease (IBD) is a chronic intestinal condition characterized by high incidence and recurrence rates, a prolonged disease course, and numerous complications. It is often referred to as "green cancer." IBD has complex etiology, and currently, there are no targeted therapies specifically for this condition. Natural products from traditional Chinese medicine (TCM) have been a significant resource in drug discovery and have garnered considerable attention in the search for new IBD therapies. However, evaluating the efficacy of active compounds from TCM in treating IBD can be challenging due to the lack of precise, real-time monitoring tools.

To address this challenge, we developed a convenient imaging method that

combines the advantages of live imaging technology with high spatiotemporal resolution to monitor the progression of IBD and assess the in vivo effectiveness of natural products derived from TCM. In our study, we synthesized a squaraine dye (SQ145), which exhibited good imaging capabilities for both the gastrointestinal tract and blood vessels in mice. After oral administration of SQ145, we were able to visualize and monitor intestinal peristalsis in real-time, and through optical flow analysis, we further quantified the intestinal movement. Then, as the integrity of the colonic barrier was compromised in colitis models, the fluorescence intensity in the blood vessels significantly increased after oral administration of the probe, allowing us to detect the intestinal barrier function *in vivo*. Next, in colitis mouse model, liver damage often accompanies the progression of the disease. Real-time tracking of liver fluorescence signals enabled non-invasive detection of liver injury associated with IBD. By integrating these three indicators, we provided a comprehensive assessment of IBD disease development. Additionally, we applied this evaluation system to screen natural products for potential IBD treatments. Our results indicated that matrine shows promising therapeutic potential, as it not only improved gut barrier function but also modulated oxidative stress processes in the liver. The near-infrared imaging method we developed provides a powerful tool for diagnosing IBD and discovering potential drugs for its treatment.

Quality assessment of the correlation of "profiling-effect-clinical application" associated with the inhibitory effect of Hyssopus volatile oil on NETs for treating steroid-resistant asthma

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Abstract:

Steroid-resistant asthma (SRA) is a significant public health concern. Hyssopi Herba, a representative herbal medicine for abnormal balgham type of asthma, lacks reported effectiveness for SRA, and quality assessment methods based on pharmacological substances are undue developed. This project identified the therapeutic efficacy of Hyssopus volatile oil(HVO) in an SRA mouse model, suggesting inhibition of NETs formation and neutrophil-related inflammation. The project proposes a scientific hypothesis for quality evaluation based on the "profiling-effect-clinical application" correlation. GC × GC Q-TOF MS will be used to identify HVO’s chemical composition spectrum, integrating multidimensional chromatography and mass spectrometry for metabolic profiling and pharmacokinetic characteristics. The study aims to clarify HVO’s efficacy components for SRA through cellular and mouse models, using them as quality markers. Quality control will involve qualitative profiling and simultaneous quantitative analysis, establishing a quality assessment method capable of "identifying efficacy." The research outcomes are expected to

guide precise clinical use of Uyghur medicine, improve quality assessment models, and contribute to innovative herbal medicine discovery.

Keywords: Hyssopus volatile oil(HVO), Steroid-resistant asthma(SRA), Profiling-effect-clinical application, Quality evaluation

Acknowledgments

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A Squaraine Probe for Quick Evaluating Drug-induced Liver Injury with NIR-II Fluorescence Imaging

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Abstract: Drug-induced liver injury (DILI) is a common adverse drug reaction, and it can lead to liver failure and even death. In China, inappropriate use of herbal medicine is a major contributor to liver injury. Thus, accurately evaluating their hepatotoxicity is of great significance for public health. Herein, a squaraine probe SQ905 for diagnosing DILI by detecting liver metabolism with NIR-II fluorescence imaging is demonstrated. The probe containing squaraine dye as accepter and a donor with butylsulfonic acid on the N atom has a good water solubility and can specifically aggregate in the liver. Further, the probe is metabolized completely within 1 hour by the liver-gallbladder-intestinal metabolic pathway under normal conditions and after the liver is damaged by drugs, the aggregation time of probe in the liver is increased and metabolism is slowed down. In addition, the mechanism of probe detecting method is further studied and explored in cells and mouse and the liver transporter protein Mrp3 is found as a significant target that affects the metabolism of the probe

in the liver. SQ905 is utilized in the acetaminophen (APAP) and triptolide-induced liver injury mouse model to quickly evaluate the liver damage dependent on probe metabolic rate. The experimental results demonstrate the SQ905 can be used in detecting chemical and herbal medicine-induced liver injury. Moreover, SQ905 has also been employed to monitor rehabilitation of liver injury during treatment process.

Therapeutic effects and potential mechanism of the Mongolian drug Tonglaga-5 on N-methyl-N′-nitro-N-nitrosoguanidine-induced chronic atrophic gastritis

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Abstract: Chronic atrophic gastritis (CAG) is a common digestive system disease worldwide, characterized by inflammation, reduction or atrophy of gastric glands, and thinning of the gastric mucosa, with or without intestinal and/or pseudopyloric metaplasia. Tonglaga-5 (TLG-5) is a Mongolian clinical prescription used for treating stomach disorders and is recorded in the Chinese Pharmacopoeia. However, the therapeutic effects of TLG-5 on CAG and its potential mechanisms remain unclear. This study aimed to investigate whether TLG-5 has a therapeutic effect on CAG and its potential mechanisms using a combination of multiple methods. A CAG model was established in Sprague-Dawley (SD) rats induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2% sodium salicylate and the hunger-satiety anomaly. We randomly divided 40 CAG rats into five groups: the model group, teprenone group, the low-dose TLG-5 group (TLG-5 L), the middle-dose TLG-5 group (TLG-5 M), and the high-dose TLG-5 group (TLG-5 H), with an additional 8 rats of the same age assigned to the control group. Pathological analysis of rat gastric tissue was performed. Enzyme linked immunosorbent assay

(ELISA) was used to measure pepsinogen I(PGI)，pepsinogen II（PGII） and gastrin-17（G-17）in the serum of each group. Network pharmacology was employed to predict the targets of TLG-5 in treating CAG. Subsequently, the metabolomics approach was used to explore specific metabolites and metabolic pathways. Finally, these predictions were validated by a “metabolite-gene” interaction network, molecular docking and real-time quantitative reverse transcription PCR (RT-qPCR). Compared with the model group, the TLG-5 group showed improvements in pathological conditions and serum levels of PGI,PGR and G-17. Serum metabolomics analysis indicated that the pathogenesis of CAG is primarily related to pyrimidine metabolism, tyrosine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and sphingolipid metabolism. The mechanism of TLG-5 in treating CAG is mainly associated with β-alanine metabolism and pyrimidine metabolism. Results from network pharmacology, molecular docking and RT-qPCR suggest that TLG-5 may exert its therapeutic effects on CAG by inhibiting the PI3K-AKT signaling pathway and HIF-1 signaling pathway.

Unravelling the Cultivar and Spatial Metabolome in Hemp Achene

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ABSTRACT:

Hemp achenes have been utilized as a source of nutrition and medicinal remedies for millennia. Rich in phytochemicals, they contain amides (phenethylamide and ligninamide), flavonoids, and cannabinoids. However, metabolomic profile in diverse hemp achene cultivars with different phenotypes remains an unresolved inquiry, and the spatial distribution of these compounds in hemp achenes is also unknown. In this research, a liquid chromatography–mass spectrometry-based targeted metabolomics technique was developed to perform a comprehensive metabolomic characterization of 6 phenylamides, 22 lignanamides, 6 flavonoids and 29 cannabinoids in hemp achenes. Furthermore, we examined the metabolomic differences in the kernel (including radicle, endosperm and cotyledon) and hull (containing testa) of 20 hemp cultivars with varying hull colors. Both lignanamides and flavonoids were more concentrated in greyish-yellow achenes than in greyish-green achenes, while cannabinoids were found at higher levels in greyish-green achenes than in greyish-yellow achenes. According to atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry imaging, phenethylamides, flavonoids, and cannabinoids spatially distributed in the hemp hulls and testa. Concurrently, the targeted metabolomics findings reveal that most of the differentially abundant compounds were found in higher concentrations within the hulls. This research provides guidance for precise breeding and effective utilization of hemp achenes.

