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Research Article

Network Pharmacology and Experimental Validation Reveal the Anti-Inflammatory Effects of *Schefflera octophylla via* Inhibition of the PI3K-AKT Pathway

Xiaoqin Zhou[1], Bin Yang[2], Yao Liang[2], Xiaobu Lan[1*]

[1]Department of Pharmacy, The Fifth Affiliated Hospital of Guangxi Medical University, Guangxi Province, China
[2]Department of Pharmacy, Guangxi Medical University, Nanning, Guangxi Province, China

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ABSTRACT

Background: Ethnopharmacological relevance shows that *Schefflera octophylla* (Lour.) Harms is an indigenous plant and traditional Chinese medicine. WaiGan mixture II, which is a complex herbal preparation contains *Schefflera octophylla* as the main herb and is commonly employed in clinical practice to treat conditions associated with wind-heat colds, such as the resolution of heat, toxins and alleviation of throat symptoms. Although these uses of *Schefflera octophylla* highlight its potential as an anti-inflammatory agent, its efficacy and underlying mechanisms remain to be thoroughly explored.

As traditional Chinese medicines contain a multitude of ingredients, it can be challenging to determine the specific small molecular compounds responsible for their medicinal effects. Here, we aim to identify the active anti-inflammatory compounds and targets of *Schefflera octophylla* through network pharmacology, evaluate their efficacy using molecular docking techniques and investigate their anti-inflammatory effects and mechanisms using *in vitro* and *in vivo* experiments.

Materials and methods: Compound-disease-target-pathway network was established through network pharmacology to determine the potential anti-inflammatory mechanism pathways in *Schefflera octophylla*. The efficacy of the medication was assessed by injecting the toes of Kunming (KM) mice with carrageenan (an inflammatory agent) to induce swelling, before measuring the swelling inhibition rate and effects on serum Interleukin (IL)-6 and Malondialdehyde (MDA). Nitrogen Monoxide (NO) content of medicated Lipopolysaccharide (LPS)-induced RAW264.7 cells was measured using the Griess method, while the levels of IL-1 Beta (β), IL-6 and IL-10 secreted by RAW264.7 cells were measured using Enzyme Linked Immunosorbent Assay (ELISA). Western blot was used to analyze the effects of the medication on protein expression of the Phosphatidylinositol 3 Kinase/protein Kinase B (PI3K/Akt) pathway. In this study, aqueous and ethanol extracts of *Schefflera octophylla* were used as medication.

Results: Aqueous and ethanol extracts of *Schefflera octophylla* significantly reduced carrageenan-induced toe swelling, and decreased IL-6 and MDA levels. The medication also demonstrated potent anti-inflammatory properties in RAW264.7 cells by decreasing the levels of the LPS-induced inflammation-related factors NO, IL-6 and IL-1β, increasing the level of the anti-inflammatory cytokine IL-10 and decreasing LPS-induced phosphorylation of PI3K-Akt pathway proteins.

Conclusion: These results provide strong evidence for further investigation into the molecular mechanisms of *Schefflera octophylla* for treating acute inflammation from an anti-inflammatory perspective.

Keywords: *Schefflera octophylla*, Network pharmacology, molecular docking, anti-inflammation, Chinese medicine, PI3K-Akt pathway

***Correspondence:** Xiaobu Lan, Department of Pharmacy, The Fifth Affiliated Hospital of Guangxi Medical University, Guangxi Province, China, E-mail: 112773956@qq.com

INTRODUCTION

Inflammation is an adaptive response triggered by specific conditions and noxious stimuli, and representing one of the most essential and prominent protective responses of an organism (Medzhitov R, 2008; Kuprash DV and Nedospasov SA, 2016). The initiation of inflammation is a response to cellular injury or the presence of pathogens which is generally mediated by resident immune cells. An inflammatory response is characterized by the engagement of Pathogen Recognition Receptors (PRRs), such as Toll Like Receptors (TLRs), leading to the synthesis and release of pro-inflammatory cytokines, which activate downstream pro-inflammatory signaling pathways (Ala A, *et al.*, 2003; Takeuchi O and Akira S, 2010; Feehan KT and Gilroy DW, 2019). The resolution of inflammation is a tightly regulated process that is driven by a complex set of mediators that regulate the cellular activities required to restore homeostasis and clear inflammatory cells from sites of infection or injury in the body (Gilroy D and de Maeyer R, 2015). However, in certain circumstances, the resolution process may become dysregulated leading to a state of chronic inflammation, which is characterized by persistent activation of immune cells and excessive secretion of chemokines, resulting in progressive tissue damage (Tang F, *et al.*, 2018). Because inflammation is widely involved in the pathological process of many diseases, anti-inflammation is an attractive field of drug development. In the current clinical context, anti-inflammatory treatments comprise a range of widely prescribed drugs, including Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), non-selective NSAIDs (nsNSAIDs) and selective Cyclooxygenase 2 NSAIDs (COXIBs), which are used for managing fever, pain and inflammation which are associated with diseases such as rheumatoid arthritis and osteoarthritis (Layton D, *et al.*, 2008; Bacchi S, *et al.*, 2012). Despite their efficacy, certain pharmaceuticals may possess limitations that restrict their widespread and sustained usage (Cunningham K, *et al.*, 2020). For example, despite their broad therapeutic effects, NSAIDs that hinder Cyclooxygenase (COX) (Mizushima T, 2010) are associated with many serious side effects, including cardiovascular risk, gastrointestinal toxicity, hepatotoxicity, kidney damage, hypertension and other minor diseases (Harirforoosh S, *et al.*, 2013; Patricio JP, *et al.*, 2013; Arfe A, *et al.*, 2016; Gunter BR, *et al.*, 2017; Bindu S, *et al.*, 2020). The quest for the discovery of safer and more effective anti-inflammatory agents continues to present a major challenge for the medical community. Chinese herbal medicine offers a potential avenue to tackle the problem of drug safety, particularly with regard to anti-inflammatory drugs. The "Manifestation theory" of Chinese medicine, proposed by Cai SQ, *et al.*, 2015, suggests that Chinese medicine

achieves therapeutic effects while reducing toxicity through a combination of single-target superposition of various manifestation forms, multi-target synergistic effects and toxic dispersion effects. Traditional Chinese medicine is a valuable resource that has been critical to disease prevention and treatment since ancient times. Chinese herbal medicine has an overwhelming advantage for treating inflammation, with low cost and few side effects. Indeed, there is a growing body of evidence supporting the anti-inflammatory effects of traditional Chinese remedies such as *Paeonia lactiflora* (Bai Shao) (Zhang L and Wei W, 2020), *Scutellaria baicalensis* (Huangqin) (Jiang M, *et al.*, 2020) and Forsythia (Lianqiao) (Chen L, *et al.*, 2018), all of which exhibit significant efficacy in controlling inflammation.

