A new strategy for analyzing the active ingredients of herbal medicines based on the public bioinformatics platforms

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Abstract

Background and Purpose The active ingredients analysis of herbal medicines is crucial for their quality control and efficacy/safety evaluation. Here, we propose a new strategy for effectively and rapidly analyzing the active ingredients of herbal medicines based on the public bioinformatics platforms. In the study, the treatment of osteoarthritis (OA) by Herba Lysimachiae (HL) was used as an example to practice this strategy. Experimental Approach Applying the public databases (PubChem BioAssay and STRING) to establish the links between herbal compounds and the biolabels (integrin alpha 2b/beta 3) of HL treating OA, from which herbal compounds that may regulate both biolabels were screened. OA model was used to confirm the analysis results. Key Results Five compounds (myricetin, fisetin, esculetin, 7-hydroxycoumarin-4-acetic acid, and caffeic acid) from HL synergistically regulated both biolabels through eleven targets, which may be the active ingredients of HL treating OA. Conclusion and Implications The experiments in the OA model verified the analysis results. With the help of the analysis strategy, the present study effectively and rapidly analyzes the active ingredients of HL treating OA. The example in this research also verifies the feasibility of this strategy, which is of reference significance to the related studies of other herbal medicines.

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Abstract

Background and Purpose

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Experimental Approach

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Key Results

Five compounds (myricetin, fisetin, esculetin, 7-hydroxycoumarin-4-acetic acid, and caffeic acid) from HL synergistically regulated both biolabels through eleven targets, which may be the active ingredients of HL treating OA.

Conclusion and Implications

The experiments in the OA model verified the analysis results. With the help of the analysis strategy, the present study effectively and rapidly analyzes the active ingredients of HL treating OA. The example in this research also verifies the feasibility of this strategy, which is of reference significance to the related studies of other herbal medicines.

Keywords: bioinformatics; herbal medicines; active ingredients; Herba Lysimachiae; osteoarthritis

Abbreviations: **CMC** , carboxymethylcellulose; **DSS** , diclofenac sodium salt; **ELISA** , enzyme-linked immunosorbent assay; **HL** , Herba Lysimachiae; **Itga2b** , integrin alpha 2b; **Itgb3** , integrin beta 3; **MIA** , monosodium iodoacetate; **OA,** osteoarthritis;

What is already known:

The present methods and strategies for analyzing the active ingredients of herbal medicines are still challenging, owing to the complex disease pathophysiology and herbal composition.

What this study adds:

We propose a new strategy for effectively and rapidly analyzing the active ingredients of herbal medicines based on the public bioinformatics platforms.

Clinical Significance:

The active ingredients analysis of herbal medicines is crucial for their quality control and efficacy/safety evaluation.

Introduction

The World Health Organization estimates that about 80% population in the developing countries apply herbal medicines for their primary health care (Li et al., 2017). Additionally, herbal medicines also play an important role in the treatment of some major diseases, such as COVID-19 (Yang et al., 2020), cardiovascular diseases (Hao et al., 2017), and cancers (Wang et al., 2020), etc. Recently, plenty of experimental studies have been carried out on the mechanism of action of herbal medicines, and the herb targets have been identified. With the massive accumulation of the previous researches (Chen et al., 2019; Liu et al., 2018b; Liu et al., 2018c), the analysis of the active ingredients that can regulate these targets will be the research focus in the future. The active ingredients analysis of herbal medicines is crucial for their quality control and efficacy/safety evaluation. However, the present methods and strategies are still challenging, owing to the complex disease pathophysiology and herbal composition. Here, we propose a new strategy for effectively and rapidly analyzing the active ingredients of herbal medicines based on the public bioinformatics platforms. This strategy may extend and develop the achievements of the previous herb targets researches, and also provide the references for the later development and clinical application of herbal medicines, which serves as a link between the preceding and the following.