Keywords: Hemp achene, Metabolomics, UPLC-MS/MS, MALDI-MSI, Cannabinoids

Study on the Therapeutic Effects and Mechanism of Mongolian Medicine Pomegranate on Hyperlipidemia

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Abstract: Hyperlipidemia (HLP) is a prevalent clinical cardiovascular disease characterized by intricate molecular mechanisms and a variety of pathogenic factors, falling under the category of metabolic disorders. Pomegranate (POG) is the fruit of the pomegranate plant, a member of the Lythraceae family, and it has a long-standing history of use in Mongolian medicine. In this study, we investigated the therapeutic effects of POG on HLP in rats and explored its mechanisms of action using metabolomics and transcriptomics approaches. Initially, we induced a HLP model in rats by feeding them a high-fat diet for eight weeks; followed by gavage administration of POG for an additional eight weeks after the model was successfully established. Subsequently, serum metabolomics was employed to analyze metabolites and associated metabolic pathways in the high-dose group demonstrating the best pharmacodynamic effects. Thirdly, liver transcriptomics was utilized to explore the pathways through which POG ameliorates HLP. The metabolomics results revealed that following POG intervention, 37 metabolites associated with HLP were regulated, such as neopentyl glycol, cholesterol sulfate, abietic acid, and glycocholic acid, among others. The pathways involved encompassed sphingolipid metabolism, tyrosine metabolism, pyrimidine metabolism, and arachidonic acid metabolism. Transcriptomic analysis indicated that POG can modulate genes involved in various lipid and inflammatory response pathways, such as the lipid and atherosclerosis signaling pathway as well as the NF-κB signaling pathway. POG effectively regulates lipid metabolism in HLP rats, which may be achieved through the modulation of fatty acid metabolism, inflammatory response, and immune homeostasis-related pathways. These findings offer new insights into the therapeutic potential of POG in managing HLP

Enhanced Bile Acid Detection and Analysis in Liver Fibrosis with Pseudo-targeted Metabolomics

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Abstract: Bile acids (BAs), as crucial signaling molecules in host and gut metabolism of microorganisms, play an essential physiological function in maintaining the stability of organisms. Liquid chromatography-mass spectrometry (LC-MS) is widely used in metabolite determination in biological samples due to its high sensitivity, excellent specificity, and low detection limits, gradually becoming the mainstream method for BAs detection and analysis. Pseudotargeted analysis combines the advantages of untargeted and targeted metabolomics methods. In this study, a comprehensive and rapid BAs detection and analysis method was established using LC-MS technology. Subsequently, this established method was applied to the detection and analysis of BAs in liver samples from bile duct-ligated (BDL) mice with liver fibrosis modeling. Firstly, a self-built database containing 488 BAs was established, and raw data of natural mixed standard (NMS) were collected using UHPLC-Q/TOF-MS, characterizing a total of 172 BAs compounds, including 74 free BAs, 27 taurine-conjugated BAs, 24 glycine-conjugated BAs, 11 glucuronic acid-conjugated BAs, 15 sulfated BAs, 1 glucoside-conjugated BAs, and 20 BAs dimeric forms. Subsequently, a total of 14 BAs failed to pass the methodological examination because they were below the detection limit, and a total of 158 BAs passed the methodological examination by using the simultaneous BAs high-coverage assay established by the UHPLC-QQQ-MS. The established BAs high-coverage assay was used in the BDL modeling liver fibrosis mouse model, and combined with statistical analysis tools, 20 differential BAs were screened in the livers of liver fibrosis mice.

Structural Features of Soluble Polysaccharide GSPA-0.3 Isolated from Panax Ginseng C. A. Meyer Root and Investigation of Its Adjuvant Activity Mechanism

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Abstract:

A novel polysaccharide (GSPA-0.3) was extracted from the root of cultivated Panax ginseng C. A. Meyer. Its structural characteristics, adjuvant activities, and mechanisms for enhancing antibody levels and the maturation of DC2.4 cells were thoroughly investigated. The molecular weight of GSPA-0.3 was determined to be 6.335×10^4 Da, with its monosaccharide composition including galacturonic acid, galactose, arabinose, glucose, rhamnose, and mannose. Structural analysis revealed that GSPA-0.3 has a homogalacturonan backbone, complemented by domains of arabinan, glucan, and type-II arabinogalactan. GSPA-0.3 demonstrated significant immunostimulatory efficacy in H1N1 vaccine-immunized mice, notably increasing levels of IgG, IgG1, IgG2a, and the IgG2a/IgG1 ratio. This was assessed through hemagglutination inhibition (HI), splenocyte proliferation, cytotoxic T lymphocyte (CTL) activity, and the promotion of GATA-3, T-bet, IFN-γ, and IL-4 production.

Furthermore, GSPA-0.3 significantly elevated the levels of neutralizing antibodies in H1N1 vaccine-immunized mice, proving to be more effective than aluminum adjuvant. Investigations into its mechanism revealed that GSPA-0.3 activated the TLR4-dependent pathway by upregulating the expressions of TLR4, MyD88, TRAF-6, and NF-κB. In conclusion, the novel polysaccharide GSPA-0.3 significantly enhanced the efficacy of the H1N1 vaccine by modulating the Th1/Th2 response through the TLR4-MyD88-NF-κB signaling pathway.

Key words: Panax ginseng C. A. Meyer; Polysaccharide; H1N1 influenza vaccine; adjuvant activity

Uncovering the effective components and underling mechanisms of Shengmai formula in early pulmonary fibrosis by integrating the serum pharmacochemistry and metabolomics aided with molecular docking

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Abstract: The worsening pulmonary fibrosis (PF) after acute lung injury leads to poor prognosis and high mortality rate，and therefore finding effective therapeutic remedies to treat and alleviate its progression especially at the early stage, has become an urgent need. Shengmai Formula (SMF), a traditional Chinese compound formula,

showed potential efficacy against LPS-induced early pulmonary fibrosis in mice characterized by alleviating inflammatory and pathologic changes occurring in the alveoli, yet its bioactive components and underlying mechanisms need to be elucidated. This study aims to elucidate the key active components and proposed mechanisms of a classical TCM formula-SMF composed of Ginseng Radix et Rhizoma Rubra (GRR), Ophiopogonis Radix (OR), and Schisandrae Chinensis Fructus (SC) in alleviating a LPS-induced early pulmonary fibrosis mice model by a strategy integrating the comprehensive phynotypes of lung index and functions, multivariate statistical analysis of the serum pharmacochemistry and metabolomics, and molecular docking of the target active natural legends to the TGR5/FXR receptors. A lipopolysaccharide (LPS)-induced early PF model in mice was established to evaluate the therapeutic effects of SMF using dexamethasone (DEX) as a positive control. The lung index, function and pathological changes including the collagen volume and inflammatory damage ratio were observed. Subsequently, UPLC-QTOF-MS/MS-based assays aided by multivariate statistical analysis between the serum pharmacochemistry and metabolomics after administration of SMF were conducted to evaluate the predominant bioactive components absorbed into blood and the proposed pathway, further speculated with molecular docking by calculating the affinity of the target components to the TGR5 and FXR receptors.

SMF significantly alleviates LPS-induced early pulmonary fibrosis in mice as evaluated by the lung index, functions and pathological changes especially the collagenation. In total 57 components exposed in serum were identified with 21 come from GRR, 6 from OR, and 30 from SCF. Serum metabolomics revealed 682 endogenous metabolites, among which 31 metabolic biomarkers can be reversed by administration of SMF. Additionally, KEGG enrichment analysis indicated that these metabolites enrolled mainly in the bile acid and arachidonic acid metabolism pathways. Furthermore, correlation analysis highlighted the predominant effective components detected in the serum of SMF administrated group, (e.g., ginsenoside Ro, 25-hydroxy Rh1, 20(S)-25-hydroxy PPT). Molecular docking using the above screened candidates show significant affinity between the target ginsenosides /

Ophiofurospiside and TGR5 and FXR receptors, which regulate bile acid homeostasis, thereby reducing bile acid secretion and alleviating the injury induced by LPS in mice. Our present study establishes a robust strategy for disclosing the target bioactive components and underling mechanisms of the complex TCM formula, using SMF against inflammatory early pulmonary fibrosis as a representative. The predominant bioactive ingredients identified may provide new insight to discover new candidates for the treatment of acute lung injury and subsequent fibrosis diseases.