Schefflera octophylla (Lour.) Harms, belonging to Araliaceae family is a frequently used plant in traditional ethnomedicine, with the bark, either fresh or dried, representing the primary source of its medicinal properties (Pang S, *et al.*, 2016). The genus *Schefflera* has been traditionally used for its medicinal properties in China and its use has been documented in various authoritative sources, including the "Chinese Dictionary of Traditional Chinese Medicine," the "Chinese Materia Medica," and the "Xinhua Materia Medica Compendium." These records indicate that the genus has not only been used for its analgesic and anti-inflammatory effects, but also for its anti-tumor and anti-viral properties (Xu J, *et al.*, 2006). The medicinal preparation "WaiGan mixture II," composed primarily of *Schefflera octophylla*, has demonstrated clinical efficacy in treating cold and flu symptoms and inflammation caused by influenza viruses (Wang Y, *et al.*, 2006). Its use at the First People's Hospital of Nanning for more than 40 years suggest that *Schefflera octophylla* has positive effect on alleviating cold- and flu-related symptoms, but its exact efficacy and underlying mechanisms warrant further investigation.

Over the past decade, molecular pharmacology has gained prominence in the field of pharmacology, leading to new avenues of research into the mechanisms of drug action, as well as uncovering innovative strategies for mediating biological systems using molecular probes, receptor signaling, drug disposition and targeting of biological systems. This advancement in molecular pharmacology has led to the development of innovative strategies for modulating biological systems and facilitated investigation into the biological processes underlying pharmacology and drug design (Tasneem S, *et al.*, 2019). Here, we employed network pharmacology to investigate the anti-inflammatory mechanism of *Schefflera octophylla* and identify specific targets. The results of this research provide a foundation for promoting the application of local Chinese herbal medicines in China.

MATERIALS AND METHODS

Network pharmacology analysis

Active ingredient screening and target prediction of *Schefflera octophylla*: After conducting a comprehensive study (Zhang H, 2014; Shuhong T, *et al.*, 2015; Wang G, 2018) on the chemical constituents present in *Schefflera octophylla*, chemical data was obtained from the PubChem database (https: //pubchem.ncbi.nlm.nih.gov/) (Kim S, *et al.*, 2021) and active ingredient targets were screened using the SWISS Absorption, Distribution, Metabolism and Excretion (ADME) criteria (http://www.swissadme.ch/) (Daina A, *et al.*, 2017), which considered high Gastrointestinal (GI) absorption and favorable drug-like properties. The potential targets of action for these active ingredients were then predicted using the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) (Daina A, *et al.*, 2019). This research established a library of chemical information on *Schefflera octophylla* and identified potential targets for further investigating its pharmacological properties.

Inflammatory disease target prediction: Online Mendelian Inheritance in Man (OMIM) (https: //omim.org/), DisGeNET (https: //www.disgenet.org/home/) (Piñero J, *et al.*, 2020) and GeneCards (https: //genecards.org/) databases were utilized to search the keyword "Inflammation" to obtain a comprehensive list of inflammation-related targets.

Common target analysis of components and diseases: Venny software version 2.1.0 (https: //bioinfogp.cnb.csic.es/tools/venny/index.html) was used to identify the overlapping active ingredients and disease targets related to inflammation. The intersection of the active ingredients and disease targets resulted in a set of common target genes, which were then subjected to further analysis.

Protein-Protein Interaction (PPI) network construction and visualization: The intersecting target genes were analyzed using the Search Tool for the Retrieval of Interacting Genes (STRING) (https: //cn.string-db.org/) (Szklarczyk D, *et al.*, 2021) database to create a PPI network of the active ingredients and inflammation-related targets of *Schefflera octophylla*. The network was processed with the aid of Cytoscape software using the CytoNCA plug-in. Centrality values, including Betweenness Centrality (BC), Closeness Centrality (CC) and Degree Centrality (DC) were calculated using the network analyzer function where the core targets were ranked based on these values.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses: GO and KEGG pathway enrichment analyses were conducted on the common target genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) database (https: //cn.string-db.org/) (Huang DW, *et al.*, 2009; Sherman BT, *et al.*, 2022). The results were visualized through correlation maps generated on a bioinformatics platform (https: //www.bioinformatics.com.cn/).

Construction of the "active ingredient-target-disease-pathway" network: Cytoscape version 3.9.1 software was used to generate a graphical representation of the 20 most significant KEGG pathways and the interconnected network of active ingredients, targets and diseases ($p<0.5$).

Validation of molecular docking technology: Subsequently, molecular docking experiments were performed to validate the network pharmacology results. The active compounds were obtained in mol2 format from the PubChem database and the protein structures of *Schefflera octophylla* and the target proteins involved in inflammation were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) database (https: //www.rcsb.org/). These structures were processed using PyMOL software for hydrogenation and dehydration, before subjecting to docking simulations using AutoDock Vina. Finally, the binding energies were visualized using heat maps generated in Origin2021.

Medication preparation

In a multifunctional extraction tank, an ethanol extract (Concentration of 8.425 g/ml) of *Schefflera octophylla* was obtained by adding the bark (Guangxi, Batch No: 20201102) of the plant to 80% ethanol and concentrating it three times. The aqueous extract (Concentration of 10.42 g/ml) was obtained by extracting the plant with pure water.

Animal experiment

Male KM mice which were 6-7 weeks old were obtained from the Experimental Animal Center of Guangxi Medical University and were maintained in a controlled environment at a temperature of 25 ± 2°C and 50% ± 5% humidity, under a 12 h light/12 h dark cycle and with ad libitum access to food and water. The study was conducted in accordance with the guidelines approved by the Guangxi Medical University Laboratory Animal Centre (Grant No: SYXK2020-0004).

Determination of toe swelling, IL-6 and MDA levels in KM mice induced by carrageenan: The animals were divided into 8 groups, including the control group, dexamethasone group (10 mg/kg) and those treated with ethanol and aqueous extracts of *Schefflera octophylla* with different

concentrations (2.5, 5 and 10 g/kg). In all cases, treatment was administered daily for 7 days. The inflammatory response was induced by injecting 2% carrageenan into the right foot of the mice and the swelling was calculated by subtracting the left foot weight from the right foot weight 4 h later.