PubChem BioAssay and STRING are the public bioinformatics platforms, both of which are open-accessed and user-friendly. PubChem BioAssay database contains the bioactive targets information of small molecules, which is generated through the experiments and literatures (Wang et al., 2017). STRING database aims to achieve a comprehensive and objective protein-protein interaction networks and allows users to visualize

the interaction networks (Szklarczyk et al., 2019). Both public resources can be applied in this strategy. At present, there are mainly two ways to obtain the information of herbal compounds. One method is directly based on the compound analysis techniques (Li et al., 2020; Liu et al., 2018a; Wang et al., 2019); the other method is based on the retrieval from the database and literature (Ding et al., 2020; Li et al., 2017; Zhang et al., 2017). Herbal medicine contains multiple compounds with different polarities. Using the different solvents for the preparation of herbal extracts will affect their composition of compounds. Therefore, the herbal compounds in each extract may not be exactly the same as those in the database and literature. Compared with the retrieval from the database and literature, the results of the compound analysis techniques (eg. LC-MS/MS analysis) may better reflect the true composition of herbal extracts (Liu et al., 2018a; Wang et al., 2019). Previous research has shown that Herba Lysimachiae (HL), the dried entire plant of *Lysimachia paridiformis* Franch. var.*stenophylla* Franch. (Primulaceae), can regulate the synovial platelet aggregation through the biolabels (integrin alpha 2b (Itga2b) and integrin beta 3 (Itgb3)) (Li et al., 2020). This is beneficial to alleviate synovial injury in osteoarthritis (OA) (Li et al., 2020). The prevalence rate of OA is 2-6% around the world and exceeds 40% in people over 70 (Hsu and Siwiec, 2019). Biolabels, also the herb targets, reflect the holistic effects of herbal medicines on the living organisms (Li et al., 2020; Li et al., 2019). Biolabels can be used not only to position the therapeutic effects of herbal medicines, but also to guide the analysis of their active ingredients (Li et al., 2020; Li et al., 2017; Zhang et al., 2017). Currently, the relevant active ingredients are still unknown. Based on previous research (Li et al., 2020), we may further use this strategy to carry out the subsequent analysis of the active ingredients. Firstly, LC-MS/MS technique is used to analyze the herbal compounds; subsequently, the compound targets are searched in PubChem BioAssay database; then, STRING database is used to analyze the association network between the biolabels and compound targets. Finally, based on the association network, the links between herbal compounds and biolabels are established, from which the herbal compounds with the potential to regulate the biolabels are screened (Figure 1). These compounds may be the active ingredients of herbal medicines in treating diseases. In the current study, the treatment of OA by HL was used as an example to practice this strategy. OA model was used to confirm the analysis results.

Materials and methods

Reagents

Myricetin (62822A, 98%), fisetin (62732B, 98%), and diclofenac sodium salt (DSS, 26107B, 98%, positive drug) were purchased from Adamas Reagent Co., Ltd (Shanghai, PR China). Monosodium iodoacetate (MIA, S104897, 98%), esculetin (D118867, 98%), and caffeic acid (C108306, 98%) were purchased from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, PR China). 7-Hydroxycoumarin-4-acetic acid (H1501, 98%) was purchased from TCI (Shanghai) Development Co., Ltd (Shanghai, PR China). Rat integrin alpha 2b (Itga2b, CD41, EL702469) and integrin beta 3 (Itgb3, CD61, EL702475) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Eterlife Co., Ltd. (Birmingham, UK). Anti-Itga2b (DF7456) and Itgb3(AF6086) antibodies were purchased from Affinity Biosciences, Inc. (Zhenjiang, PR China). Goat anti-rabbit IgG H&L (HRP) (ab205718) was purchased from Abcam plc. (Cambridge, UK).

Plant material and extraction

The herb was collected from Guanling County of Guizhou Province, PR China. A voucher specimen (GUCM-VS-2018-001) of HL is preserved in College of Pharmacy, Guizhou University of Traditional Chinese Medicine. The preparation of freeze-dried powder of HL was shown in our previous experiment (Li et al., 2020). The yield of the freeze-dried powder is 12.65% (w/w).

LC-MS/MS technique for the herbal compounds analysis of HL

The chromatographic profile of freeze-dried powder of HL was assessed by an ExionLC chromatographic system couple with a TripleTOF 5600+ Q-TOF mass spectrometer (AB SCIEX, USA). Herbal compounds were identified by Progenesis QI software (version: 2.4, Waters, MA, USA) based on the integrated MS/MS databases derived from NIST and METLIN.

