

ORIGINAL RESEARCH ARTICLE

Integrated *in silico* and *in vitro* analysis of oxyphyllacinol in low back pain–associated neuroinflammation

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Abstract

Neuroinflammation and sensitization-related mechanisms may contribute to persistent low back pain (LBP), motivating investigation of compounds that can modulate these processes. This study integrated network pharmacology, molecular docking, and *in vitro* experiments to investigate the anti-neuroinflammatory effects of oxyphyllacinol and its potential relevance to LBP-associated pathways. Candidate targets of oxyphyllacinol were predicted using the SwissTargetPrediction, BATMAN-TCM, and SuperPred databases, and the results were intersected with LBP-associated genes obtained from the GeneCards and OMIM databases. Protein–protein interaction network analysis identified PTGS2, TRPV1, CNR1, and COMT as candidate hub targets. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses indicated associations with neuroactive ligand–receptor interaction and with the regulation of TRP channels by inflammatory mediators. Molecular docking predicted favorable binding poses of oxyphyllacinol with the four candidate proteins. Biological validation was performed in lipopolysaccharide (LPS)-stimulated BV2 microglia. Oxyphyllacinol reduced LPS-stimulated PGE2, TNF- α , and IL-6 release, and decreased *PTGS2* and *TRPV1* messenger RNA expression, while increasing *CNR1* and *COMT* messenger RNA expression (all $p < 0.01$). These findings demonstrate the anti-inflammatory activity of oxyphyllacinol in an *in vitro* microglial model and support further investigation of *PTGS2*-, *TRPV1*-, *CNR1*-, and *COMT*-associated pathways. Because the experimental validation was limited to an LPS-stimulated cell model, the results should be interpreted as preliminary mechanistic evidence rather than direct evidence of therapeutic efficacy in LBP.

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1. Introduction

Low back pain (LBP) is a common musculoskeletal condition and remains a leading cause of years lived with disability worldwide.¹ The Global Burden of Disease 2021 analysis estimated more than half a billion prevalent LBP cases in 2020 and projected that the number of affected people could exceed 800 million by 2050, underscoring the continuing population-level burden of the disorder.²

Low back pain is commonly categorized according to whether a specific underlying

cause can be identified; most cases encountered in clinical practice are classified as non-specific LBP (NSLBP).³ NSLBP is multifactorial, and inflammatory and sensitization-related mechanisms may contribute to symptom persistence in a subset of patients.⁴ Inflammatory mediators have also been associated with clinical features and outcomes in chronic NSLBP.⁵ Persistent nociceptive input can promote peripheral and central sensitization, while activated microglia can amplify neuroimmune signaling through the release of pro-inflammatory mediators.⁶ Accordingly, microglial and neuroimmune mechanisms have become important experimental targets in chronic pain research.^{7,8}

The biological heterogeneity of NSLBP is particularly important when interpreting inflammatory mechanisms. The absence of a single identifiable structural cause does not imply the absence of measurable biological changes; rather, NSLBP can reflect interacting nociceptive, immune, biomechanical, psychological, and social processes. A systematic review found that inflammatory biomarkers have been investigated across acute, subacute, and chronic NSLBP, but associations vary according to disease stage, biomarker, and study design.⁴ Clinical studies have likewise reported associations between circulating cytokines and pain-related outcomes in chronic NSLBP.⁵ These observations support inflammatory signaling as one potentially relevant biological component in selected patients, but individual cytokines or other inflammatory readouts should not be interpreted as disease-specific biomarkers or as direct surrogates of clinical analgesic efficacy. This distinction is central to the present study because changes in BV2 inflammatory mediators can support a mechanistic hypothesis without demonstrating improvement of LBP itself.

Neuroimmune communication provides a mechanistic bridge between peripheral inflammatory input and persistent changes in nociceptive processing. Following tissue stress or sustained nociceptive activity, immune mediators can influence primary afferent neurons, spinal neurons, and glial cells. Microglia respond to extracellular danger signals and neuronal mediators by altering morphology, transcriptional programs, and the production of cytokines and lipid mediators. When these responses are prolonged, they can enhance neuronal excitability and contribute to central sensitization. Experimental work in LBP-related models has further implicated spinal fractalkine signaling in neuronal sensitization. Accordingly, microglia are useful for examining neuroinflammatory mechanisms that may intersect with pain sensitization, while recognizing that a microglial culture cannot reproduce the anatomical and psychosocial complexity of human LBP.