The levels of IL-6 and the antioxidant indicator MDA were measured in the serum of inflamed mice using ELISA (Elabscience, Wuhan, China) and biochemical kits (Nanjing Jiancheng Bioengineering Institute), respectively.

Cellular experiments

RAW264.7 cells were obtained from the Chinese academy of sciences cell bank and were cultured in high-glucose medium (Gibco) with 10% fetal bovine serum at 37°C, 5% Carbon dioxide (CO_2).

Effect of *Schefflera octophylla* on cell activity: The viability of RAW264.7 cells was evaluated following 24 h exposure to various concentrations of *Schefflera octophylla* ethanol and aqueous extracts (100 µg/ml, 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml and 2000 µg/ml) using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay. IC_{10} values were calculated and analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 software.

Effect of *Schefflera octophylla* on inflammatory factors and morphology of RAW264.7 cells: The RAW264.7 cells were seeded in 12-well plates at a cell density of 3×10^4 cells/well and then pretreated with *Schefflera octophylla* ethanol extract (75.5, 150 and 300 µg/ml) or aqueous extract (171.5, 343 and 686 µg/ml) for 1 h. Subsequently, the cells were challenged with Lipopolysaccharide (LPS) solution (1 µg/ml) for 24 h, before collecting the supernatant. NO content in the supernatant was determined using Griess reagent (Nanjing Jiancheng Bioengineering Institute) and the levels of IL-1β, IL-6 and IL-10 were quantified using ELISA kits (Elabscience, Wuhan, China).

Changes in the morphology were observed following successful induction of inflammation in RAW264.7 cells using LPS. The efficacy of anti-inflammatory drugs can be evaluated by their ability to restore the cells to their normal state. Therefore, the morphological assessment of RAW264.7 cells using light microscopy is a valuable tool for validating the *in vitro* inflammation model and determining the anti-inflammatory effects of *Schefflera octophylla* extract.

The cellular morphologies of the blank control, LPS and experimental groups were analyzed under a light microscope, with a field of view magnified 40 times to compare and contrast any differences. This analysis serves as a reliable method for evaluating the success of the *in vitro* inflammation model and the efficacy of *Schefflera octophylla* extract as an anti-inflammatory agent.

Western blot: RAW264.7 cells were incubated as described previously. Proteins were extracted and their concentration was determined using a Bicinchoninic Acid (BCA) assay. Subsequently, the proteins were subjected to denaturation by boiling, followed by electrophoresis in 10% Polyacrylamide Gel Electrophoresis (PAGE) gels and transfer to Polyvinylidene Difluoride (PVDF) membranes. The membranes were blocked with a blocking solution for 30 min and incubated with specific primary antibodies overnight. The primary antibodies used were as follows-

Phosphoinositide 3-Kinase (PI3K) (1:1000, #CST-4292), phosphorylated (p) PI3K (1:1000, AF3242) (Affinity Bioscience), Akt (1:1000, 10176-2-AP, Wuhan Proteintech), p-Akt (1:1000, 28731-1-AP, Wuhan Proteintech) and β-actin (1:1000, 20536-1-AP, Wuhan Proteintech). Following incubation, the membranes were washed and incubated with goat anti-rabbit IgG Heavy+Light (H+L) chains secondary antibody (1:10,000, SA5-35571, ThermoFisher Scientific).

The next day, after washing three times using Tris-Buffered Saline with 0.1% Tween (TBST) (TBS was prepared by adding 1 ml of Tween 20 dissolved in pure water) for 5 min/time, the membranes were immersed in the corresponding secondary antibody for 1 h (on a shaker, at room temperature), before washing a further three times. Finally, the protein bands were detected using two-colored infrared imaging system (Li-COR, Odysse Clx, USA).

RESULTS

Schefflera octophylla *active ingredient screening and target prediction*

The chemical composition of *Schefflera octophylla* was characterized through a comprehensive study (Zhang H, 2014; Shuhong T, *et al.*, 2015; Wang G, 2018), and PubChem database was used to obtain the Canonical Simplified Molecular Input Line Entry System (SMILES) strings and Structured Data File (SDF) format files of the chemical ingredients. Summarization of these data was used to create a *Schefflera octophylla* composition library. ADME properties of the chemical ingredients were evaluated using the SWISS ADME screening tool, which resulted in the identification of seven major active ingredients (*Table 1*). Swiss TargetPrediction database was employed to predict the potential biological targets of these active ingredients, which lead to the identification of 291 targets after de-duplication.

Inflammatory disease target prediction

To identify disease targets related to inflammation, a comprehensive search was performed using OMIM, DisGeNET and GeneCards databases were used. The results were filtered to obtain 1318 de-duplicated targets, which were associated with inflammation-related diseases.

Common target analysis of components and diseases

The overlap between the active ingredients of *Schefflera octophylla* and targets related to inflammatory disease were analyzed using Venny 2.1.0, which revealed 98 shared targets, as depicted in *Figure 1*.

PPI network construction and core target screening

PPI network was constructed between the active ingredients of *Schefflera octophylla* and the targets of inflammatory diseases using the STRING database. This network was analyzed using the CytoNCA plugin and network analyzer function in Cytoscape to determine the centrality measures (BC, CC and DC) of each target gene. Based on these calculations, the core targets were identified and ranked. The size of the circle and darkness of the center in *Figure 2*, represent the proximity of a target to other proteins within the network.

GO and KEGG pathway enrichment analysis

GO analysis was performed on the 98 common targets using DAVID software. The results were categorized into 434 Biological Process (BP), 103 Molecular Function (MF) and 47 Cellular Component (CC) categories and the top ten entries were selected and represented as a bar chart (*Figure 3*). KEGG pathway enrichment analysis was also performed, which revealed the involvement of 137 pathways in the 98 intersecting targets. The top 20 enriched pathways were visualized as a bubble map using p-value sorting.

Construction of the active ingredient-target-disease-pathway network

Cytoscape 3.9.1 software was used to generate a network map of the top 20 KEGG pathways and their associated targets, drugs, active components and diseases (*Figure 4*).