In-house compound-target database construction

The names of herbal compounds were imported into the PubChem (https://pubchem.ncbi.nlm. nih.gov). The targets' information ("Activity": Active) from the BioAssay results was downloaded to construct the in-house compound-target database.

Compound-target-biolabel link profile of HL treating OA construction

The association network of compound targets and both biolabels was assessed by STRING v11 (https://string-db.org/). The "repacxn" of compound targets and the gene names of both biolabels were imported into the input-form as "multiple proteins" and "Homo sapiens" was selected as the reference organism. The compound targets associated with both biolabels (combined score more than 0.900) were screened from the association network. Based on these targets, the relevant herbal compounds were retrieved from the in-house compound-target database. The above information was used to establish the compound-target-biolabel link profile of HL treating OA. *OA model for the validation ofthe active ingredients analysis results*Male Sprague Dawley rats (270g˜, SPF) from Tianjin Hospital of ITCWM Nankai Hospital (Tianjin, PR China) were randomly divided into control, MIA model, MIA + DSS, and MIA + herbal compounds-treated groups (MIA + low-, MIA + middle-, and MIA + high-dose), and there were 5 rats in each group. The protocol was approved by the Animal Ethical and Welfare Committee of Tianjin Hospital of ITCWM Nankai Hospital (approval No. NKYY-DWLL-2020-058) and followed by the Legislation on the Protection of Animals Used for Experiment Purposes (Directive 86/609/EEC). Rats were housed in groups in standard cages, kept under 12 h light/dark conditions, and had free access to food and water throughout. Animal studies followed with the recommendations made by the British Journal of Pharmacology.

MIA model, MIA + DSS, and MIA + herbal compounds-treated groups were anesthetized with 400 mg/kg chloral hydrate (i.p.). MIA (exactly 3 mg in 50 µl 0.9% sterile saline) was injected into the knee joint through the patellar ligament with a 26 G needle. Control rats received injections of 0.9% sterile saline after anesthesia. After 24 h of modeling, the MIA + low-, MIA + middle-, and MIA + high-dose groups were intragastrically administrated with the herbal compounds (in 0.5% carboxymethylcellulose (CMC)) at the dose of 14, 28, and 56 mg/kg in rat, respectively, once daily for 7 days. MIA + DSS group received 14 mg/kg DSS (in 0.5% CMC, p.o) once a day for 7 days. Control and MIA model groups were orally administrated with equal volume of 0.5% CMC once a day for 7 days.

The diameter of joints was measured at 24 h after the last treatment via using a digital vernier caliper (Preisser Products, Germany). Subsequently, the synovial samples were dissected from the joints for histopathological, immunohistochemical, and ELISA analyses.

Synovial sample was immediately fixed in 4% paraformaldehyde overnight, then embedded in paraffin, sectioned into 5 mm cross-sectional pieces by using a paraffin slicer (RM2016, LEICA, Shanghai, PR China). Paraffin sections were stained with hematoxylin-eosin for histopathological analysis and stained with primary and secondary (1:2000) antibodies for immunohistochemistry analysis. Primary antibodies were rabbit anti-rat Itga2b and Itgb3, at dilutions of 1:100 each. Each slide was observed in a microscope (XSP-C204, CIC, PR China). The percent areas of Itga2b and Itgb3 were assessed by ImageJ software (ver. 1.8.0; https://imagej.nih.gov/ij/). The experimental detail followed the Guidelines of British Journal of Pharmacology (Alexander et al., 2018).

Synovial samples were homogenized in ice-cold 0.9% saline. The homogenate was centrifuged at 3,000 rpm for 10 min at 4 , and the supernatant was transferred to another tube. The levels of Itga2b and Itgb3 in the tissue homogenate was assessed with the ELISA kits according to the manufacturer's instructions.

The statistical comparison of joint diameter, immunohistochemistry, and ELISA data was performed with Aov (ANOVA) and Tukey HSD (multiple comparison) in the R Programming Language (version 3.5.2). Statistical analysis data were presented as mean ± standard deviation and statistical significance was accepted if $P < 0.05$.

