Elucidating the mechanisms of Tibetan medicine Ershiwuwei Lvxue Pill in the treatment of rheumatoid arthritis via a system pharmacological approach

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Research

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Abstract

Background: Ershiwuwei Lvxue Pill (ELP, 二五威鹿血丸), a traditional Tibetan medicine preparation, has been used hundreds of years for the clinical treatment of rheumatoid arthritis (RA) in Tibet, China. Nevertheless, its chemical compositions and therapeutic mechanism are unclear.

Methods: In this work, a system pharmacological approach based on ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS), network pharmacology and molecular docking simulation was proposed for exploring the promising bioactive compounds and mechanisms for the treatment of RA.

Results: For the first time, the compounds database of ELP was successfully established and 96 compounds were identified based on UPLC-Q-TOF/MS analysis. Among them, 22 bioactive compounds were screened according to ADME, oral bioavailability and drug likeness. Screening based on relevant databases and topological analysis revealed that 22 bioactive ingredients participated in 46 potential target proteins and 12 signaling pathways in RA. The functional enrichment analysis indicated that ELP exerted the anti-RA effects via synergistically regulating multiple biological nodes of the disease network. In that, 10 targets with high degree value including IL6, TNF, TP53, AKT1, JUN, VEGFA, MAPK3, STAT3, IL1 β and PTGS2. The pathways with greater *p*-value contribution are PI3K-Akt signaling pathway, Cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, MAPK signaling pathway, TNF signaling pathway and Toll-like receptor signaling pathway. In addition, good molecular docking scores were highlighted between five promising bioactive compounds (ellagic acid, quercetin, kaempferol, galangin, coptisine) and five core targets (PTGS2, STAT3, VEGFA, MAPK3, TNF).

Conclusion: Overall, the present work revealed the material basis and potential mechanism of ELP treatment on RA and provides insight for further research. However, further studies are needed to validate the biological processes and effect pathways of ELP.

Background

Ershiwuwei Lvxue Pill (ELP), a traditional Tibetan patented prescription medicine, was officially recorded in the *Drug Standard of the Ministry of Public Health of the Peoples Republic of China* for the treatment of rheumatoid arthritis (RA) [1]. It was one of the commonly used drugs to cure gout and arthralgia, and demonstrated to effectively cure RA in clinical trials [2]. There are twenty-five kinds of medicine in ELP, including *Asini sanguis pulvis*, *lignum rhamnellae*, *Dalbergiae odoriferae lignum*, *Santali albi lignum*, *Terminaliae belliricae fructus*, *Chebulae fructus*, *Travertine*, *Phyllanthi fructus*, *Myristicae semen*, *Caryophylli flos*, *Tsaoko fructus*, *Amomi fructus rotundus*, *Cassiae semen*, *Olibanum*, *Gossampini flos*, *Ambrette seed*, *Pterocephali herba*, *Gentianae radix et rhizome*, *Saxifrage*, *Vasaka*, *Fallopia aubertii*, *Fraxini cortex*, *Moschus*, *Croci stigma* and *Bovis calculus* (Table S1). The complex chemical compositions of ELP mainly consists of phenolic acids, flavonoids and alkaloids, which have been reported to possess analgesic, anti-inflammatory and antimicrobial effects [3–6]. In addition, previous studies have shown that ELP treatment of RA may be associated with anti-inflammatory and immunosuppressive effects [7]. However, the current research on ELP contains two deficiencies: firstly, a clear relationship between ingredients and formula has not been scientifically established, largely due to its complex compound composition; secondly, in terms of the research on the drugs action mechanism, it is difficult to accurately reflect the multi-component, multi-target and multi-pathway characteristics of Tibetan medicine formula only by a single active ingredient or indicator component [8, 9]. All those obstacles the efficacy evaluation and secondary development of Tibetan medicine ELP.

At present, a system pharmacological approach integrating ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS), network pharmacology and molecular docking simulation have been widely used to study on the efficacious material basis and mechanism of traditional Chinese medicine or Tibetan medicine [10,

11]. Given the technological merit of high sensitivity, selectivity, and speed of information collection, UPLC-Q-TOF/MS can effectively solve the problems such as complex components and difficult quantification in the modern analysis of natural medicine, and has outstanding advantages in the rapid discovery and characterization of target components [12, 13]. Network pharmacology transforms a “one target, one drug” model into a “multicomponent, multitarget” model, which effectively illustrates the complex interaction between diseases and drugs from the network perspective and has been widely used in mechanism research in Tibetan medicine [14, 15]. In addition, molecular docking simulation is a method of matching a ligand (drug) to a target molecule (receptor) by producing various components in different directions, as is an excellent tool in drug discovery [16–19]. Herein, the successful combined molecular docking simulation and network pharmacology provides a powerful model for the treatment of RA with ELP.

In this work, 96 compounds in ELP were identified based on UPLC-Q-TOF/MS analysis. Then, combining network pharmacology and molecular docking simulation, the potential biological active compounds, targets and underlying mechanisms of ELP for treating RA were investigated.

Material And Methods

Materials and chemicals

ELP was obtained from Tibetan mandew Tibetan Medicine Co. Ltd (Tibet, China). The proportion of each composition in ELP was showed in Table S1. HPLC grade acetonitrile and formic acid were supplied by Fisher Scientific (Fisher, Fair Lawn, NJ, USA). Chemical reference standards of gallic acid (110831-201906), chlorogenic acid (110753-201817), esculetin (110741-201708), ellagic acid (11959-201602), rutin (100080-201811), quercetin (100081-200907), and oleanolic acid (110709-201808) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Corilagin (K-004-190509), orientin (H-044-181216), and isovitexin (Y-116-180803) were provided from Chengdu Ruifensi Biotechnology Co., Ltd. (Chengdu, China). Loganic acid (CHB190109), chebulinic acid (CHB190724), crocin I (CHB190308), ursodeoxycholic acid (CHB180626), (+)-Bicuculline (CHB190112), and β -boswellic acid (CHB190625) were purchased from Chengdu Chroma-Biotechnology Co., Ltd. (Chengdu, China). The purity of all the standards were above 98%.

Sample preparation

Accurately-weighed ELP sample (0.5 g) was placed in a conical flask for ultrasound-assisted extraction with 70% methanol (30 mL) at 25 °C for 30 min. The extracted solution was adjusted to the original weight by adding 70% methanol, and then, the extracted solution was centrifuged at 14 000 rpm for 5 min. The supernatant was filtered through a 0.22 μ m microporous membrane filter prior to injection into the UPLC-Q-TOF/MS systems. Standard stock solutions of the reference standards were prepared by dissolving appropriate amounts of the pure substances in methanol.

UPLC-Q-TOF/MS analysis

Qualitative analysis of ELP was performed on Waters acquity UPLC (Waters, Milford, MA, USA). The chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 mm $\times$ 100 mm, 1.7 μ m, Waters), and the temperature was settled at 35 °C. The mobile phase was composed of acetonitrile (A) and 0.1% aqueous formic acid solution (B), and the flow rate was 0.4 mL/min. The linear gradient program was performed as follows: 0-1 min, 5% A; 1-3.5 min, 5-20% A; 3.5-4.5 min, 20-25% A; 4.5-6 min, 25-30% A; 6-9 min, 30-50% A; 9-11 min, 50-75% A; 11-13 min, 75-90% A; 13-15 min, 90-95% A; 15-16 min, 95% A; 16-16.5 min, 95-5% A; 16.5-19 min, 5% A.

Xevo G2-XS Q-TOF (Waters, Manchester, UK) equipped with electrospray ionization ion (ESI) source was applied for mass spectrometry data's acquisition in negative and positive ionization mode ranged from m/z 100 to m/z 1200. The

parameters of the source were set as follows: the ion source temperature was set at 120 °C, cone voltage 25 V, capillary voltage 2.5 kV, collision energy 10 V, desolvation temperature 350 °C, and desolvation gas flow rate, 1000 L/h.

Acquisition of active compounds and their targets

All compounds obtained by UPLC-Q-TOF/MS analysis were retrieved on the basis of the absorption, distribution, metabolism, and excretion (ADME) parameters in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>), the following screening criteria were used: oral bioavailability (OB) $\geq 30\%$, drug-likeness (DL) ≥ 0.18 [20]. Then, the bioactive compounds selected by ADME were converted to a standard Canonical Simplified molecular input line entry specification (SMILES) format in PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), at the same time, SMILES format file was imported to Swiss Target Prediction database with properties for "Homo sapiens", and eventually ELP potential targets was built for the bioactive compounds information database [21].

Acquisition of targets for RA

Different genes related to RA were obtained from the Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>) [22]; GeneCards HUMAN GENE DATABASE <https://www.genecards.org/> [23]; and Online Mendelian Inheritance in Man (OMIM, <http://www.omim.org/>) [24]. The keyword "rheumatoid arthritis" was used to search for rheumatoid arthritis-related targets in CTD, GeneCards, and OMIM databases.

Protein-Protein Interaction Data

Protein-Protein Interaction (PPI) Data were extracted using STRING (<https://string-db.org/>) with a reasonable confidence range for PPI data scores [25]. Common targets of bioactive compounds associated with RA were inputted into the STRING database, with the species to "Homo sapiens," and the confidence scores higher than 0.7. The visualization of the PPI networks was conducted in Cytoscape 3.8.0 software (<http://www.cytoscape.org/>) [26].

Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway analyses

Based on potential common targets for this study, GO and KEGG analyses were conducted to predict the action mechanism of ELP in treating RA by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID v6.8, <https://david.ncifcrf.gov/>) [27].