Table 1: Active ingredients of *Schefflera octophylla*

Chemical name in English	MOLID/ PubChem ID	SMILES
Asiatic acid	MOL007253	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)CO)O)O)C)C)C2C1C)C)C(=O)O
Decanol	MOL003508	CCCCCCCCCCO
Hexadecanoic acid	MOL000069	CCCCCCCCCCCCCCCC(=O)O
Isovanillin	MOL001867	COC1=C(C=C(C=C1)C=O)O
Vanillin	MOL000635	COC1=C(C=CC(=C1)C=O)O
2-hydroxy-4-(octyloxy)benzophenone	Compound CID: 129820667	CCCCC(CCC)OC1=CC=CC(=C1O)C(=O)C2=CC=CC=C2
(+)-balanophonin	Compound CID: 23252258	COC1=CC(=CC2=C1OC(C2CO)C3=CC(=C(C=C3)O)OC)C=CC=O

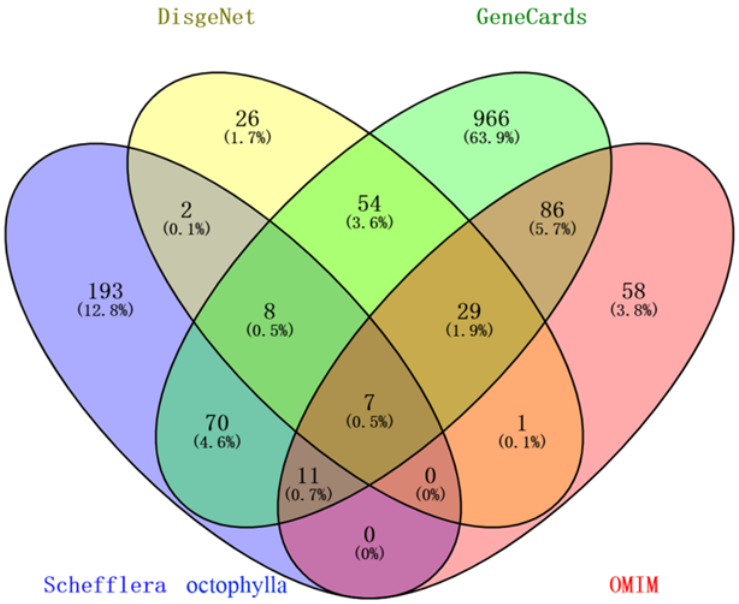


Figure 1: Common targets of inflammation and active ingredients of *Schefflera octophylla*
Note: Numbers represent number of targets while percentage (%) represents of target numbers among 1318 disease targets. The total number of graph parts where *Schefflera octophylla* overlaps with OMIM, DisGeNET, and GeneCards, is 98

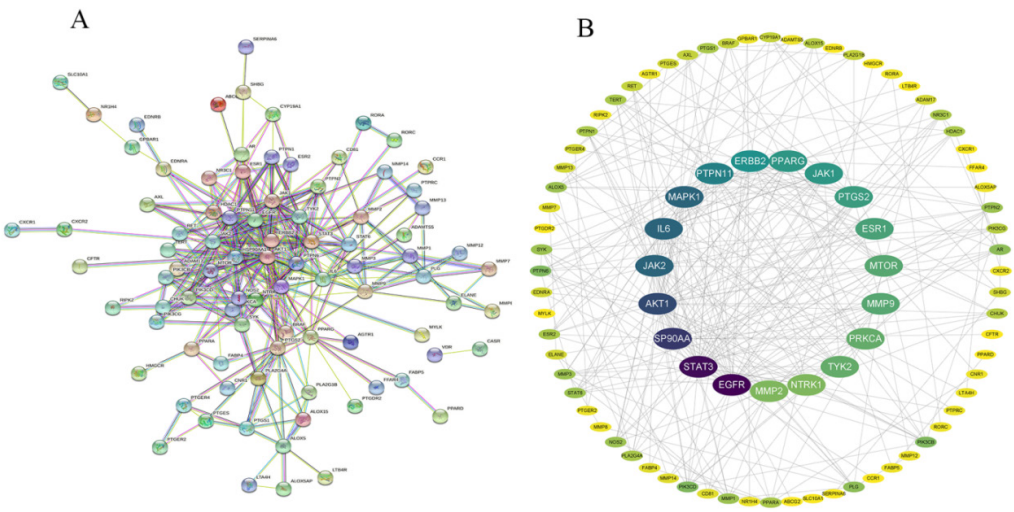
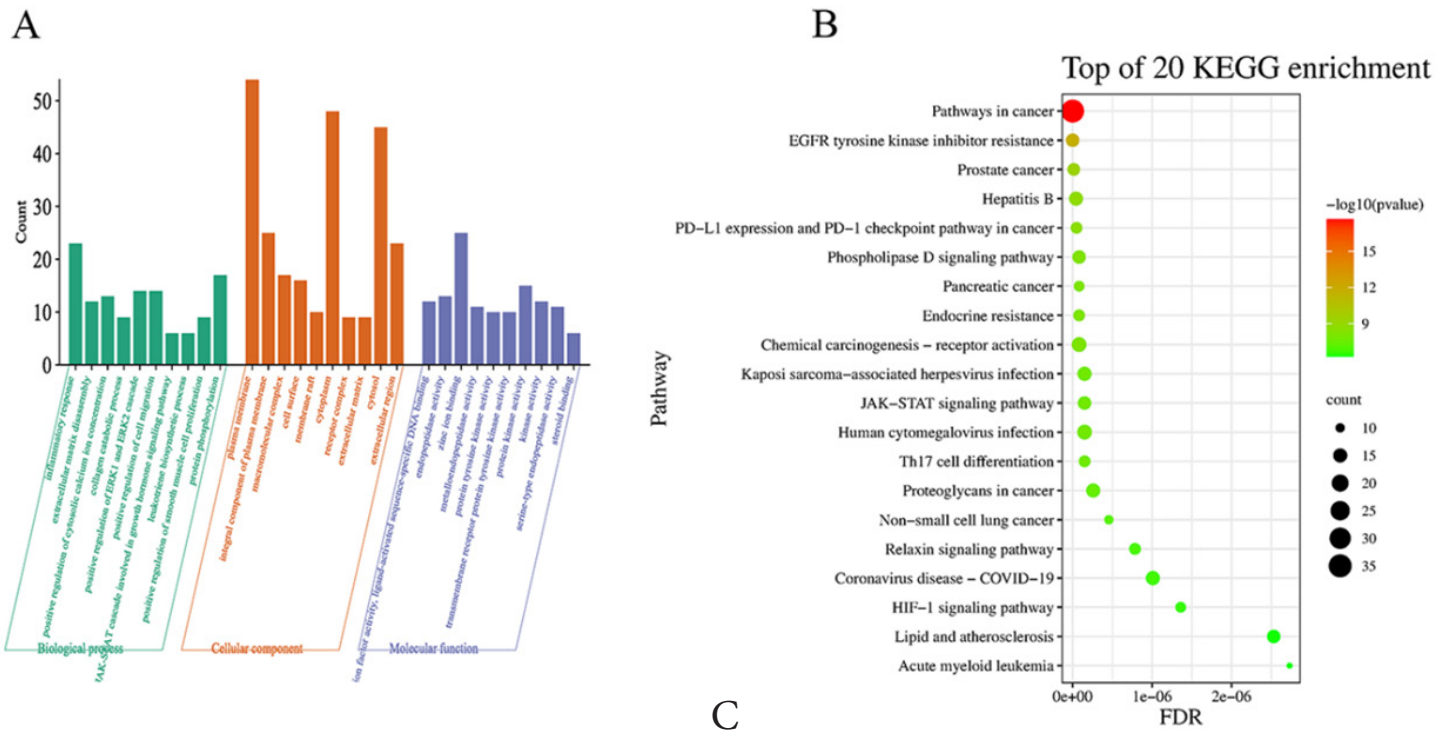


