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Regular Article

Therapeutic Potential and Mechanistic Insights of Sunflower Receptacles' Essential Oil Against Gout: An In-Depth Molecular Study

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Abstract

Gout is a common chronic disease caused by the deposition of monosodium urate crystals. Previous studies confirmed the anti-gout effects of sunflower receptacles. For example, eupatoriochromene may be one of the most important compounds for reducing uric acid and relieving gout, which can be extracted from the essential oil of sunflower receptacles (EOSR). However, the other active components in EOSR and their anti-gout molecular mechanisms remain unclear. In this work, we employed widespread methods such as network pharmacology, machine learning algorithm, molecular docking, and molecular dynamics simulation, to investigate the anti-gout molecular mechanisms of the EOSR components. The protein- protein interaction (PPI) network confirmed that the components of EOSR exert anti-gout effects mainly by targeting inflammatory targets. GO and KEGG enrichment analyses revealed that the components of EOSR play roles in several important biological pathways, potentially providing anti-gout effects through various mechanisms. Additionally, since the target URAT1 plays a critical role in the treatment of gout, we investigated the interactions between two components of EOSR, Linoleic acid (La) and Kauren-19-oic acid (Koa), and target URAT1 using machine learning algorithm, molecular docking and molecular dynamics simulation. It confirmed that La and Koa can stably bind to URAT1 and shift its conformation to the Inward-facing state. Similar to the positive control Benzbromarone (Ben), both La and Koa induce secondary structural changes in URAT1, with Koa sharing key residues with Ben. This research further indicates the molecular mechanisms of EOSR in treating gout and expands the range of therapeutic agents for it.

Key words: Essential oil of sunflower receptacles, gout, molecular dynamics simulation, Markov state model.

1. Introduction

Gout is an inflammatory disease that causes significant pain and discomfort for patients [1,2]. In the United States, approximately 12 million adults have experienced gout [3]. It is primarily caused by elevated serum uric acid levels, which lead to the deposition of excess urate crystals in joints, soft tissues, and other organs, causing inflammation and severe pain [4,5]. Current treatments for gout include drugs to relieve acute gout attacks. Medications such as etoricoxib and colchicine help reduce joint redness, swelling, and pain by inhibiting inflammation [6,7]. And for long-term management, drugs that control serum uric acid levels are used. This is primarily achieved through two mechanisms: inhibiting uric acid production and promoting uric acid excretion [8–11]. For example, allopurinol reduces uric acid production by inhibiting the key enzyme xanthine oxidase (XO), while benzbromarone reduces uric acid reabsorption in the renal tubules by inhibiting the urate transporter protein (URAT1) and other related transporters. XO and URAT1 are widely recognized in the academic literature and clinical practice as critical targets for gout treatment [12–14].

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However, previous medications can cause side effects such as liver and kidney damage and increase the risk of cardiovascular events [15]. Th erefore, research into new therapies for gout is crucial and of great importance.

As a member of the Asteraceae family, sunflower (Helianthus annuus L.) is one of the traditional crops in China, primarily cultivated for its edible seeds. In addition, the receptacles of the sunflower are rich in chemical components with anti-inflammatory, antioxidant, and hepatoprotective properties, which is of great research significance [16–18]. Converting sunflower receptacles into essential oil helps preserve their active compounds and extend their shelf life. According to our preliminary research, the components of sunflower receptacles exhibit promising anti-gout effects [19]. For example, eupatoriochromene, a compound in sunflower receptacle essential oil (EOSR), significantly reduces uric acid levels [20]. However, other active compounds in EOSR and their specific anti-gout mechanisms have yet to be thoroughly studied.

Considering the significant role of natural plant components in disease treatment and the widespread application of network pharmacology, machine learning, molecular docking, and molecular dynamics (MD) simulations, we employed these methods to study the anti-gout mechanisms of EOSR components [21–23]. We used network pharmacology to analyze the comprehensive mechanisms of EOSR components within biological systems. Then, machine learning algorithms and molecular docking were used to indicate the interactions between proteins and small molecules. Furthermore, MD simulations was used to offer dynamic modeling and reveal conformation transitions. This research not only revealed the bioactive components in EOSR and their anti-gout mechanisms but also provided theoretical foundations and references for developing new anti-gout drugs. These findings will help enhance current gout treatments, improve their effectiveness, and support the rational use of traditional medicinal resources.