Protective effect of Phellodendri Chinensis Cortex on cognitive dysfunction induced by streptozotocin combined with HFD in diabetic mice

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Abstract: Diabetic cognitive impairment (DCI) is one of the complications of diabetes mellitus, which is mainly characterized by damage to brain structure and decline in learning and memory ability. A large number of studies have confirmed that Traditional Chinese Medicine (TCM) has a significant prevention and treatment effect on DCI. In this study, the Phellodendri Chinensis Cortex (Phe) was selected as the research object to explore the protective effect of Phe on cognitive dysfunction of diabetic mice induced by streptozotocin (STZ) combined with high-fat diet (HFD). To provide theoretical basis for clinical treatment of DCI. Select male C57BL/6 mice (SPF grade) at 4 weeks of age with a body weight of 16-20 grams. After feeding a

HFD for 6 weeks, a DCI mice model was established by inducing diabetes in mice by intraperitoneal injection of STZ for 5 consecutive days. Randomly assign mice with fasting blood glucose (FGB) levels higher than 11.1 mmol/L into the following groups: the model group (Mod), the low-dose Phe group, the high-dose Phe group, and the metformin group (positive drug, Met), with 10 mice in each group. Additionally, select 10 non-modeled mice to serve as the control group (Con). After 13 weeks of drug intervention, conduct behavioral tests using the Morris water maze. Fully automated biochemistry was used to measure fasting blood glucose (FGB), total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in mice plasma. Observe pathological changes in brain tissue through brain-HE staining and brain-Nissl staining. Use of ELISA kits to determine IL-6 and TNF-α levels in brain tissue.The results of the water maze showed that after the intervention of Phe the evasion latency on the 5th day of the localisation cruise was significantly reduced in the Phe group compared with the Mod group, and the spatial exploration showed that the residence time in the platform quadrant was significantly increased in the Phe group compared with the Mod group, which indicated that Phe could improve cognitive dysfunction in diabetic mice. Biochemical results showed that the levels of FBG, TC, TG and LDL-C in the Mod group were significantly higher than those in the Con group, while the levels of FBG, TC, TG and LDL-C in the Phe group were significantly lower than those in the Mod group, which indicated that Phe could improve glucose/lipid metabolism disorders in DCI mice. The results of HE staining and Nissl staining showed that the number of neuronal cells in mice in the Mod group was significantly reduced compared with that in the Con group, and the phenomena of nuclear consolidation and inflammatory infiltration were observed. And after the intervention of Phe, the neuronal cell status of mice in Phe group was significantly improved, and the number of neuronal cells was also increased.This indicated that Phe could improve the morphology of nerve cells and increase the number of nerve cells in DCI mice.The results of the kit showed that the levels of IL-6 and TNF-α in the brain tissue of mice in the Mod group were significantly increased compared with those in the Con group, and after treatment with Phe, the Phe group showed different

degrees of regression, in which the Phe group with a high dose significantly reduced the contents of IL-6 and TNF-α in the brain tissue of DCI mice, and the above results indicated that the contents of IL-6 and TNF-α in the brain of DCI mice were abnormal The above results showed that DCI mice had abnormal levels of IL-6 and TNF-α in the brain, and there was neuroinflammation, which could be reduced after the intervention of Phe, which could effectively inhibit the release of inflammatory factors IL-6 and TNF-α. In summary, Phe can regulate glucose metabolism disorders, improve neuronal damage in brain tissue, reduce inflammatory factors in DCI mice, and thus play a protective role against diabetic cognitive dysfunction.

Study on the Beneficial Effects of Ginsenosides on Streptozotocin-induced Depressive-like Behaviors in Mice

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Abstract： In recent years, depression, a ubiquitous emotional mental disorder, has continued to escalate in its global incidence, emerging as one of the primary culprits for disease-related disabilities. Current treatments are mostly based on the monoamine neurotransmitter hypothesis, yet they are associated with pronounced side effects and limited efficacy in certain subtypes. Traditional Chinese Medicine (TCM) possesses profound insights into depression, offering therapies that effectively alleviate core symptoms with high safety profiles, garnering attention from scholars both domestically and internationally. Among numerous herbal medicines, ginseng is renowned for its abilities to tranquillize the mind, enhance mental capacity, and fortify the body's vital energy, among other restorative effects. Ginsenosides, the primary active constituents in ginseng, have been validated by numerous studies to exert extensive neuroprotective effects, particularly in ameliorating depressive-like behaviors in mice. In light of this, the present study focuses on the potential impact of

ginsenosides on depressive-like behaviors in streptozotocin (STZ)-induced diabetic mouse models, aiming to provide a scientific basis for clinical treatments of diabetes-related depression and its comorbidities. This study was meticulously designed, employing a high-fat diet combined with STZ to induce diabetes in male C57BL/6 mice. The animals were divided into several groups: a blank control group, a model group, a low-dose ginsenoside group, a high-dose ginsenoside group, and a metformin-positive control group. After 15 weeks of continuous pharmacological intervention, a series of carefully designed behavioral experiments, including the open field test (OFT), elevated plus maze (EPM), tail suspension test (TST), and forced swim test (FST), were conducted to comprehensively assess the behavioral changes in the mice. The experimental results demonstrated that ginsenosides exhibit significant efficacy in alleviating STZ-induced depressive-like behaviors. Specifically, in the TST and FST, both the low-dose and high-dose ginsenoside groups showed a significant reduction in immobility time compared to the model group, indicating their effectiveness in mitigating depressive mood in mice. In the OFT, the low-dose ginsenoside group demonstrated a significant increase in total distance traveled, reduced resting time, and increased average speed, further validating its role in enhancing spontaneous locomotor activity and improving depressive-like behaviors. Additionally, the EPM revealed that ginsenosides positively influenced exploratory behavior and enhanced activity willingness in mice. In summary, this study convincingly demonstrates that ginsenosides have a significant therapeutic effect on depressive-like behaviors in diabetic mice, providing strong support for the further application of ginsenoside in the treatment of depression in diabetes.

Beneficial effects of sweet tea on liver injury in streptozotocin-induced diabetic mice

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Abstract： Diabetes mellitus, a metabolic disorder, is marked by chronic high blood sugar levels, threatening organ health. With increasing diabetes rates, diabetic liver injury also escalates, fueled by fatty acid metabolism imbalances causing hepatic steatosis. *Lithocarpus polystachyus* Rehd. or *Lithocarpus litseifolius* (Hance) Chun., commonly known as "Sweet Tea" (ST), is rich in active compounds such as trifolin and phlorizin. Extensive modern pharmacological research has revealed that ST exhibits remarkable pharmacological effects, including hypolipidemic, antioxidant, hypoglycemic, blood pressure-regulating, antibacterial, anti-inflammatory, and anti-cerebral ischemic properties. This study aimed to investigate the beneficial effects of ST in intervening diabetic liver injury in mice, thereby providing a basis for clinical treatment of diabetic liver damage. To achieve this, a mouse model of diabetic liver injury was established through a combined approach of streptozotocin (STZ) administration and a high-fat diet, allowing for a systematic evaluation of the interventional effects of ST. According to the principle of random selection, 10 of 50 C57BL/6 mice were randomly selected as the control group with conventional diet; the rest were induced with high-fat diet combined with multiple intraperitoneal injections of low-dose STZ (50 mg/kg) to establish a diabetic mouse model. The mice model successfully established were randomly divided into four groups at random(n=10 per group): model group (Mod), low-dosage ST group(1.5 g/kg/d), high-dosage ST group(6 g/kg/d), metformin group (300 mg/kg/d), and ST was intragastric administrated for 14 weeks. After 14 weeks of treatment, the mice were fasted for 12 h. After anesthesia, the blood was taken from the heart and the plasma was collected. Then the mice were sacrificed and the liver tissue was taken for section.