Figure 2: Protein-Protein Interaction (PPI) network and core target screening



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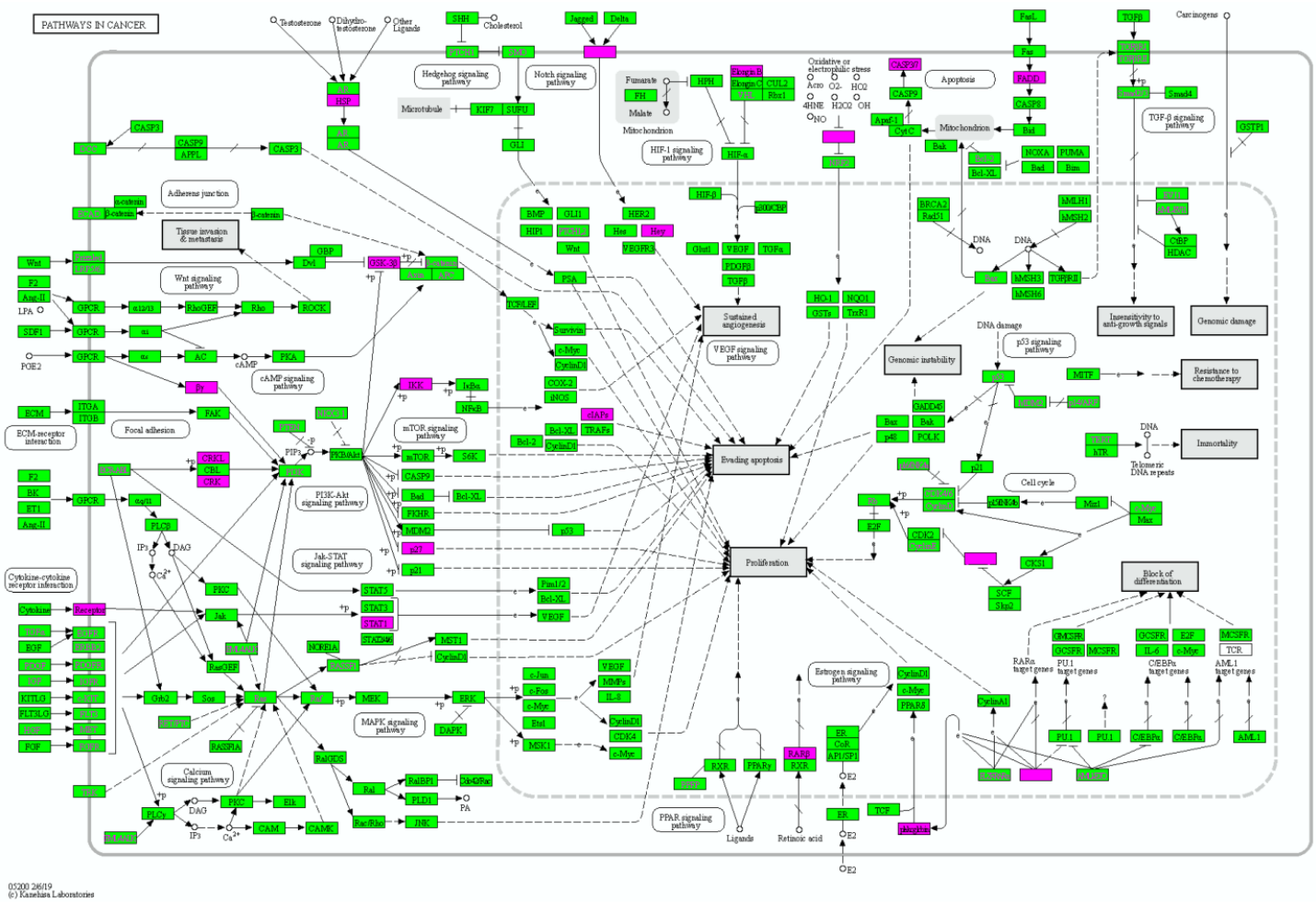

Figure 3: (A): GO function analysis; (B): KEGG enrichment analysis and (C): Pathways in cancer
Note: (■) BP; (■) CC; (■) MF

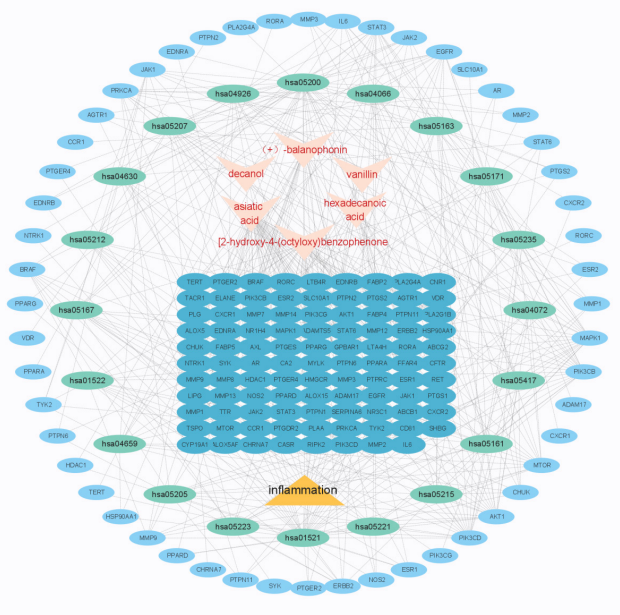

Figure 4: Active ingredient-target-disease-pathway network of *Schefflera octophylla* and inflammation

Molecular docking results

The core targets screened through network pharmacology analysis included Epidermal Growth Factor Receptor (EGFR), Signal Transducer and Activator of Transcription 3 (STAT3), Heat Shock Protein 90 Alpha family class A member 1 (HSP90AA1), Threonine Kinase 1 (AKT1), Mitogen Activated Protein Kinase 1 (MAPK1), Janus Kinase 2 (JAK2), and IL6, all of which were subjected to molecular docking to evaluate the binding affinity between the active ingredient of *Schefflera octophylla* and the predicted target genes (*Figure 5*). The results showed strong binding capacities of the active ingredient toward the core targets with AKT1, EGFR and STAT3 being among the strongest targets, as demonstrated by the molecular docking simulations (*Figure 6*). These results provide a basis for the anti-inflammatory therapeutic activity of *Schefflera octophylla* and further validate the predictions made in this study; however, further investigation into the mechanism of action and experimental validation are necessary to fully understand its effects.