Results

Herbal compounds analysis of HL

The chromatographic profile of freeze-dried powder of HL is shown in Figure 2. Ninety compounds (thirty-six organic acids, thirty flavonoids, six purines, five coumarins, four tannins, and nine others) in HL were identified (Supplementary Table S1). The MS/MS spectra data of these compounds is shown in Supplementary File S1.

In-house compound-target database construction

The in-house compound-target database constructed by PubChem BioAssay database is available in Supplementary Table S2. Sixty-four compounds from HL, such as myricetin, esculetin, and caffeic acid, etc., may act on 451 targets.

Compound-target-biolabel link profile of HL treating OA construction

The association network of compound targets and both biolabels assessed by STRING database is shown in Supplementary Figure S1. Based on the in-house database and the association network, the compound-target-biolabel link profile of HL treating OA was established (Figure 3). Five compounds from HL (myricetin, fisetin, esculetin, 7-hydroxycoumarin-4-acetic acid, and caffeic acid) synergistically regulated both biolabels (Itga2b and Itgb3) through eleven targets (CBFB, FYN, HDAC1, HSP90AA1, ITGA4, LCK, MAPK1, PIK3CA, PTPN1, RUNX1, and SYK). These five compounds may be the active ingredients of HL treating OA.

OA model for the validation of the active ingredients analysis results

Joint swelling is one clinical symptom of OA reflecting the presence of synovitis (Berenbaum, 2013) (Figure 4A). Compared to the joint diameter in the control group, that in MIA model group was increased by 47.6%. Compared to the joint diameter in MIA model group, that in MIA + DSS group was decreased by 17.2%; those in MIA + 14, 28, and 56 mg/kg myricetin groups were decreased by 21.9, 20.2, and 18.7%, respectively; those in MIA + 14, 28, and 56 mg/kg fisetin groups were decreased by 22.8, 20.6, and 21.2%, respectively; those in MIA + 14, 28, and 56 mg/kg esculetin groups were decreased by 23.7, 19.5, and 19.6%, respectively; those in MIA + 14, 28, and 56 mg/kg 7-hydroxycoumarin-4-acetic acid groups were decreased by 18.4, 16.9, and 20.0%, respectively; those in MIA + 14, 28, and 56 mg/kg caffeic acid groups were decreased by 18.0, 17.0, and 14.6%, respectively.

The change of microstructure can directly reflect the synovial damage (Figure 4B and Supplementary File S2). Synoviocytes in the control group were monolayer and arranged compactly and regularly, and there was no abnormal inflammatory cell in synovial tissue. A serious inflammatory damage occurred in the MIA model rats, and irregular synoviocytes arrangement, synovial hyperplasia, and inflammatory cells infiltration were found in synovial tissue. DSS and the herbal compounds showed the tendency to repair the synovial tissue damaged by MIA.

Itga2b and Itgb3 are abundantly and predominantly expressed in platelets and promote their aggregation. The levels of both subunits can represent the number of platelets (Li et al., 2020). In immunohistochemical analysis (Figure 5 and Supplementary File S3), compared to the percent areas of Itga2b/Itgb3 in the control group, those in the MIA model group were increased by 95.5/75.6%. Compared to the percent areas of Itga2b/Itgb3 in MIA model group, those in MIA + DSS group were decreased by 28.6/19.7%; those in MIA + 14, 28, and 56 mg/kg myricetin groups were decreased by 31.3/22.6, 37.5/31.9, and 40.8/41.8%, respectively; those in MIA + 14, 28, and 56 mg/kg fisetin groups were decreased by 37.2/27.9, 25.4/22.1, and 28.0/36.5%, respectively; those in MIA + 14, 28, and 56 mg/kg esculetin groups were decreased by 44.0/36.8, 37.2/23.1, and 31.7/22.3%, respectively; those in MIA + 14, 28, and 56 mg/kg 7-hydroxycoumarin-4-acetic acid groups were decreased by 31.8/34.6, 41.0/25.7, and 23.7/28.1%, respectively; those in MIA + 14, 28, and 56 mg/kg caffeic acid groups were decreased by 27.6/37.6, 31.2/35.3, and 29.4/22.6%, respectively.