The main chemical indicators in plasma were determined by a fully automated biochemical analyzer. The liver tissues of each group of mice were sectioned and stained by hematoxylin-eosin (HE) staining, and the histopathological changes were observed under the microscope. The experimental results revealed that compared to the model group, blood glucose in LIH and Met mice significantly decreased ($p<0.001$, $p<0.01$), while TG levels remained stable except in isolated cases. This validates sweet tea's role in glucose reduction and lipid metabolism regulation in diabetes. The model group showed elevated ALT and AST ($p<0.01$), confirming successful liver injury modeling. After 14 weeks of ST treatment, ALT and AST declined dose-dependently ($p<0.01$), mirroring metformin's effects, indicating improved liver function. HE staining confirmed reduced liver damage in ST-treated mice. These findings indicate that sweet tea has the potential to improve liver injury caused by diabetes, providing a basis for its clinical practices.

Rapid screening of antioxidant components in Huang-Lian-Jie-Du Decoction by AAPH-Incubating UPLC method

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Abstract: Modern pharmacological studies have shown that Huang-Lian-Jie-Du Decoction has obvious effects of lowering blood lipids, improving liver damage, anti-oxidation, lowering blood sugar, lowering blood pressure and anti-inflammatory. AAPH is a common azo initiator that decomposes when heated to produce one mole of nitrogen molecules and two moles of carbon radicals. Some carbon radicals can combine with each other to produce stable products, and some carbon radicals can

react with oxygen molecules to produce ROO•, which is considered to be representative of those involved in biochemical autoxidation and can be used at physiological pH (pH 7.4). It is believed that antioxidant components work by combining with ROO• to exert antioxidant effects. And when antioxidant components bind to ROO•, the conjugation system in their molecular structure is disrupted. In this study, we screened the antioxidant components in HLJDD with direct scavenging of peroxyl radicals by the change in peak area of absorption peaksin the UPLC chromatograms. Firstly, 1 mL of the crude HLJDD crude extract was incubated with different concentrations (0, 100, 200, and 400 uM) of AAPH solutions at 37 ℃ for 1 h. The objective was to assess the impact of the AAPH solution at different concentrations on the peak reduction rate in the chromatogram, and to determine the strength of the antioxidant activity by the peak reduction rate. Finally, a control test with PBS was performed for comparison to screen out the components with good ROO•scavenging activity in HLJDD. The results indicated that incubation for one hour at an APPH concentration of 200 mg/mL was the best condition for screening antioxidant compounds with ROO• scavenging activity from HLJDD. Meanwhile, it was revealed that the components showing good ROO• scavenging activity in HLJDD were mainly Baicalin and Oroxylin A-7- O-glucuronide. This experiment utilized a AAPH-Incubating UPLC method to rapidly screen for two potent antioxidant components in HLJDD, baicalin and Oroxylin A-7-O-glucuronide, providing an experimental basis for further exploring the antioxidant effects.

Exploration of beneficial effects of trilobatin against diabetic nephropathy using chemical derivatization with UPLC-Q-Orbitrap HRMS/MS analysis

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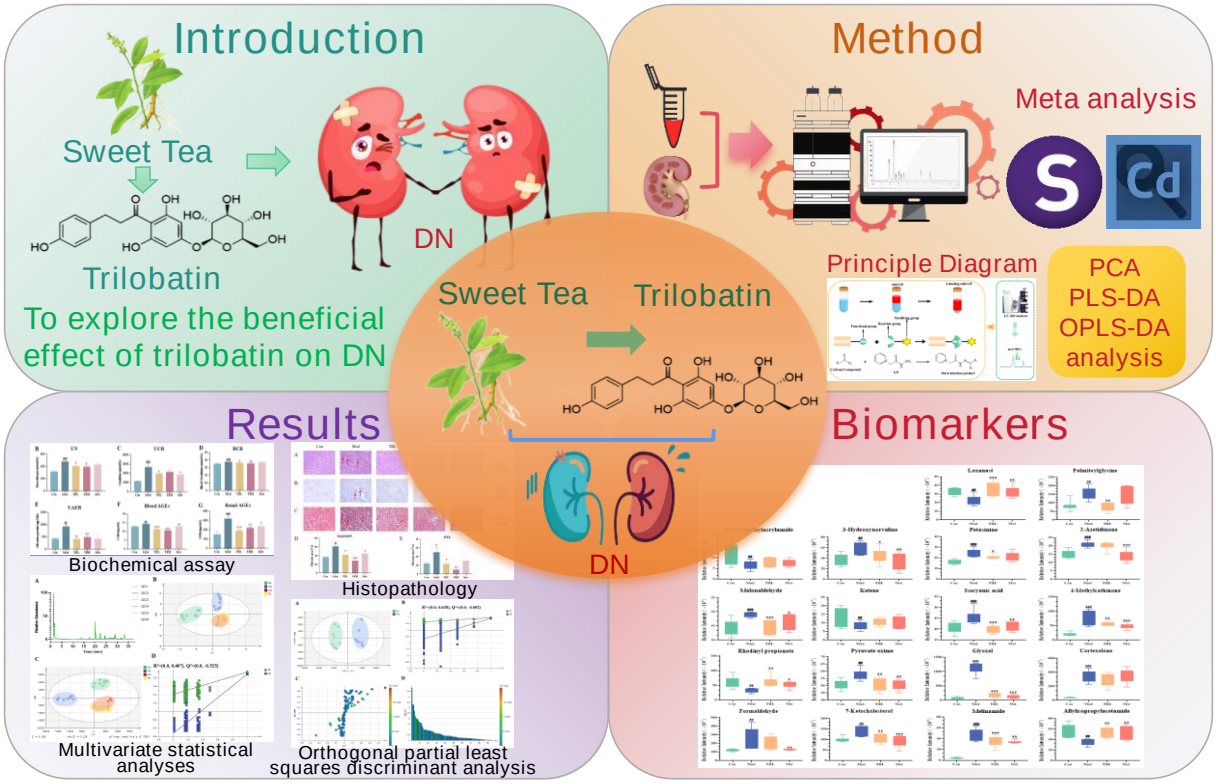
Abstract: Diabetic nephropathy is one of the most common micro-vascular complications in diabetic patients and is a metabolic disease in which chronic hyperglycemia leads to various renal cell dysfunctions, ultimately resulting in progressive renal failure. Carbonyl compounds induced carbonyl stress which further damages cellular structures and functions, exacerbating the progression of the pathogenesis of diabetes and its complications. Trilobatin, the main component of *Lithocarpus polystachyus* Rehd. or *Lithocarpus litseifolius* (Hance) Chun. commonly known as "Sweet Tea", has been shown to improve gluconeogenesis and lipid metabolism. This study explores the protective effects of trilobatin on diabetic nephropathy based on carbonyl stress, using a new metabolomics method combining chemical derivatization with UPLC-Q-Orbitrap HRMS/MS analysis. Firstly, a high-fat diet combined with multiple injections of low-dose STZ was used to establish a diabetic nephropathy mouse model. It has been shown that trilobatin improves blood glucose and lipid levels in diabetic nephropathy mice, and renal function indicators suggest that it has a regulatory effect on kidney damage following trilobatin administration. Furthermore, HE, Masson, and PAS staining were used to evaluate pathological damage in renal tissue. The results showed that trilobatin at various doses effectively improved histopathological changes, fibrosis, and glycogen accumulation in the kidneys of diabetic nephropathy mice. Finally, to further investigate the potential mechanism of trilobatin against diabetic nephropathy, a novel approach combining chemical derivatization with UPLC-Q-Orbitrap HRMS/MS analysis was employed to analyze the metabolic profiles of carbonyl compounds in

renal tissues of mice. For the metabolomic analysis, 18 carbonyl compounds including malondialdehyde, methylglyoxal, glyoxal, isocyanic acid, 7-ketocholesterol, formaldehyde were identified, which were significantly different between diabetic nephropathy and normal mice. Among them, there were 13 carbonyl compounds which had tendency to come back to normal levels after trilobatin treatment. The results of this study showed that trilobatin can protect kidney injury in diabetic by regulating carbonyl stress, making it a good prospect of developing into functional food/new drug research and development.