Effects of Schefflera octophylla *on inflammatory KM mice*

The carrageenan-induced toe swelling assay (Morris CJ, 2003) was applied to mice to assess the anti-inflammatory effects of drugs, with IL-6 (del Giudice M and Gangestad SW, 2018) being a commonly used marker of inflammation and MDA (Gaweł S, *et al.*, 2004) serving as a marker of oxidative stress and antioxidant status. As depicted in (*Figure 7*), the results showed that the carrageenan-induced toe swelling in the model group was 44.09 mg, while dexamethasone treatment, high and medium doses of *Schefflera octophylla* ethanol and high doses of aqueous extracts significantly reduced toe swelling ($p<0.05$), indicating the anti-inflammatory properties of the treatments. Additionally, all treatments resulted in a significant decrease in IL-6 and MDA levels ($p<0.05$), indicating their anti-inflammatory and antioxidant effects. These results highlight the potential of ethanol and aqueous extracts of *Schefflera octophylla* as promising anti-inflammatory agents.

Effect of Schefflera octophylla *on the content of inflammatory factors in RAW264.7 cells and their morphology*

We next conducted *in vitro* experiments to validate the anti-inflammatory mechanism of *Schefflera octophylla* as predicted by network pharmacology. The levels of relevant cytokines secreted by LPS-stimulated RAW264.7 cells were measured and the cellular proteins were extracted for Western blotting. IC_{10} concentration of *Schefflera octophylla* ethanol and aqueous extracts were 149.935 µg/ml and 343.248 µg/ml, respectively, based on the MTT results. LPS-stimulated RAW264.7 cells produced significantly higher levels of NO compared to untreated cells, while treatment with ethanol and aqueous extracts of Schefflera octophylla significantly reduced the levels of NO ($p<0.05$). Similarly, the levels of IL-1β, IL-6 and IL-10 produced by LPS-stimulated RAW264.7 cells were significantly different from those produced by untreated cells ($p<0.05$), while the extracts inhibited the secretion of IL-6 and IL-1β and increased the level of IL-10. Compared to the model group, the treatment with *Schefflera octophylla* extract inhibited the secretion of IL-6 and IL-1β and increased the level of IL-10 ($p<0.05$). These findings demonstrate that both the aqueous and ethanol extracts of *Schefflera octophylla* have anti-inflammatory effects on LPS-induced inflammation in RAW264.7 cells (*Figure 8*).

Further, we observed the morphology of RAW264.7 cells under an inverted microscope at 40X following different treatments. The control group exhibited normal cell morphology, with round or oval shapes, intact organelles and compact cell clusters. Upon LPS stimulation, the cells showed a differentiated state, with an expanded cell area and prominent branching. High and medium doses of *Schefflera octophylla* extracts, as well as LPS, induced a significant reduction in branching cells, returning most cells to a normal morphology. Similarly, low doses of the extracts and LPS resulted in a decrease in branching cells. The combination of dexamethasone and LPS effectively reduced branched cells and restored the normal morphology of most cells. Thus, these results suggest that 1 µg/ml of LPS can induce inflammation in RAW264.7 cells and that of the *Schefflera octophylla* extracts can effectively inhibit cell differentiation and alleviate the inflammatory response.

Effects of Schefflera octophylla *on the PI3K/Akt signaling pathway in RAW264.7 cells*

The anti-inflammatory effect of *Schefflera octophylla* was further investigated by examining its effect on the PI3K/Akt signaling pathway. The results showed that treatment with ethanol and aqueous extracts of *Schefflera octophylla* resulted in significant reduction of expression of phosphorylated PI3K and Akt (p-PI3K and p-Akt, respectively) compared to LPS-stimulated RAW264.7 cells ($p<0.05$) (*Figures 9A-9C*).

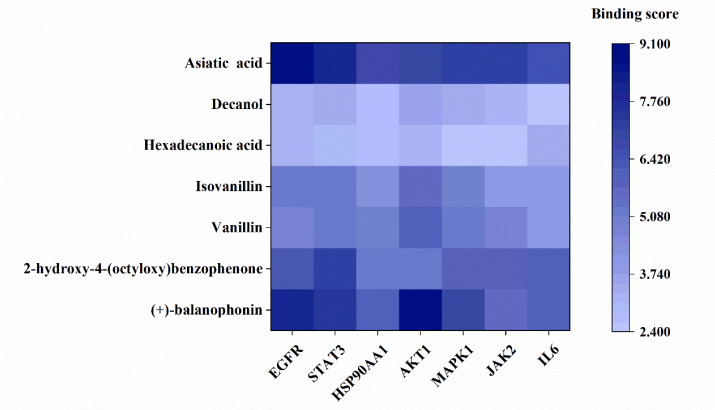

Figure 5: Docking score of bioactive ingredients binding with core genes

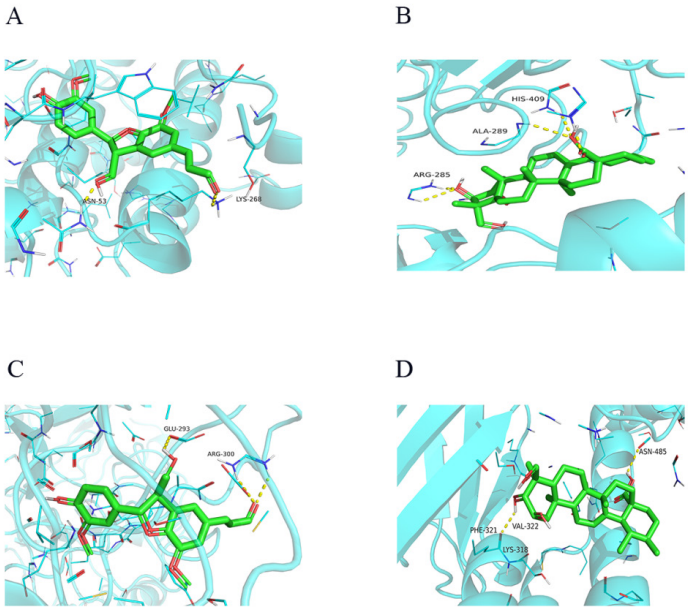

Figure 6: Molecular docking simulation of the active ingredients of *Schefflera octophylla* with core target proteins, (A): (+)-balanophonin with AKT1 (score=-9.0); (B): Asiatic acid with EGFR (score=-9.1); (C): (+)-balanophonin with EGFR (score=-8.0) and (D): Asiatic acid with STAT3 (score=-8.2)
Note: (▪): Formation of hydrogen bonds between ligand and protein