Beyond these general observations, the literature supports a neuroimmune framework for persistent pain while also emphasizing that inflammatory responses are context-dependent. Chronic LBP is heterogeneous and should not be reduced to a single inflammatory mechanism.⁹ Importantly, translational work on pain chronification has shown that the relationship between inflammation and persistent pain is not uniformly detrimental: an acute neutrophil-dominated inflammatory response was associated with protection against the transition to chronic pain in experimental and human datasets.¹⁰ This finding cautions against interpreting lower inflammatory-marker concentrations as automatically equivalent to superior clinical pain outcomes. Together with evidence for microglial signaling and sensitization in experimental pain models, these data justify studying neuroinflammatory mechanisms while maintaining a clear distinction between cellular anti-inflammatory activity and therapeutic efficacy in LBP.

Currently, the clinical pharmacological treatments for LBP primarily include nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, opioid analgesics, muscle relaxants, and antidepressants/anticonvulsants.^{11,12} However, these medications share common limitations: long-term use of NSAIDs may lead to gastrointestinal (GI) damage, cardiovascular risks, and nephrotoxicity; opioids carry serious side effects such as addiction, respiratory depression, and constipation; while other adjuvant analgesics exhibit variable efficacy and significant adverse effects.¹³ Therefore, the search for multi-target, low-toxicity, and low-side-effect lead compounds with analgesic and anti-inflammatory properties from natural products has become one of the current hot spots in drug development.

This therapeutic context motivates investigation of compounds that may influence inflammatory and sensitization-related pathways through mechanisms distinct from conventional analgesia. However, computational target prediction, network centrality, and pathway enrichment are primarily hypothesis-generating approaches: their outputs depend on database coverage, annotation density, prediction algorithms, and the prior study intensity of individual genes.¹⁴⁻¹⁶ Molecular docking can further assess whether a ligand can adopt a plausible pose within a selected protein structure, but docking scores are computational estimates rather than direct measurements of binding affinity, agonism, antagonism, or target engagement.¹⁷ Consequently, candidate targets identified using network pharmacology require orthogonal experimental validation at the protein, biochemical, or functional level. In the present work, the BV2 microglia experiments were therefore designed as preliminary

biological validation of inflammatory and transcriptional responses, not as confirmation that oxyphyllacinol directly binds the predicted targets or treats LBP.

Oxyphyllacinol is a diarylheptanoid associated with the fruits of *Alpinia oxyphylla* Miq.¹⁸ The pharmacological literature on *A. oxyphylla* supports broader neuroprotective and anti-neuroinflammatory potential, although direct evidence for oxyphyllacinol itself remains limited. Sesquiterpenoids isolated from *A. oxyphylla* fruits have shown neuroprotective effects in H₂O₂-injured SH-SY5Y cells,¹⁹ while a purified heteropolysaccharide from the fruit suppressed inflammatory mediator production in lipopolysaccharide (LPS)-stimulated BV2 microglia.²⁰ Diarylheptanoids isolated from *A. oxyphylla* fruits have also demonstrated anti-inflammatory activity.²¹ More recent studies broaden this background: *Alpiniae Oxyphyllae* Fructus was reported to influence microglial polarization in an LPS-stimulated BV2 neuroinflammation model through triggering receptor expressed on myeloid cells 2 (TREM2)-related signaling,²² an arabinoxylan isolated from *A. oxyphylla* suppressed inflammatory mediator production and toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)/nuclear factor kappa B (NF-κB) signaling in BV2 microglia,²³ and *A. oxyphylla* volatile oil reduced neuroinflammatory signaling *in vivo* through the TLR4/MyD88/NF-κB axis.²⁴ These findings strengthen the neuroinflammatory rationale for studying this plant, while also emphasizing that effects of whole extracts, polysaccharides, volatile oil, sesquiterpenoids, or other constituents cannot be attributed directly to oxyphyllacinol.