Quantification of β-elemene by GC-MS/MS and preliminary evaluation of its relationship with antitumour efficacy in cancer patients

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Abstract: The objectives of this study were to establish and validate a sensitive, robust and rapid gas chromatography-tandem mass spectrometry(GC-MS) method for the quantification of β-elemene in human plasma and to assess the correlation between antitumour effects and β-elemene exposure level in vivo. The chromatographic column was HP-5ms (30 m×0.25 mm, 0.25 μm, Agilent, USA), the carrier gas was helium (purity >99.5%), and heated using a programmed temperature. The flow rate was 1.0 mL/min and the total run time was 11.0 min. Plasma samples were pretreated with protein precipitation, and the supernatant was concentrated and subjected to liquid-liquid extraction with hexane. This study enrolled 73 malignant tumor patients and their plasma samples were collected to determine the exposure level of β-elemene. Calibration range of β-elemene was 200-20,000 ng/mL, with correlation coefficients > 0.99. The intra- and inter-day precision and accuracy were less than 1.9% and within the range of -10.38% to 6.6%. The exposure level of β-elemene in the responder group ranged from 11886.27 ng/ml to 278.13 ng/ml, with a median of 3568.91 ng/ml, while in the non-responder group, the range was from 9716.52 ng/ml to 675.92 ng/ml, with a median of 3351.94 ng/ml. No difference was found of β-elemene exposure level between the two groups(P>0.05). This method was successfully developed and validated and applied to determine the concentration of β-elemene in tumor patients, and the preliminary results proved no significant correlations between treatment outcomes and the exposure level of β-elemene.

Quality Control Study of Kunxian Capsule Based on UHPLC-MS/MS Technology and Network Pharmacology

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Abstract: Kunxian Capsule (KC) is a traditional Chinese herbal compound composed of *Kumming Shan Haitang*, *Epimedium*, *Goji berries*, and *Dodder seeds*, known for its effects in tonifying the kidneys, unblocking channels, expelling wind, and eliminating dampness. It is used in the treatment of rheumatoid arthritis (RA). The

primary chemical constituents of KC include flavonoids, alkaloids (particularly sesquiterpene alkaloids), and amides. Research has demonstrated that KC possesses anti-inflammatory, anti-tumor, immunosuppressive, and bone tissue-protective properties. While most current research on KC has focused on clinical applications, there has been relatively little investigation into its pharmacological basis, component analysis, and quality control. In this study, UHPLC-Q-TOF/MS technology was employed to identify the chemical constituents of KC. The research was combined with network pharmacology and ADME analysis to screen active components and predict their potential targets and mechanisms for treating RA. Subsequently, a detection method based on UHPLC-MS/MS was established. This method was used for the quantitative detection and quality transfer analysis of 24 active components in KC. The analysis identified 67 chemical components in KC, including 32 flavonoids, 20 alkaloids (18 of which are sesquiterpene alkaloids), 6 amides, and 5 terpenes. Network pharmacology screening identified 35 active components and predicted their potential mechanisms for treating RA. Additionally, a stable, accurate, and reliable detection method was developed, suitable for rapid high-throughput analysis. The study also determined the transfer rates of 9 key compounds from raw materials to dry extract powder and then to capsules, which are considered critical indicators of the preparation process of KC. In conclusion, this study not only reveals the complex chemical composition of KC but also proposes a multi-component, multi-target, and multi-pathway synergistic mechanism. It provides a scientific basis for the quality control of KC, ensuring the product's safety and efficacy, and lays an important foundation for further research.

Exploring the Occurrence Mechanism and Early-Warning Model of Phlebitis Induced by Aescinate Based on Metabolomics in Cerebral Infarction Patients

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Abstract: This study aims to explore the mechanism underlying the induction of phlebitis by aescinate and create an early-warning model of phlebitis based on metabolomics. Patients with cerebral infarction enrolled had been treated with aescinate. Plasma samples were collected either before administration of aescinate, upon the occurrence of phlebitis, or at the end of treatment. Non-targeted metabolomics and targeted amino acid metabolomics were carried out to analyze metabolic proffles and quantify the metabolites. Untargeted metabolomics revealed six differential metabolites in baseline samples versus post-treatment samples and four differential metabolites in baseline samples from patients with or without phlebitis. Pathways of these differential metabolites were mainly enriched in amino acid metabolism. Ten differential amino acids with a VIP value of >1 were identiffed in the baseline samples, enabling us to distinguish between patients with or without phlebitis. A logistic regression model Y ¼ 0:001*sarcosine 0:01*hippuric acid þ 2:46 was constructed (AUC 0.825) for early warning of phlebitis of grade 2 or higher. The occurrence of aescinate-induced phlebitis, which can be predicted early during onset, may be associated with perturbations of the endogenous metabolic proffle, especially the metabolism of amino acids.

Schisandrin B Exerts Anti-colorectal Cancer Effect through CXCL2/ERK/DUSP11 Signaling Pathway

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Abstract: Schisandrin B (Sch B) is an active component in Schisandra chinensis exerting anticancer effect, but the mechanism is obscure. This study was designed to explore and validate the mechanism of Sch B against colorectal cancer (CRC). Apparent experiments including cell proliferation, invasion and migration, colony formation, etc. were carried out to assess the anticancer effect of Sch B to CRC cell

lines. Results indicate that Sch B exhibits obviously inhibitory effect to cell proliferation, invasion and migration, colony formation, wound healing of CRC cell lines with a IC50 value at 75 μM. Then, the RNA-seq was performed prior to bioinformatics analysis to explore the key transcriptome alterations, furthermore, an untargeted metabolomics was carried out to profile the metabolic alterations after the treatment with Sch B and an integrated analysis and experiment validation were completed based on RNA-seq and metabolomics to find the critical mechanism. The RNA-seq and bioinformatics analysis found the ERK/MAPK pathway has been significantly suppressed by the Sch B treatment, while the chemokine, CXCL2, could activate the ERK pathway when binding to its receptor CXCR2. The metabolomics revealed the metabolic profile of CRC cell was remarkably influenced by the Sch B, focusing on the arginine and proline metabolism, ubiquinone and other terpenoid-quinone biosynthesis, etc. Importantly, the integrated analysis found the DUSP11 connected the ERK pathway and the glycometabolism and pyrimidine metabolism, may mediate the anticancer effect of Sch B; and subsequent experiments confirmed this finding. In summary, Sch B showed obviously anticancer effect to the CRC through inhibiting CXCL2/ERK/DUSP11 axis, and our findings provides a potential therapeutic medicine for CRC.

RadpidMass: A Graphical Data Processing Application Designed for Rapid Detection Mass Spectrometry for Identification and Characterization Based on Database

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Abstract: Rapid detection mass spectrometry techniques, such as direct infusion mass

spectrometry (DI-MS), atmospheric solids analysis probe mass spectrometry (ASAP-MS), and direct analysis in real-time mass spectrometry (DART-MS), offer high-throughput approaches for a wide range of analytical applications. However, the inherent diversity and complexity of mass spectrometry data present significant challenges, exposing the limitations of current databases and analytical methodologies. Traditional methods, including manual spectral analysis and chemometric techniques, are increasingly inadequate for the demands of high-throughput and automated data processing. To address these challenges, we introduce RapidMass, a versatile data management and analysis tool specifically designed to enhance the processing of rapid detection mass spectrometry data. In a rigorous evaluation, we selected 78 easily confusable flower varieties, comprising 540 batches of samples, and analyzed them under both positive and negative ion modes using DI-QDa and DI-QTOF instruments. RapidMass demonstrated exceptional performance, achieving Top1 identification rates exceeding 97%.The application of RapidMass facilitated accurate general origin identification of *Dendrobium* species, precise detection of *Rhodiola crenulata* and its adulterants, and showed promise in the geographical origin identification of *Chrysanthemum morifolium*, indicating broad applicability across various fields. Additionally, RapidMass features an intuitive visualization interface and supports the creation and management of personalized databases, thus enabling more tailored and efficient data analysis and research applications. In conclusion, RapidMass partially bridges the gap in general analytical tools for rapid detection mass spectrometry, offering a robust solution for the establishment of mass spectrometry databases and high-throughput analysis.