2. Theoretical method

2.1 Identification of gout-related targets

We screened for gout target genes from DisGeNET (https://www.disgenet.org/) [24], GeneCards (https://www.genecards.org/) [25], and PharmGKB (https://www.pharmgkb.org/) [26]. We identified 196 potential targets in DisGeNET databases, 928 potential targets in GeneCards and 7 potential targets in PharmGKB. After performing a union operation on the potential targets from three databases, 996 unique potential targets were obtained.

2.2 Prediction of targets of EOSR components

We screened SwissTargetPrediction (http://swisstargetprediction.ch/) [27] for potential targets of EOSR components. We identified 551 potential targets in the 104 components.

2.3 Identification of common targets of gout and EOSR components and construction of the PPI Network

We utilized the online tool jvenn (https://jvenn.toulouse.inra.fr/app/example.html) [28] to assess the overlap of targets of gout and EOSR components. The construction of the PPI network relied on data sourced from the STRING database (http://string-db.org/) [29] to evaluate potential interactions among the identified targets. We used Cytoscape 3.9.1 [30] to visually represent these interactions. Subsequently, using the cyto-Hubba plugin, we analyzed the topological properties of the network through the MCC method and identified the top 15 ranked targets.

2.4 GO and KEGG enrichment analysis

We employed several R packages-clusterProfiler, AnnotationHub, org.Hs.eg, enrichplot, pathview, dplyr, and ggplot2-for conducting enrichment analyses on GO biological processes and KEGG based on common targets of gout and EOSR components. Utilizing a significance threshold of $p = 0.01$ and $q = 0.01$, we retrieved GO information from org.Hs.eg.Db and KEGG information from clusterProfiler. The outcomes were presented through bar charts and bubble charts to offer a comprehensive visualization of the final results.

2.5 Molecular docking and machine learning algorithms

Due to the lack of a resolved crystal structure for URAT1, we utilized the URAT1 structure constructed by AlphaFold2. Additionally, the small molecule structures of Ben, La, and Koa were obtained from PubChem. The Flexible Docking module in Discovery Studio 2019 was used for the docking process. For selecting the docking site of URAT1, although its crystal structure has not yet been resolved, several studies have identified key residues. Yanyu Chen et al. verified that mutating the Ser35 and Phe241 residues to alanine significantly impairs the urate transport function of URAT1, providing a theoretical basis for the docking of URAT1 [31]. Based on the report, we selected Ser35 and Phe241 as the approximate positions for the docking box. The results of molecular docking were visualized using PyMOL 2.5.7 [32] and Discovery Studio 2019. Additionally, in this study, we applied the machine learning algorithm ConPLex [33], which integrates pretrained protein language models (PLMs) with contrastive learning techniques [34]. ConPLex predicted the binding potential of the components to the target protein based on their chemical structure, which were represented as SMILES strings.

2.6 Construction of membrane protein system using Charmm-GUI

Using Charmm-GUI (https://www.charmm-gui.org/?doc=input/membrane.bilayer), we constructed the membrane protein system for URAT1. In the Orientation Options, we selected PPM2.0 [35] to determine the position of the phospholipid bilayer relative to the protein. We set the water thickness to 15 Å (the minimum distance between the protein and the edge is 15 Å), the lipid type to POPC, and the lengths of X and Y to 110 Å, with NaCl as the equilibrating ion. Other settings were kept as default. For the Force Field Options, we selected the AMBER force field: FF14SB [36] for the protein, Lipid21 for the phospholipids, TIP3P [37] for water, and GAFF2 [38] for the small molecules [39].

2.7 Molecular dynamics simulations

Using AMBER 16's PMEMD engine, we conducted MD simulations on our systems, employing periodic boundary conditions to prevent edge effects. Based on the mdin files provided by Charmm-GUI, we conducted steps involving energy minimization, equilibration and production.