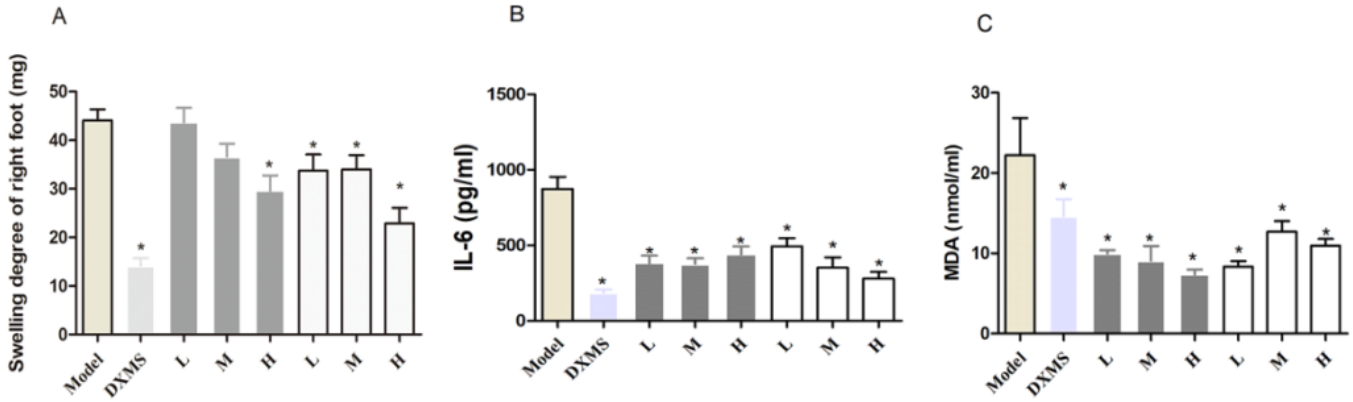

Figure 7: Effect of high, medium and low doses (10 g/kg, 5 g/kg and 2.5 g/kg, respectively) of medication on toe swelling and serum levels of IL-6 and MDA in KM mice
Note: (▬): Aqueous and (▭): Ethanol extract and *p<0.05 compared to the model group

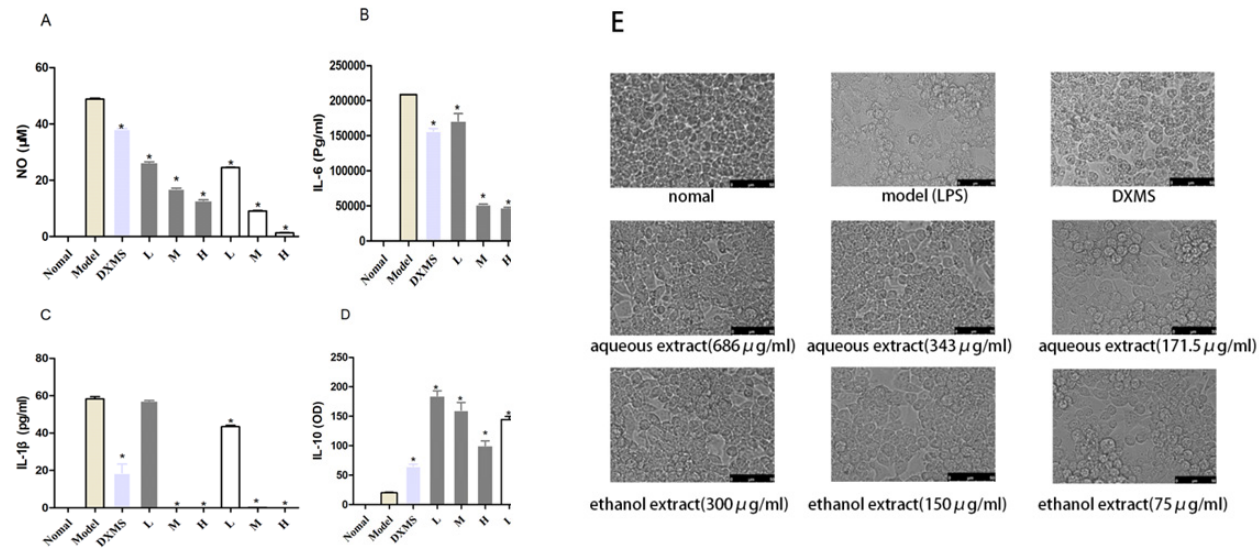

Figure 8: Effect of medication on LPS-induced levels of (A): NO, (B): IL-1β, (C): IL-6 and (D): IL-10 in RAW264.7 cells
Note: (▬): Aqueous and (▭): Ethanol extract

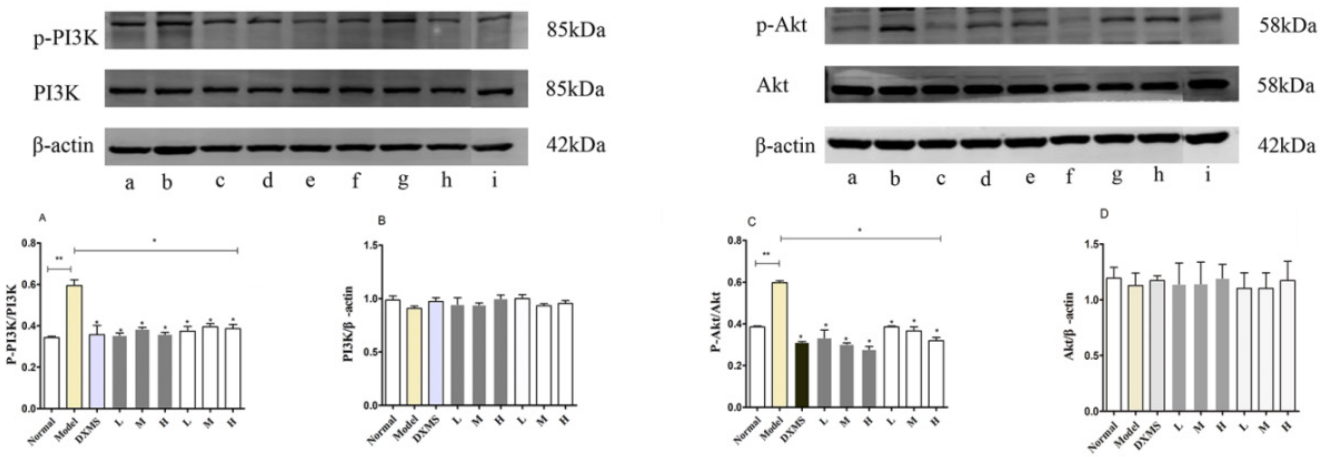

Figure 9: Effect of medication on the LPS-induced PI3K/Akt signalling pathway in RAW264.7 cells
Note: (▬): Aqueous and (▭): Ethanol extract