In ELISA analysis (Figure 6), compared to the levels of Itga2b/Itgb3 in the control group, those in the MIA model group were increased by 111.3/113.0%. Compared to the levels of Itga2b/Itgb3 in MIA model group,

those in MIA + DSS group were decreased by 37.7/35.5%; those in MIA + 14, 28, and 56 mg/kg myricetin groups were decreased by 28.7/46.0, 40.3/51.8, and 26.4/51.1%, respectively; those in MIA + 14, 28, and 56 mg/kg fisetin groups were decreased by 38.4/49.1, 23.9/29.6, and 26.1/40.3%, respectively; those in MIA + 14, 28, and 56 mg/kg esculetin groups were decreased by 43.0/45.0, 36.2/29.9, and 25.3/25.2%, respectively; those in MIA + 14, 28, and 56 mg/kg 7-hydroxycoumarin-4-acetic acid groups were decreased by 70.8/28.7, 34.4/27.9, and 25.3/44.8%, respectively; those in MIA + 14, 28, and 56 mg/kg caffeic acid groups were decreased by 25.1/24.4, 25.3/17.7, and 23.9/21.6 %, respectively.

Discussion

The current study provides a new strategy for effectively and rapidly analyzing the active ingredients of herbal medicines based on the public bioinformatics platforms, which fills the gap in existing research. With the help of the public resources (PubChem BioAssay and STRING), this analysis strategy has the advantages of simplicity, convenience, efficiency, rapidness, and low-cost. In the following sections, we briefly discussed this strategy using the treatment of OA by HL as an example. Itga2b and Itgb3 are two subunits of integrin, both of which heterodimerize to form a complex (Cordeiro et al., 2016). The expression of both subunits may be influenced by each other. As shown in Figure 3, eleven compound targets (CBFB, FYN, HDAC1, HSP90AA1, ITGA4, LCK, MAPK1, PIK3CA, PTPN1, RUNX1, and SYK) are combined with both biolabels to form a synergistic regulation network with Itga2b and Itgb3 as the core. According to Reactome pathway database (https://reactome.org), CBFB, RUNX1, and HDAC1 may regulate Itga2b gene transcription; LCK interacts with SYK to bind to Itga2b and Itgb3; PTPN1 interacts with Itga2b and Itgb3. PIK3CA, MAPK1, and both biolabels participate in platelet activation pathway annotated by KEGG (https://www.kegg.jp/kegg/). PIK3CA and MAPK1 may activate Itga2b and Itgb3 through a series of cascade reactions. In platelet activation pathway of KEGG database, FYN modulates the downstream signal to activate Itga2b as well. Through the prediction of SRTING database, HSP90AA1 and ITGA4 are very likely to bind to Itgb3 and regulate this subunit. In Figure 3, each compound may only modulate the biolabels by acting on two to four targets. Therefore, if some of the targets are mutated, it may lead to the single compound's efficacy drop, and even failure. HL is a complex system composed of multi-compounds, five of which (myricetin, fisetin, esculetin, 7-hydroxycoumarin-4-acetic acid, and caffeic acid) may synergistically regulate the expression of both biolabels through their interventions on eleven targets. Thus, compared with the intervention mode of a single compound, the multi-compounds and multi-targets synergistic mode of action of herbal medicines is more capable of producing stable efficacy in the treatment of diseases and may reduce the possibility of drug resistance. The above analysis indicates that myricetin, fisetin, esculetin, 7-hydroxycoumarin-4-acetic acid, and caffeic acid may be the active ingredients of HL treating OA. To verify the analysis results, we examined the effects of these compounds on the joint swelling, synovial pathological injury, and synovial platelet aggregation in the OA rat model induced by MIA. Previous studies have shown that all of these compounds, except for 7- hydroxycoumarin-4-acetic acid, may play the protective roles in OA cartilage (Huang et al., 2018; Pan et al., 2019; Yamada et al., 1999; Zheng et al., 2017). Besides cartilage, the synovium in OA also suffers significant damage, and accompanied by synovial platelet aggregation (Alunno et al., 2014; Balbaloglu et al., 2014; Li et al., 2020). Subsequently, the platelet was activated to trigger thrombus formation in synovial microcirculation and participate in cartilage damage (Balbaloglu et al., 2014). However, to our knowledge, no study has shown that these compounds can protect OA synovium by regulating the platelet aggregation. Therefore, this study also provides a new reference for their mechanism of action in the treatment of OA. In the MIA-induced OA rat model (Figure 4), the synovium displays obvious damage, including irregular synoviocytes arrangement and synovial hyperplasia. Meanwhile, the expression of Itga2b and Itgb3 in the damaged synovium was significantly up-regulated (Figures 5 and 6), indicating a massive aggregation of platelets at this site. This is consistent with the previous studies (Alunno et al., 2014; Balbaloglu et al., 2014; Li et al., 2020). The results of the current experiment show that these compounds may reverse the overexpression of both biolabels induced by MIA, which may protect OA synovium against the platelet aggregation (Figures 5 and 6).