Species-specific identification of Colla corii asini and its non-donkey adulterating ingredients based on liquid chromatography–tandem mass spectrometry proteomics

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Abstract: Colla corii asini (CCA) is a protein-rich traditional Chinese medicine prepared from the dried or fresh skins of donkeys with high nutritional and medicinal value, so it is of great significance to identify CCA and its non-donkey (sheep, horse, pig, camel, and cattle) adulterating ingredients. In this work, liquid chromatography–tandem mass spectrometry proteomics technology combined with bioinformatics was applied to discover the specific peptide biomarkers in CCA and its non-donkey adulterating ingredients. Firstly, liquid chromatography–tandem mass spectrometry proteomics technology combined with bioinformatics was applied to discover the specific peptide biomarkers in CCA and its non-donkey adulterating ingredients. Secondly, we screened out the specific peptide biomarkers with higher content and higher specificity using an ultrahigh-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) multireaction monitoring (MRM) method. Thirdly, synthesizing these specific peptide biomarkers, a UPLC–MS/MS MRM analysis method was established and the limits of specific peptides of common non-donkey adulterating ingredients content in CCA were proposed. Finally, a total of nine specific peptide biomarkers (one in CCA, two in sheep skin gelatin, one in horse skin gelatin, one in pig skin gelatin, two in camel skin gelatin, and two in cow skin gelatin) with good signal responses were screened, a UPLC–MS/MS MRM method was established for them, and the limits of specific peptides of common non-donkey adulterating ingredients content in CCA were formulated. This work provides a rapid and simple method with high sensitivity and specificity for the authentication and

evaluation of the authenticity of CCA, improving its quality, ensuring the quality and safety of this product, and providing new ideas for the quality control research of foods and drugs with high protein content.

Comprehensive Authentication of Six *Bupleurum* Species and Their Tracebility in Herbal Formulations via a Novel Integrated Strategy

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The issue of multi-origin species and adulterants presents significant challenges to the safety and efficacy of herbal medicines. Bupleuri Radix, a renowned diaphoretic and febrifuge, is included in over 100 commercial products. However, numerous counterfeit products with similar appearances and chemical compositions create substantial market confusion. Currently, no practical method exists to reliably distinguish between authentic and adulterated samples. In response to this challenge, a novel integrated strategy was developed for the precise identification of six *Bupleurum* species and their traceability in commercial products. Multiple analytical technologies, including HPTLC, HPLC, and LC-HRMS, were employed to cross-validate data from different analytical dimensions. Additionally, an innovative R language-based feature ion screening algorithm was introduced to searching for

distinctive chemical markers. A systematic chemical component analysis of the six *Bupleurum* species revealed 489 compounds, allowing for the identification of these markers. The markers were then transferred from LC-HRMS to the more cost-effective LC-QDa instrument, enhancing the method's suitability for routine testing. The results demonstrated that the simultaneous authentication of *Bupleurum* species could not be achieved solely using TLC, HPLC, or LC-MS profiles, highlighting the need for a multi-technique approach to improve authentication accuracy. Through an in-depth analysis of LC-HRMS data using an integrated workflow, 13 differential markers were identified. These markers were applied to trace *Bupleurum* species in 68 batches of products using LC-QDa MS, revealing up to 45% adulteration in the market. This approach prioritizes both accuracy and practicality, offering a readily adaptable solution for the precise differentiation of other closely related and frequently confused herbal materials.

An integrated data filtering and identification strategy for rapid analysis of chemical constituents in *Dictamnus dasycarpus* Turcz.

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ABSTRACT: In this study, a comprehensive data filtering and identification strategy was developed. Firstly, a five-point mass defect filtering (MDF) screening was conducted for three subtypes of alkaloids and limonoids, respectively. Then, based on representative reference standards and literature, fragmentation patterns were explored to determine diagnostic ions for each type of compound. By using the in-house database of Dictamnus L., a total of 113 compounds were identified efficiently and accurately from the root bark of Dictamnus dasycarpus Turcz. (DD) extract, including 95 quinoline alkaloids and 18 limonoids. Simultaneously, the comparison of the polarity of isomers and the determination of their elution order was aimed at using CLogP values and dipole moments. The metabolite profiling of 36 batches of DD from 12 production regions was analyzed through untargeted metabolomics. Significant differences were observed in DD samples from different geographical origins, and 27 differential metabolites were identified (21 quinoline alkaloids and 6 limonoids). The results indicated that this method was an efficient, accurate, and promising approach for classifying and exploring compounds in the complex system of natural products, providing a basis for evaluating the quality of DD from different sources.

KEYWORDS: *Dictamnus dasycarpus* Turcz.; UHPLC-QTOF-MS; Mass defect filtering; Diagnostic ion database; Untargeted metabolomics

An integrated LC-MS/MS strategy for quantitative profiling multiple energy metabolites of tricarboxylic acid cycle，glycolysis and Oxidative phosphorylation pathway in mice and human

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Abstract: The central carbon energy metabolism, which are crucial metabolic pathway in almost all living organisms and in the regulation of responses to various kinds of stress. In this study, we established a method for the simultaneous quantification of 31 metabolites involved three pathways in the tricarboxylic acid (TCA) cycle, glycolysis and oxidative phosphorylation metabolic pathway by using the AB SCIEX 6500 QTRAP LC-MS/MS system. This LC-MS/MS method for profiling 31 endogenous metabolites offers significant advantages including simple and fast preparation of a wide range of mice and human biological samples, with good sensitivity and stability. The method was successfully validated with satisfactory linearity, sensitivity, accuracy, precision, matrix effects, recovery and stability for all analytes. Corresponding metabolomics analysis was performed in different tissues and organs of mice were demonstrated to display metabolic differences in different biological samples. Nonalcoholic fatty liver (NAFLD) disease is a metabolic disease of excess energy. By comparing the TCA, glycolysis and oxidative phosphorylation metabolome in high-fat diet-induced NAFLD mice with that of control mice, and NAFLD human with healthy human, we demonstrated a substantial metabolic disparity among different groups of mice and human, further verifying the applicability and reliability of our method.

The biosynthesis and evolutionary origin of indigo in *Isatis* plants

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Abstract: Indigo, a plant-originated blue dye, has a long and well-documented

history of extensive human use. In ancient China, several species of medical plants have been used for blue (indigo) dyeing, while also showing detoxifying activities. Such plants were referred to as “Lan” (indigo plant). Only *Isatis indigotica* is recorded as the original plant of Banlangen and Daqingye in Chinese Pharmacopoeia (2020). While genus *Isatis* has been a significant resource for indigo production, the biosynthetic pathway responsible for indigo remains unknown. Through phylogenetic and metabolic analyses of *Isatis* taxa, it appears that the capacity of indigo producing was apparently lost in certain taxa. Subsequent to de novo genome sequencing, assembly, and comparative analysis between *I. indigotica* and *I. cappadocica*, the origin and evolution of indigo biosynthesis were investigated. The involvement of multiple oxidase families, including flavin-containing monooxygenase (FMO) and cytochrome P450 protein (CYP), in indigo biosynthesis suggests that it is a metabolic innovation derived from the oxime pathway in plants. Additionally, the emergence of indigo biosynthesis in various plant taxa could be attributed to convergent evolution.