Compound-Target-Pathway network

Based on the results in DAVID database, the compound-target-pathway (C-T-P) network was created in Cytoscape 3.8.0 software. In a complex associative network, nodes and edges represent compounds/entities and their direct interactions, respectively. A high degree value equates to a prominent node status.

Molecular docking simulation

The molecular docking simulation was performed on the selected targets and corresponding compounds by using Maestro version 11.5 from the Schrodinger software suite. The lowest/minimum energy conformation was used for molecular docking via the default parameters. The docking score was the negative logarithm of the experimental dissociation/inhibition constant (pKd/pKi) and usually ranges from 0 to 10 (weak to strong combination force) [28]. The human protein structures with the highest resolution related to RA were selected at the UniProt database (<https://www.uniprot.org/>). Meanwhile, the X-ray crystal structures of these proteins were obtained at the RCSB PDB database (<https://www.rcsb.org/>).

Results

Identification of chemical compounds in ELP by UPLC-Q-TOF/MS

A total of 96 compounds were identified based on UPLC-Q-TOF/MS in the positive and negative ion mode, and the total ion chromatogram was analyzed based on the chemical standard, fragmentation patterns, previous literature data and the UNIFI software (v1.8 Waters Corp. Milford MA USA) (Fig. 1 and Fig. S1). Meanwhile, the following public databases were consulted: ChemSpider (<http://www.chemspider.com>), SciFinder Scholar (<https://scifinder.cas.org>). Finally, in the positive ion mode, 14 chromatographic peaks, mainly alkaloids, were characterized; and in the negative ion mode, 82 chromatographic peaks, mainly tannins, flavonoids, phenylpropanoids, terpenoids, quinonoids, steroids, alkaloids, and other compounds (Table 1).

Fig. 1. UPLC-Q-TOF-MS total ion chromatogram of ELP. (A) negative mode; (B) positive mode.

Table 1. Characterization of the chemical constituents in ELP by UPLC-Q-TOF-MS.

Tannins

Phenolic acid

Peak 2 was characterized as quinic acid with $[M-H]^-$ at m/z 191.0561 ($C_7H_{12}O_6$). It displayed fragments at m/z 173 $[M-H-H_2O]^-$, 127 $[M-H-2H_2O-CO]^-$ [29]. Quinic acid is an important secondary metabolite formed by shikimic acid pathway [30]. Peak 3 with $[M-H]^-$ at m/z 355.0310 ($C_{14}H_{12}O_{11}$) was identified as chebulic acid [31]. By comparison with the reference standard, peaks 5 and 38 with fragment ions $[M-H]^-$ at m/z 191.0561 ($C_7H_6O_5$) and m/z 300.9973 ($C_{14}H_6O_8$), were characterized as gallic acid and ellagic acid, respectively [32]. Quinic acid was esterified mainly with caffeic acid, and five chlorogenic acid isomers were identified by combining the authentic standard and the fragment ion information. Peaks 12, 17, and 25 had same ion $[M-H]^-$ at m/z 353.0887 ($C_{16}H_{18}O_9$), and fragment ions at 191 $[M-H-caffeoyl]^-$, 179 $[caffeic\ acid-H]^-$, 173 $[M-H-caffeoyl-H_2O]^-$, 135 $[caffeic\ acid-H-CO_2]^-$, which were identified as neochlorogenic acid (3-O-caffeoyl quinic acid), chlorogenic acid (5-O-caffeoyl quinic acid), and cryptochlorogenic acid (4-O-caffeoyl quinic acid), respectively [33]. Likewise, peaks 50 and 51 were isomers with $[M-H]^-$ at m/z 515.1195 ($C_{25}H_{24}O_{12}$), and fragment ions at 353 $[M-H-caffeoyl]^-$, 191 $[M-H-2caffeoyl]^-$, 135 $[caffeic\ acid-H-CO_2]^-$, which were tentatively identified as isochlorogenic acid A, isochlorogenic acid B, respectively [34].

Simple Gallic Acid Esters

The basic structural units of tannin compounds are gallic acid and glucose, which contain or lose $-C_7H_5O_5$ (m/z 169), $-C_7H_4O_4$ (m/z 152), $-C_6H_5O_3$ (m/z 125) ion fragments in the MS/MS spectrum. Peak 4 had ion $[M-H]^-$ at m/z 331.0674 ($C_{13}H_{16}O_{10}$), and fragment ions at m/z 313 $[M-H-H_2O]^-$, 271 $[M-H-C_2H_4O_2]^-$, 211 $[M-H-C_4H_8O_4]^-$, 169 $[M-H-glc]^-$, 124 $[M-H-glc-CO_2]^-$, thus peak 4 was tentatively characterized to be monogalloyl glucose [32] (Fig. S2-1). Peaks 10, 14 and 15 had same ion $[M-H]^-$ at m/z 483.0776 ($C_{20}H_{20}O_{14}$), were identified as digalloylglucose/ isomer [30]. In this way, peaks 22, 26, and 27 were characterized as trigalloylglucose/isomer [30], and the peaks 33 and 37 were characterized to be tetragalloylglucose/isomer, and peak 47 were identified to be 1,2,3,4,6-pentagalloylglucose [29, 31, 32, 35].

Ellagitannins

Ellagitannins, hydrolyzable tannin class of polyphenols, hydrolyzes with acids or bases to produce hexahydroxy diphenolic acid (HHDP), which were further spontaneously esterified into ellagic acid [36]. Peak 6 presented a precursor $[M-H]^-$ ion at m/z 781.0555 ($C_{34}H_{22}O_{22}$), and ions at m/z 601 $[M-H-glc]^-$, 301 $[HHDP]^-$, showing the existence of a gallic acid group, which

was identified as punicalin [37]. Comparing the retention time and fragmentation patterns of reference standard, peaks 24 and 44 were assigned as corilagin and chebulinic acid, respectively [37,38]. Peaks 32 and 45 had identical ion $[M-H]^-$ at m/z 953.0884 ($C_{41}H_{30}O_{27}$), and fragment ions at 454 $[M-H-COOH]^{2-}$, 476 $[M-2H]^{2-}$, 301 $[HHDP-H]^-$, were identified as chebulagic acid, and 3-galloyl-2,4-chebuloyl-1,6-HHDP-glucose, respectively [39,40].

Flavonoids

ESI-MS analysis of flavonoids exhibited some diagnostic features such as loss of methyl, methoxyl, H_2O (18 Da), CO (28 Da), CO_2 (44 Da), C_2H_2O (42 Da) [41]. Meanwhile, for the identification of flavonoid aglycones, the retro-Diels-Alder (RDA) reaction provides structurally relevant information on the number and types of substituents in the A- and B-rings from the two C-C bonds in Cleavage of the C-ring [42]. In addition, flavonoids C-glycosides in ESI-MS/MS spectra, ions of $[M-H-C_2H_4O_2 (60\text{ Da})]^-$, $[M-H-C_3H_6O_3 (90\text{ Da})]^-$, $[M-H-C_4H_8O_4 (120\text{ Da})]^-$ are considered as characteristic of C-glycoside flavonoids under low collision energy. Unlike C-glycosides, O-glycosides generated the most intense aglycone ion after loss of sugar molecule under low collision energy. The presence of O-glucoside in flavonoids could be revealed by MS/MS experiment in product ion scan mode indicating neutral loss of a glucose moiety (162 Da), from precursor ions [43].

Flavones

Peaks 30 and 41 with $[M-H]^-$ ion at m/z 447.0933 ($C_{21}H_{20}O_{11}$) and m/z 431.0988 ($C_{21}H_{20}O_{10}$), lost $C_3H_6O_3$ and $C_4H_8O_4$ in the MS^2 spectrum, were confirmed as orientin and isovitexin by comparison with the authentic standard [44,45]. Likewise, peaks 18, 20 and 46 could be tentatively identified as lutonarin, Isovitexin-7-O-glucoside, and vitexin 2"-O-rhamnoside, respectively [46–48]. Peak 65 showed a deprotonated ion $[M-H]^-$ at m/z 269.0831 ($C_{15}H_{10}O_5$) and was determined to be apigenin. It lost CO and H_2O , and formed base peaks at m/z 251, 241 in the MS^2 spectrum [13].

Flavonols

Peaks 35 and 40 gave identical ion $[M-H]^-$ at m/z 609.1447 ($C_{27}H_{30}O_{16}$). By losing glucose (162 Da) and H_2O , peaks 35 showed ions $[M-H]^-$ at m/z 285, 267, which was assigned as kaempferol-3-O-gentiobioside [47]. Peaks 40 were identified as rutin by comparison with their authentic standard [49] (Fig. S2-2). Peak 66 displayed $[M-H]^-$ ion at m/z 301.0364 ($C_{15}H_{10}O_7$), and was accurately identified as quercetin by a reference standard and mass spectrometry [47]. Quercetin displayed fragment ions at m/z 151 $[M-H-C_8H_6O_3]^-$, 121.0431 $[M-H-C_8H_6O_3-CH_2O]^-$ resulted from RDA cleavage [50]. Similarly, the other peaks 71, 82, 86, and 87 could be preliminarily identified as kaempferol, kaempferide, galangin, and rhamnazin, respectively [13,50].

Isoflavones

Peaks 31 and 72 were preliminarily characterized as cajanin and formononetin with $[M-H]^-$ at m/z 299.0568 ($C_{16}H_{12}O_6$) and m/z 267.0657 ($C_{16}H_{12}O_4$), respectively. Peak 31 displayed fragments at m/z 284 $[M-H-CH_3]^-$, 256 $[M-H-CH_3-CO]^-$ [51].