Joint swelling reflects the presence of synovitis (Berenbaum, 2013). Histopathological analysis in Figure 4 also shows that a large number of inflammatory cells infiltrated into the damaged synovium induced by

MIA. Previous studies have shown that these compounds have anti-inflammatory effects (Chang and Chiang, 1995; Hou et al., 2018; Huang et al., 2018; Tubaro et al., 1988; Zheng et al., 2017), which is conducive to the recovery of joint swelling and the inflammatory damage in the synovium (Figure 4). Additionally, platelet aggregation and activation are also the markers of synovial inflammation in OA (Alunno et al., 2014). From this view, their anti-inflammatory effects may also contribute to the inhibition of platelet aggregation by these compounds in OA synovium.

Conclusions

With the help of the analysis strategy, the present study effectively and rapidly analyzes the active ingredients of HL treating OA. The example in this research also verifies the feasibility of this strategy, which is of reference significance to the related studies of other herbal medicines. Meanwhile, the results also display the multi-compounds and multi-targets synergistic mode of action of herbal medicines and provide the important reference information for their basic researches and clinical applications. In addition, based on these results, we also propose that researchers should use an active ingredients group instead of a single compound to control the quality of herbal medicines according to the pathogenesis of different diseases.

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Declaration of interest

The authors declare that they have no conflict of interest.

Author Contributions

Xu-zhao LI and Shuai-nan ZHANG conceived and designed the study, developed the methodologies, acquired the data, analyzed and interpreted the data, and wrote the paper.

References

Alexander SPH, Roberts RE, Broughton BRS, Sobey CG, George CH, Stanford SC, et al. (2018). Goals and practicalities of immunoblotting and immunohistochemistry: A guide for submission to the British Journal of Pharmacology. Br J Pharmacol 175: 407-411.

Alunno A, Petito E, Mirabelli G, Falcinelli E, Luccioli F, Momi S, et al. (2014). THU0194 Role of Platelets in the Pathogenesis of Osteoarthritis and Biological Effects of Hyaluronic Acid: in Vivo and in Vitro Study. Ann Rheum Dis 73: 248-249.

Balbaloglu O, Korkmaz M, Yolcu S, Karaaslan F, Beceren NG (2014). Evaluation of mean platelet volume (MPV) levels in patients with synovitis associated with knee osteoarthritis. Platelets 25: 81-85.

Berenbaum F (2013). Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage 21: 16-21.

Chang WS, Chiang HC (1995). Structure-activity relationship of coumarins in xanthine oxidase inhibition. Anticancer Res 15: 1969-1973.

Chen IC, Chang CN, Chen WL, Lin TH, Chao CY, Lin CH, et al. (2019). Targeting Ubiquitin Proteasome Pathway with Traditional Chinese Medicine for Treatment of Spinocerebellar Ataxia Type 3. Am J Chin Med 47: 63-95.

Cordeiro OG, Chypre M, Brouard N, Rauber S, Alloush F, Romera-Hernandez M, et al. (2016). Integrin-Alpha IIb Identifies Murine Lymph Node Lymphatic Endothelial Cells Responsive to RANKL. PLoS One 11: e0151848.