DISCUSSION

The traditional Chinese herb *Schefflera octophylla* has a rich history of medicinal use, as documented in ancient medical texts. Previous research on *Schefflera octophylla* has focused on the isolation and characterization of its constituent components. For instance, Song M, *et al.*, 2019; Sung TV and Adam G, 1991; Sung TV, *et al.*, 1991; Sung TV, *et al.*, 1991; Sung TV, *et al.*, 1992; van Sung T and Adam G, 1992 conducted a study on the isolation of terpenoids in *Schefflera* J. R.Forst and G.Forst. Subsequently, Liu X, *et al.*, 2019 established a method for the efficient extraction of triterpenoids in *Schefflera octophylla*. Studies examining the pharmacological properties of *Schefflera octophylla* have reported its antiviral, antioxidant (Li YL, *et al.*, 2004; Zheng YJ, 2009), analgesic, and anti-inflammatory effects (Chen *et al.*, 2015), which were evaluated using alcoholic extracts and active fractions of different polarities. The structure of the active components was also analyzed using spectroscopic data. However, there remains limited research on the underlying mechanism of action of *Schefflera octophylla*.

This study represents advancement in the exploration of the pharmacology and pharmacodynamics of *Schefflera octophylla*. Utilizing a network pharmacology approach, the previously identified active ingredients were integrated to predict the targets of action and molecular docking techniques which were employed to determine the specific pathways responsible for the anti-inflammatory effects of *Schefflera octophylla*. Experimentally, aqueous and ethanol extracts of *Schefflera octophylla* were compared to assess their efficacies in different treatment groups. The results indicate that the compounds of *Schefflera octophylla* impact multiple targets, with some instances of overlapping targets identified among different components. This finding suggests that the anti-inflammatory effects of *Schefflera octophylla* may be due to the synergistic actions of its compounds, which aligns with the "Manifestation theory" of Chinese medicine proposed by Cai SQ, *et al.*, 2015 and others.

The compounds of *Schefflera octophylla* are known to affect multiple targets, including core targets such as AKT1, EGFR, and STAT3. Results obtained from molecular docking analysis verified that the active ingredients of *Schefflera octophylla* bind to these targets. The anti-inflammatory effects of *Schefflera octophylla* were confirmed through *in vivo* and *in vitro* experiments, which confirmed that the inhibition of various inflammatory cytokines was the primary mechanism. Moreover, KEGG enrichment analysis revealed that *Schefflera octophylla* primarily interferes with the onset and

progression of inflammation through cancer signaling pathway, specifically PI3K/Akt, as well as through EGFR, JAK/STAT and other pathways.

The current study was conducted to investigate the anti-acute inflammatory effects of *Schefflera octophylla*. The results indicated that both aqueous and ethanol extracts of *Schefflera octophylla* inhibited toe swelling in animals, with more pronounced effect observed at higher doses. Additionally, the extracts down-regulated the inflammatory factor, IL-6 and reduced inflammatory peroxidation. The results of *in vitro* studies demonstrated that the extracts were equally effective in reducing inflammation in LPS-induced RAW264.7 cells, with high doses of the extracts demonstrating a significant reduction in inflammation and the state of cell differentiation. Overall, *Schefflera octophylla* shows promising anti-inflammatory properties, making it a potential candidate for future drug development.

PI3K, an upstream kinase of the serine/threonine protein kinase AKT (Song M, *et al.*, 2019), promotes the release of pro-inflammatory cytokines and mediates the secretion of IL-6 through Nuclear Factor Kappa B (NFkB) activation downstream of AKT (Koorella C, *et al.*, 2014). Studies have demonstrated that PI3K plays a regulatory role in certain innate immune responses (Hawkins PT and Stephens LR, 2015; Stark AK, *et al.*, 2015), while AKT is central to immune regulation by down-regulating inhibitory signals and promoting reactivation of immune cells (Tang F, *et al.*, 2018). The results of KEGG pathway enrichment analysis implicate that PI3K/Akt pathway as the most significant pathway involved in mediating anti-inflammatory activity, which was further validated through Western blot experiments.

Regarding the limitations of the study, the database-based analysis and screening represent only part of the approach and may not fully reflect the components, and targets that exert efficacy. Therefore, further research is needed to fully validate the specific anti-inflammatory pathways of *Schefflera octophylla* through *in vivo* and *in vitro* experiments.

CONCLUSION

In this study, we demonstrate the anti-inflammatory effects of *Schefflera octophylla* using both animal and cellular experiments. Both the aqueous and ethanol extracts of *Schefflera octophylla* showed potent anti-inflammatory properties. *In vivo*, *Schefflera octophylla* reduced carrageenan-induced toe swelling and decreased serum levels of IL-6 and MDA, while *in vitro*, *Schefflera octophylla* inhibited LPS-induced secretion of NO, IL-6, and IL-1β by RAW264.7 cells and up-regulated IL-10. The anti-inflammatory mechanism of *Schefflera octophylla* was further substantiated by the results of Western blotting, which showed decreased phosphorylation of PI3K and Akt, as predicted by network pharmacology. However, other potential anti-inflammatory mechanisms require further investigation in the future.

AUTHOR'S CONTRIBUTIONS

Zhou Xiaoqin was involved in writing the original draft and performed the experiments. Lan Xiaobu contributed in formal analysis, data curation and analyzing the data. Liang Yao was included in data curation and performed the experiments. Yang Bin investigated, writing, reviewing and editing of the paper.

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DATA AVAILABILITY

The research presented in this study requires continued accumulation of data. The data that has been used is confidential.

ETHICAL STATEMENT

The research was carried out in strict conformity with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of China. The protocol was approved by the Guangxi Medical University Laboratory Animal Centre (Guangxi, China).

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