Aurone

Peak 34 with a deprotonated ion $[M-H]^-$ at m/z 287.0559 ($C_{15}H_{12}O_6$) was determined to be maesopsin. By losing neutral molecules H_2O and CO, it formed base peaks at m/z 251, 241 in the MS^2 spectrum [52].

Phenylpropanoids

Peaks 13, 19, and 29 were characterized as esculin, esculetin, and fraxetin with deprotonated ions $[M-H]^-$ at m/z 339.0716 ($C_{15}H_{16}O_9$), m/z 177.0194 ($C_9H_6O_4$), and m/z 207.0297 ($C_{10}H_8O_5$), respectively. Peak 19 displayed fragments at m/z 149

[M-H-CO]⁻, 133[M-H-CO₂]⁻, the same as the authentic standard [34,53,54].

Terpenoids

Monoterpenoids. Peak 16 with [M-H]⁻ ion at m/z 375.1297 (C₁₆H₂₄O₁₀) lost a glucose moiety, CO₂, and H₂O in the MS² spectrum, and it was confirmed as loganic acid by comparison with the authentic standard [12]. Peaks 23, 49, and 55 were preliminarily characterized as genipin-1-O-gentiobioside, sylvestroside I, and cantleyoside with deprotonated ions [M-H]⁻ at m/z 549.1807 (C₂₃H₃₄O₁₅), m/z 747.2738 (C₃₃H₄₈O₁₉), and m/z 745.2544 (C₃₃H₄₆O₁₉), respectively [55,56].

Diterpenoids

Peaks 62 and 67 were tentatively characterized as diosbulbin J-glucoside and diosbulbin L-glucoside with deprotonated ions [M-H]⁻ at m/z 539.1772 (C₂₆H₃₆O₁₂) and m/z 523.1837 (C₂₅H₃₂O₁₂), respectively. Peak 62 displayed fragments at m/z 377 [M-H-glc]⁻, 349 [M-H-glc-CO]⁻ [57]. Peaks 56 and 73 were isomers with ions [M-H]⁻ at m/z 975.3721 (C₄₄H₆₄O₂₄), and fragment ions at 813 [M-H-glc]⁻, 651 [M-H-2glc]⁻, respectively. Peak 56 was confirmed as crocin I by comparison with the authentic standard [38]. In the same manner, peaks 64 and 74 were tentatively identified as crocin II and cis-crocin II, respectively [58].

Pentacyclic triterpenoids

Peaks 76, 77, 78, 84, 85, 88, 89, 90, 91, 92, 94, 95, and 96 are preliminarily classified as triterpenoids from *Olibanum*. Among them, comparing the retention time and fragmentation patterns of reference standard, peaks 91, 92 and 96 had same ion [M-H]⁻ at m/z 455.3532 (C₃₀H₄₈O₃), and fragment ions along with RDA at 393 [M-H-H₂O-CO₂]⁻, 239 [B^{8,9}]⁻, 217 [D^{8,9}]⁻, which were accurately identified as oleanolic acid, α-boswellic acid and β-boswellic acid (Fig. S2-3), respectively [12, 59]. Peaks 76 and 77 were isomers with ion [M-H]⁻ at m/z 503.3388 (C₃₀H₄₈O₆), and were tentatively identified as trihydroxy-α-boswellic acid, trihydroxy-β-Boswellic acid, respectively [60]. Peak 78 presented a deprotonated ion [M-H]⁻ at m/z 501.3236 (C₃₀H₄₆O₆) and was preliminarily determined to be dihydroxy-11-Keto-β-boswellic acid [60]. Peaks 84 and 85 were isomers with ion [M-H]⁻ at m/z 471.3478 (C₃₀H₄₈O₄), and were tentatively identified as dihydroxy-α-boswellic acid, dihydroxy-β-boswellic acid, respectively, which had fewer hydroxyl group than compounds 76 and 77 [60]. Peaks 88, 89, and 90 were isomers with ion [M-H]⁻ at m/z 471.3478 (C₃₀H₄₈O₄), and were tentatively identified as 11-hydroxy-α-boswellic acid, 11-hydroxy-β-boswellic acid, and 2α-hydroxy-oleanolic acid, respectively [12]. Peak 88 displayed fragments at m/z 409 [M-H-H₂O-CO₂]⁻, 239 [B^{8,9}]⁻, 233 [D^{8,9}]⁻. Peaks 94 and 95 were preliminarily determined to be Acetyl-11-keto-β-boswellic acid and 3-Acetyl-β-boswellic acid, respectively [12, 59–62].

Quinonoids

Anthraquinones

The main cleavage method of anthraquinones is to lose CO step by step and retain p-π conjugated system in the parent nucleus group when induced by selective ion collision by mass spectrometry. In addition, side chain substituents are easily lost in the form of small neutral molecules such as H₂O, CH₃, CH₂ and CO₂ [63]. Peaks 59, 68, and 75 were characterized as aurantio-obtusin-6-O-β-D-glucoside, obtusin, and aurantio-obtusin with deprotonated ions [M-H]⁻ at m/z 491.1199 (C₂₃H₂₄O₁₂), m/z 343.0824 (C₁₈H₁₆O₇), and m/z 329.0667 (C₁₇H₁₄O₇), respectively [64,65]. Peak 59 displayed fragments at m/z 476 [M-H-CH₃]⁻, 461 [M-H-2CH₃]⁻, 329 [M-H-glc]⁻, 286 [M-H-glc-CH₃-CO]⁻.

Steroids

Peak 83 with $[M-H]^-$ ion at m/z 3391.2856 ($C_{24}H_{40}O_4$) lost H_2O and $COOH$ in the MS^2 spectrum, and it was confirmed as ursodeoxycholic acid (UDCA) by comparison with the authentic standard [66]. Likewise, peaks 80 and 81 were tentatively characterized as taurochenodeoxycholic acid (TDCA) and cholic acid (CA), respectively [10].

Alkaloids

Alkaloids are a kind of basic organic compounds containing nitrogen, which have good response in ESI (+) mode and mainly appear in the positive mode as $[M+H]^+$. In all, 14 alkaloids were identified. Peak 21 presented a deprotonated ion $[M+H]^+$ at m/z 314.1368 ($C_{18}H_{19}NO_4$) and fragment ions at m/z 299 $[M+H-CH_3]^+$, 177 $[M+H-CH_3-C_8H_9O]^+$, 121.0646 $[C_8H_9O]^+$, which was tentatively identified as N-trans feruloyltyramine [67]. Peak 7 exhibited a quasi-molecular ion $[M+H]^+$ at m/z 192.0992 ($C_{11}H_{13}NO_2$). Meanwhile fragment ions at m/z 177 $[M+H-CH_3]^+$, 149 $[M+H-CH_3-CO]^+$ were also observed, which were consistent with the fragmental data of N-methyl-2,3-dioxole-tetrahydroisoquinoline reported by literature [68]. Peak 28 presented a ion $[M+H]^+$ at m/z 342.1706 ($C_{20}H_{23}NO_4$) and fragment ions at m/z 311 $[M+H-CH_3NH_2]^+$, 297 $[M+H-(CH_3)_2NH_2]^+$, 279 $[M+H-CH_3NH_2-CH_3OH]^+$, which was tentatively identified as isocorydine [69]. Peaks 39 and 42 were tentatively characterized as scoulerine and tetrahydropalmatine with quasi-molecular ions $[M+H]^+$ at m/z 328.1553 ($C_{19}H_{21}NO_4$) and m/z 356.1857 ($C_{21}H_{25}NO_4$), respectively [70]. Peak 42 firstly loses the side chain CH_3 to generate fragment ion at m/z 341 $[M+H-CH_3]^+$, and RDA fragments at m/z 192 $[M+H-C_{10}H_{12}O_2]^+$, 165 $[M+H-C_{11}H_{13}NO_2]^+$ [71]. Based on the RDA reaction of the C-ring and typical RDA-cleavage characteristics, peak 54 with ion $[M+H]^+$ at m/z 354.1339 ($C_{20}H_{19}NO_5$) was identified as protopine [71]. Similarly, peaks 61, 63, and 70 were tentatively characterized as coptisine, jatrorrhizine and dehydrocorydaline [71,72]. Peak 43 was tentatively characterized as (-)-Corlumine [73,74]. Peak 48 was confirmed as (+)-Bicuculline by comparison with the authentic standard (Fig. S2-4), and peak 52 was characterized as (-)-Bicuculline from previous literature [73,75]. Peaks 60 and 69 were characterized as (-)-chelidonine and dihydrochelerythrine with ions $[M+H]^+$ at m/z 354.1339 ($C_{20}H_{19}NO_5$) and m/z 350.1361 ($C_{21}H_{19}NO_4$), respectively [68].

Other compounds

Peaks 1 and 9 were characterized as L-arginine and phenylalanine with $[M-H]^-$ at m/z 173.1041 ($C_6H_{14}N_4O_2$) and m/z 164.0721 ($C_9H_{11}NO_2$), respectively [76,77]. Peaks 11 and 36 were characterized as glucosyringic acid and syringic acids [33]. Peak 57 presented a deprotonated ion $[M-H]^-$ at m/z 269.0831 ($C_{16}H_{14}O_4$) and was preliminarily determined to be medicarpin [78]. Peaks 53 and 58 were characterized as cassiaside B2 and cassiaside with $[M-H]^-$ at m/z 919.2737 ($C_{39}H_{52}O_{25}$) and m/z 403.1398 ($C_{20}H_{20}O_{10}$), respectively [64]. Peak 93 with a deprotonated ion $[M-H]^-$ at m/z 227.2017 ($C_{14}H_{28}O_2$) was determined to be myristic acid.