Ding Z, Zhong R, Yang Y, Xia T, Wang W, Wang Y, et al. (2020). Systems pharmacology reveals the mechanism of activity of Ge-Gen-Qin-Lian decoction against LPS-induced acute lung injury: A novel strategy for exploring active components and effective mechanism of TCM formulae. Pharmacol Res 156: 104759.

Hao P, Jiang F, Cheng J, Ma L, Zhang Y, Zhao Y (2017). Traditional Chinese Medicine for Cardiovascular Disease: Evidence and Potential Mechanisms. J Am Coll Cardiol 69: 2952-2966.

Hou W, Hu S, Su Z, Wang Q, Meng G, Guo T, et al. (2018). Myricetin attenuates LPS-induced inflammation in RAW 264.7 macrophages and mouse models. Future Med Chem 10: 2253-2264.

Hsu H, Siwiec RM (2019). Knee Osteoarthritis. In StatPearls [Internet]. StatPearls Publishing: Treasure Island (FL).

Huang X, Xi Y, Pan Q, Mao Z, Zhang R, Ma X, et al. (2018). Caffeic acid protects against IL-1beta-induced inflammatory responses and cartilage degradation in articular chondrocytes. Biomed Pharmacother 107: 433-439.

Li XZ, Huang HJ, Zhang SN, Liu Q, Wang YM (2020). Label-free quantitative proteomics positions the effects and mechanisms of Herba Lysimachiae on synovial diseases based on biolabel-led research pattern. J Chromatogr B Analyt Technol Biomed Life Sci 1138: 121969.

Li XZ, Zhang SN, Yang XY (2017). Combination of cheminformatics and bioinformatics to explore the chemical basis of the rhizomes and aerial parts of Dioscorea nipponica Makino. J Pharm Pharmacol 69: 1846-1857.

Li XZ, Zhu KY, Zhang SN (2019). Exploration of Biolabel: A New Pattern For The Study On The Nature Of Chinese Materia Medica. World Sci Tech-Modernization Trad Chin Med Mater Med 21: 14-18.

Liu Q, Liu J, Fan S, Yang D, Wang H, Wang Y (2018a). Rapid discovery and global characterization of multiple components in corn silk using a multivariate data processing approach based on UHPLC coupled with electrospray ionization/quadrupole time-of-flight mass spectrometry. J Sep Sci 41: 4022-4030.

Liu S, Tian G, Chen J, Zhang X, Wu A, Li M, et al. (2018b). Traditional Chinese Medicine for Bradyarrhythmia: Evidence and Potential Mechanisms. Front Pharmacol 9: 324.

Liu T, Ding Y, Wen A (2018c). Traditional Chinese medicine for ischaemic stroke. Lancet Neurol 17: 745.

Pan X, Chen T, Zhang Z, Chen X, Chen C, Chen L, et al. (2019). Activation of Nrf2/HO-1 signal with Myricetin for attenuating ECM degradation in human chondrocytes and ameliorating the murine osteoarthritis. Int Immunopharmacol 75: 105742.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 47: D607-d613.

Tubaro A, Del Negro P, Ragazzi E, Zampiron S, Della Loggia R (1988). Anti-inflammatory and peripheral analgesic activity of esculetin in vivo. Pharmacol Res Commun 20 Suppl 5: 83-85.

Wang J, Qi F, Wang Z, Zhang Z, Pan N, Huai L, et al. (2020). A review of traditional Chinese medicine for treatment of glioblastoma. Biosci Trends 13: 476-487.

Wang Y, Bryant SH, Cheng T, Wang J, Gindulyte A, Shoemaker BA, et al. (2017). PubChem BioAssay: 2017 update. Nucleic Acids Res 45: D955-d963.

Wang Y, Liu Q, Fan S, Yang X, Ming L, Wang H, et al. (2019). Rapid analysis and characterization of multiple constituents of corn silk aqueous extract using ultra-high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry. J Sep Sci 42: 3054-3066.