The method development for the identification and quantitative detection of antler and its adulterants based on droplet digital PCR

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Abstract： In this study, an efficient and sensitive droplet digital polymerase chain reaction (dd PCR) approach was developed for the quantitative analysis of *Cervi Cornu Pantotrichum* and its adulterants, including *Rangifer tarandus Linnaeus*. Firstly, the specific primer-probes for the detection were designed, and screened for

the best one based on the specificity and efficiency. Moreover, several important conditions were also optimized, such as the annealing temperature, the concentrations of primer-probe sets, the amount of DNA quantity, and so on. Furthermore, the methodology validation was conducted according to Chinese Pharmacopeia 2020 and ICH Q2. With this method, *Cervi Cornu Pantotrichum* could be specifically identified from *Rangifer tarandus Linnaeus*. The amplifying efficiency of this method was 0.95. It showed a strong linear correlation between the DNA contents and DNA copy numbers, with *Cervi Cornu Pantotrichum* in the range of 0.78-50.00 pg/mL (R2=0.9961), and the counterfeits in the range of 3.91-250.00 pg/mL (R2=0.9991). The average of recovery rate was 100.3% when the adulteration level was as low as 5%. In conclusion, this dd PCR method was specific, sensitive and accurate, and could be used for the precisely identifying and quantifying *Cervi Cornu Pantotrichum* and adulterants in the mixture.

Screening and validation of anti-inflammatory active ingredients in Wuwei Qingzhuo pill based on spectral-effect correlation

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Abstract: Wuwei Qingzhuo Pill(WQP) is a traditional Mongolian medicine used to treat chronic atrophic gastritis; however, its anti-inflammatory mechanism remains unclear. In this study, we conducted the anti-inflammatory active ingredients screeningbased on spectral-effect correlation. Firstly, a quality evaluation of WQP using fingerprinting combined with pattern recognition was evaluated to analyze different batches of the medicine. Then, the spectral-effect correlations were established wtih common peaks and inflammatory concentrations of NO, TNF-α,

IL-1β in RAW264.7 cells. Subsequently, candidate anti-inflammatory quality markers in WQP were screened based on the “five principles” of quality criteria. Additionally, molecular docking technology and in vitro cellular experiments were employed to verify the efficacy of these quality markers on inflammation. Ultimately, ellagic acid, gallic acid, and punicalagin glycosides were identified as the three active anti-inflammatory components in WQP. This study contributes to the scientific understanding of the material foundation as well as anti-inflammatory properties of WQP .

Systematic characterization of sesquiterpenes from *Dendrobium nobile* through offline two-dimensional chromatography tandem mass spectrometry and target isolation

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Abstract: *Dendrobium nobile* is the species of the *Dendrobium* genus, which can be used as both medicinal herb and healthy food. The sesquiterpenes in *D. nobile* have attracted extensive attention in recent years. In this study, Amide × RP offline two-dimensional chromatography separation tandem high resolution mass spectrometry combined with GNPS (Global Natural Product Social Molecular Networking) was developed for the characterization of sesquiterpenes in *D. nobile.* After first-dimensional Amide separation, the 70% ethanol extract of *D. nobile* was

divided into forty fractions, which were analyzed by second-dimensional reverse phase system separation and LTQ-Orbitrap detection. The raw data was imported into GNPS, resulting in efficient clustering of similar substances. Finally, 594 sesquiterpene compounds were characterized and twenty-five compounds were isolated based on molecular network analysis, including six new compounds. In vitro bioassays, the isolated compounds can decrease NO production in LPS-induced microglial BV-2 cells model and the content of MDA in PC12 cells, demonstrating neuroprotective activity. These findings unraveled the underlying material basis and provided valuable insights for quality control of *D. nobile*.

Rosmarinic acid enhances autophagy-lysosome pathways via ULK2-VPS35 signaling to mitigate Alzheimer's disease progression

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Abstract

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, driven by amyloid-beta (Aβ) plaque accumulation and tau protein tangles. Dysregulation of the autophagy-lysosome pathway is a key contributor to these pathologies. In this study, we identified rosmarinic acid (RosA), a natural polyphenolic compound, as a potent activator of autophagy. RosA enhances

autophagic flux by inhibiting the RNA helicase DDX3X, a critical autophagy regulator, as confirmed through molecular assays including immunoprecipitation and protein microarrays, showing its activation of the DDX3X/ULK2/VPS35 complex. In vitro experiments demonstrated that RosA reduced $A\beta_{(1-42)}$-induced neuronal cell death and enhanced neuronal survival. Structure-activity relationship analysis highlighted the importance of RosA's polyphenolic hydroxyl groups for its biological efficacy. In AD mouse models, chronic RosA administration not only significantly decreased Aβ plaque deposition but also improved synaptic density and enhanced hippocampal neurogenesis. Behavioral tests indicated obvious improvements in spatial memory and learning abilities in RosA-treated mice. Additionally, RosA treatment reduced neuroinflammation, decreases levels of pro-inflammatory cytokines (IL-1β, TNF-α), and normalized metabolic markers in these models. These findings suggested that RosA, through the modulation of the autophagy-lysosome pathway, could be a promising therapeutic candidate for effectively slowing AD progression and potentially reversing cognitive impairments associated with the disease.

The P-P-P system based on hydrochar of sustainably utilised *Panax pseudoginseng* residues (PHC) combined with self-assembled perylene diimide (PDI) organic supramolecular photocatalyst activated peroxodisulfate (PDS) for visible light removal of minocycline

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Abstract: Traditional Chinese medicine (TCM) is widely used in medicine and health

care, but the low extraction efficiency leaves a large amount of waste residue. With the continuous development of the TCM industry, the waste residues generated from TCM processing are also increasing, but most of the TCM residues are disposed of by direct landfill or incineration, causing environmental problems such as carbon emissions and global warming. Many attempts have been made to utilize these TCM residues, such as converting the residues into biogas, adsorbents, and sugar. Another possible option is to convert them into biochar, which is a porous and lightweight material. It has the prospect of various applications. In this study, PHC/PDI was firstly prepared by one-pot water bath heating method and electrostatic self-assembly method, and PDS was activated under visible light. The catalytic performance of the PHC/PDI/PDS (P-P-P) system was evaluated under visible light by eliminating minocycline in the aqueous solution. The effects of catalyst dosage, PDS concentration, pH value and other parameters on the degradation of minocycline were investigated. Based on the characterization results, free radical quenching experiments and electron paramagnetic resonance (EPR) measurements, a possible reaction mechanism for the P-P-P system was proposed. The intermediates of minocycline were identified using liquid chromatography mass spectrometry (LC-MS), and the attack sites of free radicals were confirmed by density-functional theory (DFT) calculations. Toxicity assessment was carried out using the Ecological Structure Activity Relationship (ECOSAR) program. By this method, a novel visible-light photocatalytic degradation system for the efficient degradation of minocycline was constructed.

Exploring the efficacy and mechanism of action of Puerarin in improving diabetes mellitus based on HIF-1α

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Abstract

Background and Aims: Diabetes mellitus is a complex polygenic disease that is

influenced by a combination of genetic and environmental factors and has a high morbidity and mortality rate, which can lead to complications such as coronary heart disease, stroke, retinopathy, cognitive dysfunction, heart failure and other related complications. According to a survey by the International Diabetes Federation (IDF), there are about 464 million people with diabetes worldwide. Among them, type 2 diabetes mellitus (T2DM) accounts for more than 90% of the diabetic population.T2DM is mainly caused by defective insulin secretion from pancreatic β-cells and Insulin Resistance (IR). First, β-cells are responsible for insulin production, and high glucose concentration triggers insulin release, which can also be induced by amino acids, fatty acids, and hormones. And inflammation, oxidative stress, endoplasmic reticulum stress, glucotoxicity, and lipotoxicity can lead to β-cell dysfunction. When β-cell dysfunction leads to abnormal insulin secretion, which triggers diabetes. Currently, the main clinical drugs used to treat T2DM are oral hypoglycemic agents and insulin analogs. Although oral hypoglycemic agents are effective in controlling blood glucose, they have certain limitations and side effects. Therefore, there is an urgent need to find effective, mild and safe effective drugs for diabetes.