Network pharmacology analysis

Potential bioactive compounds and targets of ELP in the treatment of RA

After ADME screening, 22 potential bioactive compounds ($OB \geq 30\%$, $DL \geq 0.18$) in ELP were identified (Table 2). Firstly, 145 potential targets of the 22 bioactive compounds were obtained from the Swiss Target Prediction platform (Fig. 2 and Fig. 3) (Table S2). Secondly, 361 RA-related targets were retrieved from GeneCards, CTD, and OMIM databases. Finally, 46 targets associated with RA were obtained by precisely matching the two steps.

Table 2. Potential bioactive compounds ADME values of ELP.

Fig. 2 Structures of 22 potential bioactive compounds of ELP.

Fig. 3 Compound-target network of ELP. In the network, there are 167 nodes and 292 edges. 22 bioactive compounds have interactions with 145 protein targets. The red circles represent the bioactive compounds, and the blue squares represent the targets.

Protein-Protein Interaction (PPI) network

The PPI relationship of 46 target genes was obtained by using STRING tool, and the visualization was realized by Cytoscape 3.8.0 software. The network of PPI relationships contained 46 nodes and 563 edges when a combined score of > 0.4 was used (Fig. 4 and Table S3). The 10 target genes with the highest connectivity degree were selected as the hub genes for RA. So, the hub genes, which might play a crucial role in RA progression, were IL6, TNF, TP53, AKT1, JUN, VEGFA, MAPK3, STAT3, IL1B and PTGS2.

Fig. 4. Protein-protein interaction (PPI) network of ELP for the treatment of rheumatoid arthritis. In the network, there are 46 nodes and 563 edges. Each node represents the relevant gene, the size and color of the node represent the value of the free degree. (the larger the node, the redder the color, the greater the free degree).

GO function and KEGG pathway enrichment analysis

GO function and KEGG pathway enrichment analysis of the 46 candidate targets were conducted by DAVID v6.8 to explore the molecular mechanism of ELP in treating RA. GO evaluation were illustrated using biological process (BP), cell component (CC), and molecular function (MF) terms (Fig. 5A, 5B and 5C). A total of 79 enrichment results in the related items of BP, involving regulation of apoptosis, protein amino acid phosphorylation, defense response, and intracellular signaling cascade; 20 enrichment results are related to CC, which includes membrane-enclosed lumen, organelle lumen, cytosol, and cell fraction; 39 enrichment processes are related to the MF which cover the nucleoside binding, ATP binding, protein kinase activity, cytokine activity, etc. Enrichment results of each p -value were calculated ($p < 0.01$ was considered as significant enrichment). Subsequently, a total of 29 signaling pathways were obtained ($p < 0.05$) and 12 major signaling pathways closely associated with RA were screened (Fig. 5D). Therefore, ELP probably produced the anti-RA effects by synergistically regulating many biological pathways, such as PI3K-Akt signaling pathway, Cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, MAPK signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway, etc.

Fig. 5. The results of Gene Ontology enrichment and KEGG pathway analysis by DAVID. A: biological process (BP), B: cell component (CC), C: molecular function (MF), D: KEGG pathway analysis. The P value of each biological process was less than 0.01.

Network construction and analysis

A compound-target-pathway network (C-T-P) of ELP for treating RA was constructed using Cytoscape 3.8.0 software (Fig. 6). The network showed that the relationships among 22 bioactive compounds, 46 protein targets and 12 signaling pathways. With the excavation of the C-T-P network, the mechanism of ELP in treating RA was preliminarily understood.

Fig. 6. Compound-target-pathway Network (C-T-P) of ELP for treating RA. In the network, there are 80 nodes and 274 edges. The interaction relationship was shown between 22 bioactive compounds, 46 protein targets, and 12 signaling pathways. The green triangles represent the bioactive compounds, red circles represent targets, and blue squares represent signaling pathways.

Molecular docking simulation

Molecular docking simulation was performed using Maestro11.5 (Schrodinger Suites) between the 22 potential bioactive compounds and the top 10 targets from PPI network. The 3D structures of the 10 selected targets were obtained from the

PDB database (<https://www.rcsb.org/>), which is a single global archive that includes experimentally determined atomic-level 3D structures of biological macromolecules (DNA, RNA, and proteins) [79]. The docking scores were depicted in Fig. 7A and Table S4. The hydrogen bonding and π - π stacking were involved between the targets and the potential compounds. Finally, according to the heat map analysis, good molecular docking scores were highlighted between five promising bioactive compounds (ellagic acid, quercetin, kaempferol, galangin, coptisine) and five core targets (PTGS2, STAT3, VEGFA, MAPK3, TNF). The typical schematic representation of three-dimensional (3D) and 2D molecular docking patterns of target proteins and compounds are shown in Fig. 7B, including PTGS2 with coptisine, STAT3 with ellagic acid, MAPK3 with quercetin and VEGFA with kaempferol. For instance, the binding mode of coptisine in the active site of PTGS2 had been represented in its 3D and 2D mode. Coptisine showed a H-bond interaction and three π - π stacking. Accurately, the oxygen of coptisine forms a hydrogen bond with ASN382, and three Pi-Pi bonds were formed by binding the six-membered ring to HIE388 and HIS207, respectively.

Fig. 7. Molecular docking results between 10 targets and 22 bioactive compounds from ELP. A: Heatmap for docking score. Docking score represents a negative logarithm of experimental dissociation/inhibition constant value (pKd/pKi). B: The typical Schematic representation of the molecular docking (Including 2D structure and 3D structure). Droplet shapes represent amino acid groups that interact with compounds in 3Å. Capital letters represent the abbreviation for amino acids. (Color Fig. can be accessed in the online version.)

Discussion

As a common systemic inflammatory autoimmune disease, with high disability and incidence, RA can severely impair physical function and quality of life [80]. Recently discoveries have improved understanding of the rheumatoid inflammation and their consequences [81]. RA is characterized by infiltration of the synovial membrane in joints with T cells, B cells, and monocytes and the common symptoms are musculoskeletal pain, swelling, and stiffness in clinical practice [82]. The pathophysiology of RA involves chronic inflammation of the synovial membrane, which can destroy articular cartilage and juxta articular bone [83]. As reported that the innate immune response produces some cytokines, among that, IL-1, IL-6 and TNF- α played important parts in progress of RA [84]. In addition, the process of RA involves the activation of multiple inflammatory signaling pathways and interactions with inflammatory cytokines [85]. Nevertheless, given the uncertainty and complexity of its pathogenesis, there's no specific medicine that can effectively treat RA. Modern therapies such as nonsteroidal anti-inflammatory drugs or pain medications only improve symptoms, but do not prevent damage progression and irreversible disability [83]. The mainly therapeutic strategy is applied disease-modifying antirheumatic drugs for patient with RA at present. For example, methotrexate, one of the therapeutic drugs, is the most effective and commonly used first-line drug [86]. Unsatisfactory, it has certain limitations and large side effects. Consequently, it is urgent to discover and develop safe and effective therapeutic from traditional medicine.

ELP, a traditional Tibetan patented prescription medicine, which was one of the commonly used drugs to cure RA in clinical trials [2]. Hence, for the first time, a system pharmacological approach based on UPLC-Q-TOF/MS, network pharmacology and molecular docking simulation was applied to the prediction of promising bioactive compounds and mechanisms of ELP treating RA. In this work, it was found that 22 potential active compounds of ELP probably perform anti-RA effects via synergistically regulating multiple biological processes, the important six such as PI3K-Akt signaling pathway, Cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, MAPK signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway. Concurrently, good molecular docking scores were highlighted between five promising bioactive compounds (ellagic acid, quercetin, kaempferol, galangin, coptisine) and five core targets (PTGS2, STAT3, VEGFA, MAPK3, TNF).

Excessive proliferation of fibroblast-like synoviocytes (FLSs) is one of the critical features of RA, leading to cartilage and bone destruction [87]. Studies have suggested that the PI3K/Akt signaling pathway contributes to excessive cell proliferation, migration, and invasion in RA-FLSs [88, 89]. Bone marrow MSCs (BMSCs), play a key role in the healing of

bone defects, has been applied for the treatment of RA via activation of the PI3K/AKT signaling pathway [90]. Consequently, according to the result of network analysis, one of the ELP mechanisms in anti-RA effects may be associated with regulating the PI3K/AKT signaling pathway. Cytokine-cytokine receptor interaction plays a vital role in both innate and adaptive inflammatory host defenses, and development and repair processes aimed at the restoration of homeostasis, which is involved in the pathogenesis of inflammatory and autoimmune diseases including RA [91]. Notably, there are plenty of studies also suggesting cytokine-cytokine interaction involved in the pathogenesis of RA [92]. Also, large post-injury increases in RA-associated markers, and differential upregulation of the cytokine-cytokine receptor interaction pathway that is closely associated with inflammation [93]. JAK-STAT signaling pathway is a key player in RA progression. [94]. In previous studies, the levels of serum cytokine TNF- α and IL-1 β are increased in RA patients. Meanwhile, activation of the JAK2-STAT3 pathway is regulated by inflammatory cytokines stimulation during the progression of RA [95, 96]. The MAPK family includes p38MAPK, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), which were activated in the synovium of RA patients [97, 98]. Accumulating studies indicated that the MAPK signal transduction pathway can regulate the inflammatory cytokine and downstream cell transduction pathways, thereby affecting the inflammation and destruction of joints [99]. It was reported that CCR5 silencing suppresses inflammatory response, inhibits viability, and promotes apoptosis of synovial cells in RA rats by inhibiting MAPK pathway [100]. TNF- α is a major cytokine implicated in RA [101]. Recently, an article revealed that miR-17 overexpression inhibited TRAF2 expression and its association with cIAP2, thereby suppressing TNF- α signaling pathways and downstream inflammatory proteins in RA SFs [102]. Emerging evidence shows that the activation of Toll-like receptor signaling pathway can initiate the perpetual cycle of inflammation in the arterial wall and the joint synovium in patients with RA [103]. It is demonstrated that functional suppression of RGS1 inhibits the inflammatory response and angiogenesis by inactivating the TLR signaling pathway in rats with collagen-induced arthritis (CIA) [104]. Above investigations improved that the efficacy of ELP against RA via those inflammation-associated pathways. However, it is necessary to verify them through further experimental research.