Yamada H, Watanabe K, Saito T, Hayashi H, Niitani Y, Kikuchi T, et al. (1999). Esculetin (dihydroxycoumarin) inhibits the production of matrix metalloproteinases in cartilage explants, and oral administration of its prodrug, CPA-926, suppresses cartilage destruction in rabbit experimental osteoarthritis. J Rheumatol 26: 654-662.

Yang Y, Islam MS, Wang J, Li Y, Chen X (2020). Traditional Chinese Medicine in the Treatment of Patients Infected with 2019-New Coronavirus (SARS-CoV-2): A Review and Perspective. Int J Biol Sci 16: 1708-1717.

Zhang SN, Li XZ, Yang XY (2017). Drug-likeness prediction of chemical constituents isolated from Chinesemateriamedica Ciwujia. J Ethnopharmacol 198: 131-138.

Zheng W, Feng Z, You S, Zhang H, Tao Z, Wang Q, et al. (2017). Fisetin inhibits IL-1beta-induced inflammatory response in human osteoarthritis chondrocytes through activating SIRT1 and attenuates the progression of osteoarthritis in mice. Int Immunopharmacol 45: 135-147.

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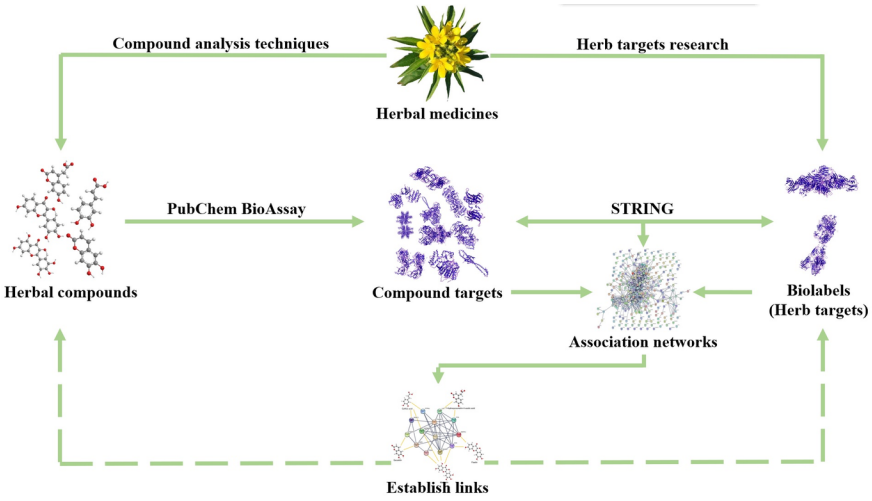

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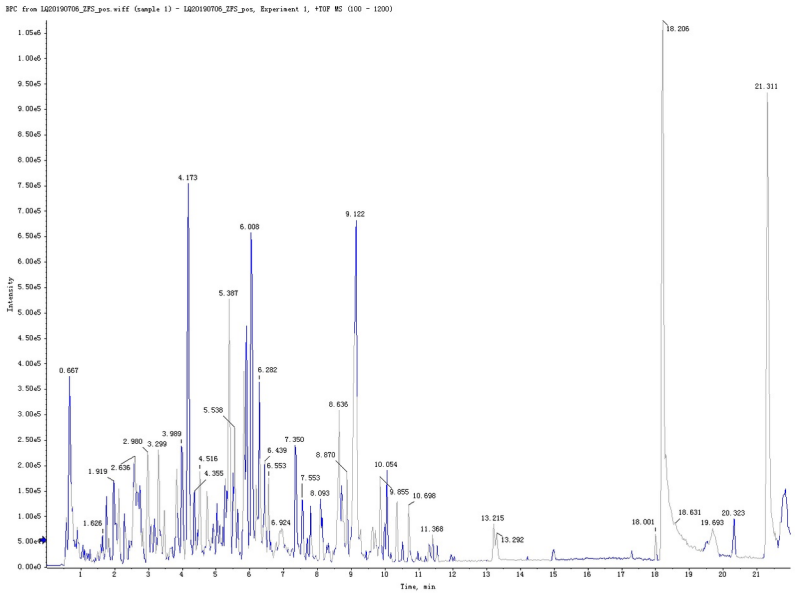

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