Insulin resistance is the insensitivity of insulin-sensitive tissues (liver, adipose, muscle tissues) to insulin, preventing insulin from exerting its biological effects. The liver, as an important organ in maintaining the body's glucose-fat metabolism, is the first to develop insulin resistance. When monosaccharides cannot be converted to glycogen, they are converted to triglycerides (TG) and free fatty acids (FFA), and when there is an excess of TG and FFA in the blood, they accumulate in the liver and cause lipid deposition in tissues. The accumulation of hepatic lipid ectopic deposition further contributes to the onset and development of IR and T2DM. Therefore, it is important to study abnormalities in hepatic lipid metabolism for the development and progression of IR and T2DM.

Hypoxia inducible factor-1 (HIF-1) is a major transcription factor adapted to the hypoxic response, and is composed of two basic helix-loop-helix structures of the PAS (Per/Arnt/Sim) family, HIF-1α and HIF-1β.HIF-1α, the active subunit of HIF-1,

is the main part of HIF-1 that exerts its function to affect disease by agitating the transcription of genes for angiogenesis, erythropoiesis, glucose transport, glycolysis, and ceramide synthase. A high-fat diet has been reported to lead to increased expression of HIF-1α in the liver, which causes steatosis in hepatocytes, which in turn induces hepatic steatosis. Sphingolipid metabolism is closely related to the development of diabetes, in which ceramide is the center of sphingolipid metabolism and one of the most important causes of insulin resistance and diabetic process. Ceramide, as a harmful lipid, accumulates in obese and dyslipidemic individuals, triggering tissue dysfunction that is associated with diabetes and cardiovascular disease. Studies have shown that ceramide is a key player in the induction of β-cell apoptosis, insulin resistance and reduced insulin gene expression. Therefore, intervening in HIF-1α signaling to regulate ceramide may be one of the effective strategies for the treatment of diabetes.

Puerarin (8-beta-D-glucopyranose-4',7-dihydroxyisoflavone) is a natural compound of isoflavonoids extracted from the roots of the legume Pueraria thomsonii Benth, which is high in content and activity in Pueraria lobata. Puerarin may protect pancreatic β-cells by regulating the PI3K/Akt signaling pathway, enhancing the GLP-1 signaling pathway, attenuating oxidative stress injury, and inhibiting β-cell apoptosis. Puerarin may also ameliorate diabetic hepatic lipid deposition and liver injury, and inhibit iron death and inflammation to ameliorate metabolic dysfunction in fatty liver.

The association between puerarin and HIF-1α, liver tissue lipid metabolism and ceramide in T2DM is not clear, and the underlying mechanisms are unknown. The aim of this study was to investigate the potential mechanism of action of puerarin to improve diabetes and to provide a scientific basis for the treatment of diabetes by puerarin.

METHODS AND RESULTS:

(1) In vivo, a diabetic mouse model was constructed by using a high-fat and high-sugar diet combined with streptozotocin (STZ), and the successfully modeled

mice were divided into the normal group, the model group, the puerarin high-dose group, the puerarin low-dose group, and the positive-medicine group. Citric acid-sodium citrate buffer was used in the normal group, and metformin was used in the positive drug group. Fasting blood glucose (FBS), oral glucose tolerance (OGTT), and fasting insulin (FINS) were tested in mice with different administration doses, and we found that Puerarin had a significant effect on reversing hyperglycemia. The results showed that Puerarin administration significantly reduced the blood glucose concentration in diabetic model rats. The significant difference between the model group and the administered group increased and was dose-dependent as the administration time increased. We found that Puerarin had a significant effect on protecting the liver and regulating blood lipids by using kits for liver injury indicators and lipid metabolism indicators. Puerarin administration significantly reduced the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in diabetic mice, while total cholesterol (TC), triglycerides (TG), and lipoproteins (HDL, LDL) were significantly down-regulated.

(2) By constructing an insulin-resistant HepG2 cell model and detecting the glucose consumption of IR-HepG2 cells in different groups, we found that Puerarin improved insulin resistance in IR-HepG2 cells. By Drug Affinity Response Target Stability Technique (DARTS), we found that Puerarin retained HIF-1α protein. Then using Dimethyloxallyl Glycine (DMOG) to inhibit the degradation of HIF-1α in the insulin-resistant HepG2 cell model, we found that the effect of Puerarin to improve glucose depletion in IR-HepG2 was reversed.

(3) In vivo, a diabetic mouse model was constructed using a high-fat, high-sugar diet combined with streptozotocin (STZ), and we administered DMOG to the mice to inhibit the degradation of HIF-1α, and detected FBS, OGTT, FINS, liver injury and lipid indices of the mice in different groups. The results showed that the DMOG-Puerarin administration group significantly elevated the blood glucose concentration in diabetic mice. Detection of liver injury indexes and lipid metabolism indexes showed that DMOG-Puerarin administration group elevated ALT, TC, TG levels in diabetic mice. Histopathological stained sections revealed that liver injury

and hepatic lipid droplet deposition were significantly increased after administration of DMOG to inhibit the degradation of HIF-1α. Western blot results showed that Puerarin administration significantly reduced HIF-1α and SPTLC2 protein expression. The effect of Puerarin in reducing HIF-1α and SPTLC2 protein expression was reversed when DMOG was given. The content of ceramide in the liver of mice was determined and the ceramide content in the liver tissue of mice in the Puerarin administration group was significantly reduced compared to the model group.

CONCLUSION: In summary, we demonstrated that Puerarin has a significant role in reversing hyperglycaemia, regulating blood lipids and protecting the liver in diabetes. By DART S technology, an interaction relationship between Puerarin and HIF-1α was found. In vitro experiments showed that DMOG inhibited the effects of puerarin in reducing the expression of HIF-1α protein in IR-HepG2 and puerarin in improving insulin resistance in IR-HepG2 cells. It reversed the effects of puerarin in reducing HIF-1α and SPTLC2 protein expression and ceramide content and inhibited the effects of puerarin in improving blood glucose, oral glucose tolerance, liver injury, and hepatic lipid deposition in diabetic mice by in vivo administration of DMOG. These evidences suggest that puerarin ameliorates diabetes by regulating ceramide synthesis through the HIF-1α pathway, providing a scientific basis for the treatment of diabetes by Puerarin. **Key Words:** Diabetes mellitus; Puerarin; HIF-1α; SPTLC2; ceramide

Dual-Channel Fluorescent Probe for the Simultaneous Monitoring of Nitric oxide and Methylglyoxal *in vivo*

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Abstract: The fluctuations in the concentrations of the signaling molecules nitric oxide (NO) and methylglyoxal (MGO) are closely associated with the development of various diseases, including drug-induced liver injury (DILI), chronic inflammatory conditions like diabetes, and tumors. It is imperative to continuously monitor these two molecules to gain insights into the pathogenic mechanisms underlying these diseases. Herein, a dual-responsive probe TPA-4T was developed to simultaneously monitor NO and MGO levels by harnessing its dual color imaging capability. Under the influence of NO, the probe TPA-4T transformed from an o-phenylenediamine (OPD) structure to a benzothiadiazole structure TPA-4T-NO (channel 1, λ_{abs}=640nm, λ_{em}=940nm). Conversely, MGO oxidation led to the red-shifted conversion of TPA-4T to the NIR-II dye of TPA-4T-MGO (channel 2, λ_{abs}=740nm, λ_{em}=1040nm). The use of probe TPA-4T as a combined NO and MGO probe was demonstrated in cellular imaging studies. Under the treatment of AML 12 with LPS, resulted in increasing signal intensity in channel 1, and similar fluorescence changes were seen in channel 2 in the presence of an MGO donor. Notably, real-time imaging of endogenous MGO and NO generation in two independent channels without spectral cross-interference was achieved in acute or chronic inflammatory diseases, including DILI, subcutaneous tumors, and type II diabetes. In conclusion, the dual-responsive probe TPA-4T targeting MGO and NO could be an efficient and reliable tool for monitoring inflammation-related pathological processes.

Keywords: Metabonomics, Serotonin, Ischemic stroke, Naomaitong, Tryptophan, Akkermansia muciniphila