There are many literatures analysis found that the potential targets such as PTGS2, STAT3, VEGFA, MAPK3 and TNF in the above rheumatoid arthritis signaling pathway network, had been reported. A literature for understanding the development of inflammatory arthritis revealed that LL-37 and IL17A can significantly enhance PTGS2 and TNF gene expression, then release its downstream proinflammatory cytokines, PGE2 and TNF, contributing to enhancement of pathogenesis mechanisms of inflammatory arthritis [105]. Meanwhile, PTGS2, IL1 β , IL6, TNFA and CCL20 have been shown that mediators transformed RA-FLS to be a major source of pro-inflammatory in the pathology of RA [106]. The STAT proteins are usually inactive cytoplasmic proteins. Excess continuous exposure of STAT proteins induced pro-inflammatory cytokines or growth factors causes the development of RA [107]. STAT3, one of the STAT family members, has been proposed as an early pathophysiological event in RA. It was demonstrated that three STAT3-regulated genes BCL-2, SOCS3 and PIM1 could induce pathological lesion in RA via altered T cell effector function [108]. VEGF is a multifunctional cytokine that expressed in macrophages and neutrophils, inducing leukocyte accumulation and collagen deposition [109]. There is an evidence indicated that VEGFA promotes the migration and proliferation of endothelial cells, as well as inducing vascular permeability and mediating inflammation. VEGFA is a key factor in the development of pannus in RA [110]. Therefore, VEGFA inhibitors could disrupt new vessels and inhibit the delivery of nutritional proteins to sites of inflammation in RA [111]. Mitogen-activated protein kinases (MAPKs) are closely correlated with inflammatory diseases. MAPK3, one of the MAPKs has the same function participated in MAPK signaling pathway in RA [112]. Genes in the TNF family have been associated with RA, and may be a potential therapeutic target [113]. A plenty of evidences illustrated that TNF perpetuate synovial inflammation via activates RA-FLS inducing a constellation of genes [101]. All these provide strong evidences for the prediction of ELP treat RA via intervention these key targets, but it is necessary to further validate the claims using molecular biological methods.

Molecular docking analysis showed that five promising bioactive compounds, ellagic acid, quercetin, kaempferol, galangin and coptisine, that docking own stable. The above compounds mainly belong to polyphenols, flavonoids, and alkaloids,

and a large of investigation explicated that all these compounds are efficiency in RA [114–116]. Previous studies have shown that ellagic acid alleviated adjuvant induced arthritis model in mice by modulation of pro- and anti-inflammatory cytokines (IL-1 β , TNF- α , IL-17, IL-10, and IFN- γ) [117]. Quercetin could diminish myeloperoxidase activity, and ROS level to aid control of the autoimmune inflammation in patients with RA [118]. Kaempferol inhibits the migration and invasion of fibroblast-like synovial cells in rheumatoid arthritis by blocking activation of MAPK pathway [119]. Experiments showed that galangin improved human RA FLS by inhibition of the NF- κ B/NLRP3 pathway activation [120]. Coptisine has been reported to possess anti-inflammatory activity, that significantly inhibited the IL-1 β -induced NF- κ B activation in human RA chondrocytes [121]. All above evidences proved that the prediction of bioactive compounds in ELP ant-RA is reasonable and reliable.

Conclusion

In this study, it was the first time that an integrative strategy based on UPLC-Q-TOF/MS coupled with UNIFI informatics platform was applied for chemical profile analysis of ELP. A sum of 96 compounds were identified or tentatively characterized from 70% methanol extraction of ELP by comparing retention times, mass spectra to authentic standards, fragmentation behaviors and data previously reported, including, tannins, flavonoids, phenylpropanoids, terpenoids, quinonoids, steroids, alkaloids, and other compounds. Furthermore, based on the traditional Tibetan clinical efficacy and identified compounds of ELP in the treatment of RA, a compound-target-pathway network was constructed by relying on the network pharmacology method. In addition, good molecular docking scores were highlighted between five promising bioactive compounds (ellagic acid, quercetin, kaempferol, galangin, coptisine) and five core targets (PTGS2, STAT3, VEGFA, MAPK3, TNF). These targets were further related with the key signaling pathways such as PI3K/Akt, JAK-STAT, MAPK, TNF, Cytokine-cytokine receptor interaction, and Toll-like receptor, which could explain the anti-RA effects of ELP.

In summary, a system pharmacological approach using UPLC-Q-TOF/MS and network pharmacology with molecular docking simulation was applied to the prediction of promising bioactive compounds in ELP and mechanisms for the treatment of RA, which provides a new idea for the research of other Tibetan medicine prescriptions. Nevertheless, the bioactive compounds, biological targets and signaling pathways predicted needed to be confirmed and validated using CIA rat model in the further studies.

Abbreviations

ELP: Ershiwuwei Lvxue Pill; RA: rheumatoid arthritis; UPLC-Q-TOF/MS: ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry; ADME: absorption, distribution, metabolism, and excretion; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; OB: oral bioavailability; DL: drug-likeness; SMILES: Simplified molecular input line entry specification; PPI: Protein-Protein Interaction; GO: Gene Ontology analysis; KEGG: Kyoto Encyclopedia of Genes and Genomes; DAVID: Annotation, Visualization, and Integrated Discovery; C-T-P: compound-target-pathway network; BP: biological process; CC: cell component; MF: molecular function

Declarations

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Author Contributions

YZ and YT conceived and designed the experiments; CL performed the experiments and wrote the manuscript; CL, F-FF and LZ processed the data; LZ, YSZ, JS, QC and CT performed the partial experiments; CL, FFF, NL, YT and YZ provided critical review and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors consent to publication of this study in the journal Chinese Medicine.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Characterization of the chemical constituents in ELP by UPLC-Q-TOF-MS.

NO.	Rt (min)	Compound	Molecular formula	Molecular weight (Da)	Detected ion (m/z) [M-H] ⁻ /[M+H] ⁺	Error (ppm)	MS ² fragment ion (m/z)
1	0.53	L-arginine	C ₆ H ₁₄ N ₄ O ₂	174.1117	173.1041	-1.5	-
2	0.61	Quinic acid	C ₇ H ₁₂ O ₆	192.0634	191.0561	-0.3	173.0456[M-H-H ₂ O] ⁻ , 127.907[M-H-2H ₂ O-CO] ⁻
3	0.78	Chebolic acid	C ₁₄ H ₁₂ O ₁₁	356.038	355.031	0.8	337.0201[M-H-H ₂ O] ⁻ , 293.0590[M-H-H ₂ O-CO ₂] ⁻ , 275.0197[M-H-2H ₂ O-CO ₂] ⁻
4	0.80	Monogalloyl glucose	C ₁₃ H ₁₆ O ₁₀	332.0743	331.0674	-0.9	313.0020[M-H-H ₂ O] ⁻ , 271.0459[M-H-C ₂ H ₄ O ₂] ⁻ , 211.0951[M-H-C ₄ H ₈ O ₄] ⁻ , 169.0142[M-H-glc] ⁻ , 124.9060[M-H-glc-CO ₂] ⁻
5	1.00	Gallic acid*	C ₇ H ₆ O ₅	170.0215	169.0142	0.9	125.0243[M-H-CO ₂] ⁻ , 107.0132[M-H-CO ₂ -H ₂ O] ⁻
6	1.28	Punicalin	C ₃₄ H ₂₂ O ₂₂	782.0603	781.0515	2.9	600.9894[M-H-glc] ⁻ , 300.8809[DPPH]
7	1.33	N-Methyl-2,3-dioxole-Tetrahydroisoquinoline	C ₁₁ H ₁₃ NO ₂	191.0946	192.0992	-3.3	177.0765[M+H-CH ₃] ⁺ , 149.0807[M+H-CH ₃ -CO] ⁺
8	1.55	5-Galloylshikimic acid	C ₁₄ H ₁₄ O ₉	326.0638	325.0566	1.8	169.0140[gallic acid-H] ⁻ , 153.0200, 125.0244[gallic acid-H-CO ₂] ⁻
9	1.63	Phenylalanine	C ₉ H ₁₁ NO ₂	165.079	164.0721	0.9	-
10	2.11	1,2-di-O-galloyl-D-glucose	C ₂₀ H ₂₀ O ₁₄	484.0853	483.0776	-1.5	331.0671[M-H-galloyl] ⁻ , 313.0562[M-H-galloyl-H ₂ O] ⁻ , 271.0448, 211.0246, 169.0139[gallic acid-H] ⁻
11	2.13	Glucosyringic Acid	C ₁₅ H ₂₀ O ₁₀	360.1057	359.0981	0.8	197.0452[M-H-glc] ⁻ , 153.0535[M-H-glc-CO ₂] ⁻
12	2.23	Neochlorogenic acid	C ₁₆ H ₁₈ O ₉	354.0951	353.0879	1.5	191.0553[M-H-caffeoyl] ⁻ ,

							179.0349[caffeic acid-H] ⁻ , 173.0480[M-H-caffeoyl-H ₂ O] ⁻ , 135.0492[caffeic acid-H-CO ₂] ⁻
13	2.52	Esculin	C ₁₅ H ₁₆ O ₉	340.0794	339.0716	0	177.0189[M-H-glc] ⁻ , 149.0711[M-H-glc-CO] ⁻ , 133.0295[M-H-glc-CO ₂] ⁻ , 121.0625[M-H-glc-CO-CO] ⁻ ,
14	2.58	Digalloylglucose isomer I / 2,3-di-O-galloyl-D-glucose	C ₂₀ H ₂₀ O ₁₄	484.0853	483.0776	-1.5	331.0673, 313.0565, 271.0456, 211.0247, 169.0139
15	2.65	Digalloylglucose isomer II / 3,6-di-O-galloyl-D-glucose	C ₂₀ H ₂₀ O ₁₄	484.0853	483.0779	-1.5	331.0671, 313.0563, 271.0458, 211.0245, 169.0139
16	2.72	Loganic acid*	C ₁₆ H ₂₄ O ₁₀	376.1369	375.1297	-0.8	213.0766[M-H-glc] ⁻ , 169.0864[M-H-glc-CO ₂] ⁻ , 151.0761[M-H-glc-CO ₂ -H ₂ O] ⁻ ,
17	2.89	Chlorogenic acid*	C ₁₆ H ₁₈ O ₉	354.0951	353.0877	1.5	191.0554[M-H-caffeoyl] ⁻ , 179.0347[Caffeic acid-H] ⁻
18	3.11	Lutonarin	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1458	1.8	447.0933[M-H-glc] ⁻ , 357.0616[M-H-glc-90] ⁻ , 327.0512[M-H-glc-120] ⁻ ,
19	3.12	Esculetin*	C ₉ H ₆ O ₄	178.0266	177.0194	-0.7	149.0240[M-H-CO] ⁻ , 133.0288[M-H-CO ₂] ⁻ , 121.0732[M-H-CO-CO] ⁻
20	3.16	Isovitexin-7-O-glucoside	C ₂₇ H ₃₀ O ₁₅	594.1584	593.1512	1.3	503.1197[M-H-90] ⁻ , 473.1085[M-H-120] ⁻ , 341.0661[M-H-glc-90] ⁻ , 311.0551[M-H-glc-120] ⁻ ,
21	3.17	N-trans Feruloyltyramine	C ₁₈ H ₁₉ NO ₄	313.1314	314.1368	-0.6	299.1100[M+H-CH ₃] ⁺ , 177.0520, 121.0646[C ₈ H ₉ O] ⁺
22	3.19	1,2,6-Trigalloylglucose	C ₂₇ H ₂₄ O ₁₈	636.0963	635.0891	-1.9	483.0780[M-H-galloyl] ⁻ , 465.1779[M-H-galloyl-H ₂ O] ⁻ , 331.1322[M-H-2galloyl] ⁻ , 313.0762[M-H-2galloyl-H ₂ O] ⁻ , 178.9124[M-H-3galloyl] ⁻ ,

23	3.29	Genipin-1-O-Gentiobioside	C ₂₃ H ₃₄ O ₁₅	550.1898	549.1807	-1.2	517.1517[M-H-CH ₃ OH] ⁻ , 323.0980 [M-H-genipin] ⁻ , 225.0791[M-H-2glc] ⁻ , 207.0599[M-H-2glc-H ₂ O] ⁻ , 123.0478 [M-H-2glc-H ₂ O-C ₄ H ₄ O ₂] ⁻
24	3.37	Corilagin*	C ₂₇ H ₂₂ O ₁₈	634.086	633.0733	2.2	481.0671[M-H-galloyl] ⁻ , 463.0520[M-H-galloyl-H ₂ O] ⁻ , 300.9992[M-H-galloyl-H ₂ O-Hex] ⁻
25	3.40	Cryptochlorogenic acid	C ₁₆ H ₁₈ O ₉	354.0951	353.0887	1.5	191.0553[M-H-caffeoyl] ⁻ , 179.0349[caffeic acid-H] ⁻ , 173.0480[M-H-caffeoyl-H ₂ O] ⁻ , 135.0492[caffeic acid-H-CO ₂] ⁻
26	3.49	1,2,6-Trigalloylglucose	C ₂₇ H ₂₄ O ₁₈	636.0963	635.0891	-1.9	483.0780[M-H-galloyl] ⁻ , 465.1779[M-H-galloyl-H ₂ O] ⁻ , 331.1322[M-H-2galloyl] ⁻ , 313.0762[M-H-2galloyl-H ₂ O] ⁻ , 178.9124[M-H-3galloyl] ⁻
27	3.55	1,2,6-Trigalloylglucose	C ₂₇ H ₂₄ O ₁₈	636.0963	635.0891	-1.9	483.0780[M-H-galloyl] ⁻ , 465.1779[M-H-galloyl-H ₂ O] ⁻ , 331.1322[M-H-2galloyl] ⁻ , 313.0762[M-H-2galloyl-H ₂ O] ⁻ , 178.9124[M-H-3galloyl] ⁻
28	3.68	Isocorydine	C ₂₀ H ₂₃ NO ₄	341.1627	342.1706	0.1	311.1422[M+H-CH ₃ NH ₂] ⁺ , 297.1134[M+H-(CH ₃) ₂ NH ₂] ⁺ , 279.1079[M+H-CH ₃ NH ₂ -CH ₃ OH] ⁺
29	3.69	Fraxetin	C ₁₀ H ₈ O ₅	208.0372	207.0297	-0.8	192.0062[M-H-CH ₃] ⁻ , 164.0111[M-H-CH ₃ -CO] ⁻
30	3.81	Orientin*	C ₂₁ H ₂₀ O ₁₁	448.1006	447.0933	0.5	357.0618[M-H-90] ⁻ , 327.0511[M-H-120] ⁻
31	3.83	Cajanin	C ₁₆ H ₁₂ O ₆	300.0634	299.0568	1.2	284.0324[M-H-CH ₃] ⁻ (100%), 256.0[M-H-CH ₃ -CO] ⁻

32	3.92	Chebulagic acid	C ₄₁ H ₃₀ O ₂₇	954.0975	953.0884	-0.2	454.2341[M-H-COOH] ²⁻ , 476.3053 [M-2H] ²⁻ , 300.9994[HHDP-H] ⁻ ,
33	3.99	1,2,3,6-Tetragalloylglucose	C ₃₄ H ₂₈ O ₂₂	788.1072	787.1003	0.9	635.0890[M-H-galloyl] ⁻ , 483.0776[M-H-2galloyl] ⁻ , 331.0931[M-H-3galloyl] ⁻ ,
34	4.01	Maesopsin	C ₁₅ H ₁₂ O ₆	288.0637	287.0559	1.1	269.0461[M-H-H ₂ O] ⁻ , 259.0622[M-H-CO] ⁻ , 215.0702[M-H-CO-CO ₂] ⁻
35	4.03	Kaempferol-3-O-gentiobioside	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1461	1.8	285.0783[M-H-2glc] ⁻ , 267.0585[M-H-2glc-H ₂ O] ⁻ ;
36	4.05	Syringic acid	C ₉ H ₁₀ O ₅	198.0528	197.0456	0.8	182.0568[M-H-CH ₃] ⁻ , 179.0347[M-H-H ₂ O] ⁻ , 135.0033[M-H-CO ₂] ⁻
37	4.09	1,3,4,6-Tetragalloylglucose	C ₃₄ H ₂₈ O ₂₂	788.1072	787.1003	0.9	635.0890[M-H-galloyl] ⁻ , 483.0776[M-H-2galloyl] ⁻ , 331.0931[M-H-3galloyl] ⁻ ,
38	4.39	Ellagic acid*	C ₁₄ H ₆ O ₈	302.0063	300.9973	-1.1	283.9963[M-H-H ₂ O] ⁻ , 273.0189[M-H-CO] ⁻ , 257.0082[M-H-CO ₂] ⁻ , 229.0141[M-H-CO ₂ -CO] ⁻ ,
39	4.13	Scoulerine	C ₁₉ H ₂₁ NO ₄	327.1471	328.1553	0.1	296.1267[M+H-CH ₃ OH] ⁺ , 178.0829[M+H-C ₉ H ₁₀ O ₂] ⁺ , 163.0598[M+H-C ₉ H ₁₀ O ₂ -CH ₃] ⁺ ,
40	4.16	Rutin*	C ₂₇ H ₃₀ O ₁₆	610.1534	609.1447	1.8	300.9992[M-H-Rutinose] ⁻ , 151.0054[A ^{1,3}]-
41	4.23	Isovitexin*	C ₂₁ H ₂₀ O ₁₀	432.1057	431.0988	0.6	341.0671[M-H-90] ⁻ , 311.0563[M-H-120] ⁻
42	4.25	Tetrahydropalmatine	C ₂₁ H ₂₅ NO ₄	355.1783	356.1857	-0.6	341.1520[M+H-CH ₃] ⁺ , 192.0992[M+H-C ₁₀ H ₁₂ O ₂] ⁺ , 177.0754[M+H-C ₁₀ H ₁₂ O ₂ -CH ₃] ⁺ , 149.1734[M+H-C ₁₀ H ₁₂ O ₂ -CH ₃ -CO] ⁺

							165.0915[M+H-C ₁₁ H ₁₃ NO ₂] ⁺ ,
43	4.26	(-)-Corlumine	C ₂₁ H ₂₁ NO ₆	383.1396	384.1447	0	368.1106[M+H-H ₂ O] ⁺ , 249.0520
44	4.29	Chebulinic Acid*	C ₄₁ H ₃₂ O ₂₇	956.1631	955.1034	0.2	803.2881[M-H-galloyl] ⁻ , 651.3185[M-H-2galloyl] ⁻ , 633.1791[M-H-2galloyl-gallic] ⁻ , 499.4882[M-H-3galloyl] ⁻
45	4.37	3-galloyl-2,4-chebuloyl-1,6-HHDP-glucose	C ₄₁ H ₃₀ O ₂₇	954.0975	953.0885	-0.2	454.2341[M-H-COOH] ²⁻ , 300.9994[Hexahydroxydiphenoyl-H] ⁻ ,
46	4.38	Vitexin 2"-O-rhamnoside	C ₂₇ H ₃₀ O ₁₄	578.1636	577.1554	-0.3	457.1158[M-H-120] ⁻ , 431.0808[M-H-Rha] ⁻ , 413.0879[M-H-Rha-H ₂ O] ⁻ , 341.3668[M-H-Rha-90] ⁻ , 311.0567[M-H-Rha-120] ⁻ ,
47	4.42	1,2,3,4,6-Pentagalloylglucose	C ₄₁ H ₃₂ O ₂₆	940.1182	939.1083	-2.1	469.0504[M-2H] ²⁻ , 787.0979[M-H-galloyl] ⁻ , 635.2447[M-H-2galloyl] ⁻ , 483.3546[M-H-3galloyl] ⁻ , 331.4498[M-H-4galloyl] ⁻
48	4.49	(+)-Bicuculline*	C ₂₀ H ₁₇ NO ₆	367.1056	368.1145	1.1	307.0586[M+H-NH ₂ CH ₃ -H ₂ O-CH ₃] ⁺ , 277.0499, 249.0520, 190.0849
49	4.65	Sylvestroside I	C ₃₃ H ₄₈ O ₁₉	748.279	747.2738	2.6	585.2177[M-H-glc] ⁻ , 423.1231[M-H-glc-glc] ⁻
50	4.65	Isochlorogenic acid A	C ₂₅ H ₂₄ O ₁₂	516.1268	515.1195	0.8	353.0879[M-H-caffeoyl] ⁻ , 191.0553[M-H-2caffeoyl] ⁻ , 179.0343[M-H-2caffeoyl-H ₂ O] ⁻ , 135.0446[caffeic acid-H-CO ₂] ⁻
51	4.68	Isochlorogenic acid B	C ₂₅ H ₂₄ O ₁₂	516.1268	515.1195	0.8	353.0879[M-H-caffeoyl] ⁻ , 191.0553[M-H-2caffeoyl] ⁻ , 179.0343[M-H-2caffeoyl-H ₂ O] ⁻ , 135.0446[caffeic acid-H-CO ₂] ⁻
52	4.86	(-)-Bicuculline	C ₂₀ H ₁₇ NO ₆	367.1056	368.1145	1.1	307.0586[M+H-

							NH ₂ CH ₃ -H ₂ O-CH ₃] ⁺ , 277.0499, 249.0520, 190.0849
53	5.01	Cassiaside B2	C ₃₉ H ₅₂ O ₂₅	920.2798	919.2737	-1.7	271.0601[M-H-4glc] ⁻ , 257.0369[M-H-4glc- CH ₃] ⁻
54	5.09	Protopine	C ₂₀ H ₁₉ NO ₅	353.1263	354.1339	-0.3	336.1216[M+H-H ₂ O] ⁺ , 188.0672[M+H- C ₉ H ₈ O ₂ -H ₂ O] ⁺ , 149,0582[M+H- C ₁₁ H ₁₁ NO ₃] ⁺ ,
55	5.15	Cantleyoside	C ₃₃ H ₄₆ O ₁₉	746.2633	745.2544	-1.5	583.2032[M-H-gluc] ⁻ , 421.1159[M-H-2glc] ⁻
56	5.21	Crocin I*	C ₄₄ H ₆₄ O ₂₄	976.3787	975.3721	1.3	813.3179[M-H-gluc] ⁻ , 651.2274[M-H-2glc] ⁻
57	5.26	Medicarpin	C ₁₆ H ₁₄ O ₄	270.0892	269.0831	-0.2	159.0242[M-H- C ₆ H ₆ O ₂] ⁻
58	5.28	Cassiaside	C ₂₀ H ₂₀ O ₁₀	404.4257	403.1398	-0.4	257.0454[M-H-gluc] ⁻
59	5.36	Aurantio-obtusin-6-O-β-D-glucoside	C ₂₃ H ₂₄ O ₁₂	492.1268	491.1199	-1.4	476.0960[M-H-CH ₃] ⁻ , 461.0760[M-H-2CH ₃] ⁻ , 329.0694[M-H-gluc] ⁻ , 314.0418[M-H-gluc- CH ₃] ⁻ , 286.0182[M-H- gluc-CH ₃ -CO] ⁻ , 258.0227[M-H-gluc-CH ₃ - 2CO] ⁻
60	5.37	(-)-Chelidonine	C ₂₀ H ₁₉ NO ₅	353.1263	354.1339	-0.3	336.1216[M+H-H ₂ O] ⁺ , 322.1068[M+H- NH ₂ CH ₃] ⁺ ,
61	5.39	Coptisine	C ₁₉ H ₁₄ NO ₄ ⁺	320.0923	320.0899	2.4	292.0939[M-CO] ⁺ , 262.0863[M-CH ₂ O] ⁺
62	5.46	Diosbulbin J- glucoside	C ₂₆ H ₃₆ O ₁₂	540.1843	539.1772	-0.5	377.1246[M-H-gluc] ⁻ , 349.0827[M-H-gluc-CO] ⁻
63	5.51	Jatrorrhizine	C ₂₀ H ₂₀ NO ₄ ⁺	338.1392	338.1395	0.5	323.1228[M-CH ₃] ⁺ , 308.0904[M-2CH ₃] ⁺ , 280.0947[M-2CH ₃ - CO] ⁺ ,
64	5.63	Crocin II	C ₃₈ H ₅₄ O ₁₉	814.3259	813.3167	-1.4	651.2267[M-H-gluc] ⁻ , 489.2274[M-H-2glc] ⁻
65	5.77	Apigenin	C ₁₅ H ₁₀ O ₅	270.0892	269.0831	-0.4	251.0343[M-H-H ₂ O] ⁻ , 241.4335[M-H-CO] ⁻

66	6.04	Quercetin*	C ₁₅ H ₁₀ O ₇	302.0427	301.0364	-0.4	283.1690[M-H-H ₂ O] ⁻ , 273.0665[M-H-gluc-CO] ⁻ , 163.0189[A ^{0,2}] ⁻ , 151.0033[A ^{1,3}] ⁻
67	6.17	Diosbulbin L- glucoside	C ₂₅ H ₃₂ O ₁₂	524.1894	523.1837	2.1	361.2174[M-H-gluc] ⁻ , 333.0882[M-H-gluc-CO] ⁻
68	6.34	Obtusin	C ₁₈ H ₁₆ O ₇	344.0896	343.0824	-0.7	328.0227[M-H-CH ₃] ⁻ , 313.0284[M-H-2CH ₃] ⁻ , 285.0477[M-H ₂ CH ₃ -CO] ⁻ , 270.9883[M-H-3CH ₃ -CO] ⁻ , 242.9933[M-H-3CH ₃ -2CO] ⁻
69	6.37	Dihydrochelerythrine	C ₂₁ H ₁₉ NO ₄	349.1314	350.136	-3.2	334.1060[M+H-CH ₃] ⁺ ,
70	6.87	Dehydrocorydaline	C ₂₂ H ₂₄ NO ₄ ⁺	366.1705	366.1695	-1	366.1695[M] ⁺ , 351.1484[M-CH ₃] ⁺ , 334.1060[M-2CH ₃] ⁺ ,
71	7.10	Kaempferol	C ₁₅ H ₁₀ O ₆	286.0477	285.0397	-0.2	257.0296[M-H-CO] ⁻ , 243.0245[M-H-C ₂ H ₂ O] ⁻ , 227.0345[M-H-CO-CHOH] ⁻ , 151.0076[A ^{1,3} -H] ⁻ , 133.0318 [B ^{1,3}] ⁻
72	7.46	Formononetin	C ₁₆ H ₁₂ O ₄	268.2604	267.0657	0	252.0417[M-H-CH ₃] ⁻
73	7.68	Crocin I / Cis-trans isomer	C ₄₄ H ₆₄ O ₂₄	976.3787	975.3695	1.3	813.3179[M-H-gluc] ⁻ , 651.2274[M-H-2gluc] ⁻
74	7.99	Cis- Crocin II / Cis-trans isomer	C ₃₈ H ₅₄ O ₁₉	814.3259	813.3153	-1.4	651.2267[M-H-gluc] ⁻ , 489.2274[M-H-2gluc] ⁻
75	8.13	Aurantio-obtusin	C ₁₇ H ₁₄ O ₇	330.0739	329.0667	-1.4	314.0415[M-H-CH ₃] ⁻ , 299.0197[M-H-2CH ₃] ⁻ , 285.0402[M-H-CO ₂] ⁻ , 271.0520[M-H-2CH ₃ -CO] ⁻
76	8.25	Trihydroxy-α-boswellic acid	C ₃₀ H ₄₈ O ₆	504.3451	503.3388	1.6	-
77	8.30	Trihydroxy-β-Boswellic acid	C ₃₀ H ₄₈ O ₆	504.3451	503.3388	1.6	-
78	8.43	Dihydroxy-11-Keto-β-boswellic acid	C ₃₀ H ₄₆ O ₆	502.3294	501.3236	2	-
79	8.55	Isorhamnetin	C ₁₆ H ₁₂ O ₇	316.0583	315.0514	1.3	300.0275[M-H-CH ₃] ⁻ , 272.0320[M-H-CH ₃ -CO] ⁻ , 151.0469[A ^{1,3}] ⁻ , 163.0424[B ^{1,3}] ⁻

80	8.60	Taurochenodeoxycholic acid (TDCA)	C ₂₆ H ₄₅ NO ₆ S	499.2968	498.2893	-1.1	480.3216[M-H-H ₂ O] ⁻ , 427.3157[M-H-HSO ₃] ⁻ , 373.2737[M-H-HSO ₃ -C ₂ H ₅ N] ⁻
81	9.21	Cholic Acid (CA)	C ₂₄ H ₄₀ O ₅	408.2876	407.2807	-1.6	389.2747[M-H-H ₂ O] ⁻ , 362.2839[M-H-COOH] ⁻ , 343.2640[M-H-COOH-H ₂ O] ⁻ ,
82	9.28	Kaempferide	C ₁₆ H ₁₂ O ₆	300.0634	299.0562	0.3	284.0323[M-H-CH ₃] ⁻ , 271.0606[M-H-CO] ⁻ , 151.0076[A ^{1,3} -H] ⁻
83	9.56	Ursodeoxycholic acid* (UDCA)	C ₂₄ H ₄₀ O ₄	392.2927	391.2856	0.3	373.2657[M-H-H ₂ O] ⁻ , 346.0562[M-H-COOH] ⁻ , 328.0393[M-H-COOH-H ₂ O] ⁻ ,
84	9.77	Dihydroxy-α-boswellic acid	C ₃₀ H ₄₈ O ₅	488.3502	487.3443	1.7	-
85	10.05	Dihydroxy-β-boswellic acid	C ₃₀ H ₄₈ O ₅	488.3502	487.3443	1.7	-
86	10.20	Galangin	C ₁₅ H ₁₀ O ₅	270.0528	269.0454	0.3	251.1652[M-H-H ₂ O] ⁻ , 225.0555[M-H-CO ₂] ⁻ , 151.1026[A ^{1,3} -] ⁻
87	10.33	Rhamnazin	C ₁₇ H ₁₄ O ₇	330.074	329.0668	0.5	314.0431[M-H-CH ₃] ⁻ , 299.0197[M-H-CH ₃ -CH ₃] ⁻ , 271.02487[M-H-2CH ₃ -CO] ⁻ , 165.1026[A ^{1,3}] ⁻
88	11.35	11-hydroxy-α-boswellic acid	C ₃₀ H ₄₈ O ₄	472.3553	471.3478	2	409.2831[M-H-H ₂ O-CO ₂] ⁻ , 239.2327[B ^{8,9}] ⁻ , 233.2012[D ^{8,9}] ⁻
89	11.38	11-hydroxy-β-boswellic acid	C ₃₀ H ₄₈ O ₄	472.3553	471.3476	2	409.2831[M-H-H ₂ O-CO ₂] ⁻ , 239.2327[B ^{8,9}] ⁻ , 233.2012[D ^{8,9}] ⁻
90	11.48	2α-hydroxy-oleanolic acid	C ₃₀ H ₄₈ O ₄	472.3553	471.3478	2	453.3402 [M-H-H ₂ O] ⁻ , 407.3328 [M-H-H ₂ O-CO ₂] ⁻ ,
91	12.78	Oleanolic acid*	C ₃₀ H ₄₈ O ₃	456.3604	455.3528	-1.8	393.3420[M-H-H ₂ O-CO ₂] ⁻ , 239.1201[B ^{8,9}] ⁻ , 217.2012[D ^{8,9}] ⁻
92	12.87	α-boswellic acid	C ₃₀ H ₄₈ O ₃	456.3604	455.3532	-1.8	393.3420[M-H-H ₂ O-

							CO ₂], 239.1201[B ^{8,9}], 217.2012[D ^{8,9}]
93	13.21	Myristic acid	C ₁₄ H ₂₈ O ₂		227.2017	0.1	-
94	12.89	Acetyl-11-keto-β-boswellic acid	C ₃₂ H ₄₈ O ₅	512.3502	511.3469	0.5	469.8469[M-H-C ₂ H ₂ O], 407.3417[M-H-C ₂ H ₂ O- H ₂ O-CO ₂], 280.1628[B ^{8,9}], 231.8353[D ^{8,9}]
95	13.82	3-Acetyl-β-boswellic acid	C ₃₂ H ₅₀ O ₄	498.3709	497.3632	1.6	455.2737[M-H-C ₂ H ₂ O], 393.8599[M-H-C ₂ H ₂ O- H ₂ O-CO ₂], 280.8910[B ^{8,9}], 217.8976[D ^{8,9}]
96	14.70	β-boswellic acid*	C ₃₀ H ₄₈ O ₃	456.3604	455.3532	-1.8	393.3420[M-H-H ₂ O- CO ₂], 239.1201[B ^{8,9}], 217.2012[D ^{8,9}]

* Components identified with standard substance.

Table 2. Potential bioactive compounds ADME values of ELP.

Number	Molecule ID	PubChem CID	Molecular name	OB (%)	DL
N1	MOL006826	71308174	chebolic acid	72.00	0.32
N2	MOL009038	3082301	Genipin 1-gentiobioside	45.58	0.83
N3	MOL001002	5281855	Ellagic acid	43.06	0.43
N4	MOL002322	162350	Isovitexin	31.29	0.72
N5	MOL000098	5280343	Quercetin	46.43	0.28
N6	MOL006475	155380	Obtusin	81.43	0.4
N7	MOL000422	5280863	Kaempferol	41.88	0.24
N8	MOL000392	5280378	Formononetin	69.67	0.21
N9	MOL006472	155011	Aurantio-obtusin	31.55	0.37
N10	MOL000354	5281654	Isorhamnetin	49.6	0.31
N11	MOL004564	5281666	Kaempferide	73.41	0.27
N12	MOL002563	5281616	Galangin	45.55	0.21
N13	MOL008647	5280537	Moupinamide	86.71	0.26
N14	MOL001467	10143	Luteanin	55.63	0.55
N15	MOL000217	439654	(S)-Scoulerine	32.28	0.54
N16	MOL004071	5417	Tetrahydropalmatine	73.94	0.64
N17	MOL000791	185838	(-)-Bicuculline	69.67	0.88
N18	MOL001481	978315	(-)-Chelidone	48.32	0.86
N19	MOL001458	72322	Coptisine	30.67	0.86
N20	MOL006397	72323	Jatrorrhizine	30.44	0.75
N21	MOL001461	485077	Dihydrochelerythrine	32.73	0.81
N22	MOL004204	34781	Dehydrocorydaline	41.98	0.68

Supplemental Information

Supplementary Materials

The following are available online. Figure S1: Chemical structures of the compounds identified from ELP. Figure S2: The fragmentation pathways of representative compounds from ELP. Table S1: The composition of ELP. Table S2: Targets of 22 potential bioactive compounds. Table S3: Target information of ELP in the treatment of RA. Table S4: Results of molecular docking simulation.

Figures

Figure 1

UPLC-Q-TOF-MS total ion chromatogram of ELP. (A) negative mode; (B) positive mode.

Figure 2

Structures of 22 potential bioactive compounds of ELP.

Figure 3

Compound-target network of ELP. In the network, there are 167 nodes and 292 edges. 22 bioactive compounds have interactions with 145 protein targets. The red circles represent the bioactive compounds, and the blue squares represent the targets.

Figure 4

Protein-protein interaction (PPI) network of ELP for the treatment of rheumatoid arthritis. In the network, there are 46 nodes and 563 edges. Each node represents the relevant gene, the size and color of the node represent the value of the free degree. (the larger the node, the redder the color, the greater the free degree).

Figure 5

The results of Gene Ontology enrichment and KEGG pathway analysis by DAVID. A: biological process (BP), B: cell component (CC), C: molecular function (MF), D: KEGG pathway analysis. The P value of each biological process was less than 0.01.

Figure 6

Compound-target-pathway Network (C-T-P) of ELP for treating RA. In the network, there are 80 nodes and 274 edges. The interaction relationship was shown between 22 bioactive compounds, 46 protein targets, and 12 signaling pathways. The green triangles represent the bioactive compounds, red circles represent targets, and blue squares represent signaling pathways.

Figure 7

Molecular docking results between 10 targets and 22 bioactive compounds from ELP. A: Heatmap for docking score. Docking score represents a negative logarithm of experimental dissociation/inhibition constant value (pKd/pKi). B: The typical Schematic representation of the molecular docking (Including 2D structure and 3D structure). Droplet shapes represent amino acid groups that interact with compounds in 3Å. Capital letters represent the abbreviation for amino acids. (Color Fig. can be accessed in the online version.)

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