This distinction is important for defining the knowledge gap addressed here. Direct mechanistic evidence for oxyphyllacinol in microglial neuroinflammation remains limited, and it is not known whether this individual compound modulates molecular pathways relevant to LBP-associated pain sensitization. At the chemical-class level, recent experimental work has shown that selected diarylheptanoids can suppress LPS-stimulated inflammatory mediators through α7 nicotinic acetylcholine receptor (nAChR)–Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3)/NF-κB–related signaling,²⁵ supporting anti-inflammatory potential for the scaffold without establishing the activity or targets of oxyphyllacinol. Transient receptor potential vanilloid 1 (TRPV1) and cannabinoid receptor 1 (CB1, encoded by *CNR1*) signaling are implicated in pain modulation and therefore provide biologically relevant candidate pathways for investigation.²⁶ Network pharmacology offers a systems-level framework for generating drug–target–disease hypotheses, while molecular docking can prioritize

structurally plausible ligand–protein interactions for experimental follow-up. Accordingly, computational predictions in the present study were treated as hypothesis-generating evidence rather than proof of direct target engagement.

The four candidate genes emphasized in this study are biologically relevant to different components of nociception and neuroinflammation. *PTGS2* encodes cyclooxygenase-2, an inducible enzyme involved in prostaglandin synthesis. TRPV1 is a non-selective cation channel that integrates noxious and inflammatory stimuli; experimental evidence has linked increased *TRPV1* expression in dorsal root ganglia with LBP-related phenotypes, and broader reviews identify TRP channels as major transducers of nociception.^{27,28} *CNR1* encodes CB1, a major component of endogenous pain-modulatory signaling,²⁹ whereas *COMT* participates in catecholamine metabolism and has been linked to pain sensitivity.³⁰ Because the products of these genes occupy distinct positions within inflammatory, sensory, and neuromodulatory systems, their convergence in a network analysis is potentially informative. At the same time, network centrality and docking scores cannot establish target engagement in living cells; experimental validation is therefore required.

Accordingly, this study combined computational prediction with *in vitro* validation. Network pharmacology was used to identify candidate oxyphyllacinol targets overlapping with LBP-associated genes, and molecular docking was used to evaluate plausible interactions with selected hub proteins. An LPS-stimulated BV2 microglial model was then used to assess inflammatory mediator release and the messenger RNA (mRNA) expression of selected candidate genes. The objective was to generate and experimentally examine mechanistic hypotheses linking the anti-neuroinflammatory activity of oxyphyllacinol to pathways potentially relevant to pain sensitization.

2. Materials and methods

2.1. Main experimental reagents

The main experimental reagents included: oxyphyllacinol standard (purity ≥ 98%, CAS number: 87657-77-0, Chengdu Must Bio-technology Co., Ltd., China); LPS (derived from *Escherichia coli* O111: B4; L2630, Sigma-Aldrich, United States of America [USA]); Dulbecco's Modified Eagle Medium/Ham's F-12 (DMEM/F12) nutrient mixture (11330032), fetal bovine serum (FBS; 10099141), and 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) (25200056), all purchased from Gibco, USA; Cell-Counting Kit-8 (CCK-8) cell proliferation–toxicity assay kit (C0037), mouse tumor necrosis factor alpha (TNF-α) enzyme-linked immunosorbent assay (ELISA)

kit (PT512), mouse interleukin (IL)-6 ELISA kit (PI326), mouse prostaglandin E2 (PGE2) ELISA kit (PE206), all purchased from Biotek Biotechnology, China; TRIzol® Reagent (15596026, Invitrogen, USA); PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A), TB Green® Premix Ex Taq™ II (Tli RNaseH Plus) (RR820A), all purchased from TaKaRa, Japan; and dimethyl sulfoxide (DMS; analytical grade; D2650, Sigma-Aldrich).

2.2. Main experimental instruments

The primary experimental instruments included: CO₂ incubator (HERAccl 150i, Thermo Scientific, USA), laminar flow hood (SW-CJ-2FD, Suzhou Antai Air Technology Co., Ltd., China), class II biosafety cabinet (1300 Series A2, Thermo Scientific), inverted phase-contrast microscope (CKX53, Olympus, Japan), micropipette (Eppendorf, Germany), centrifuge (5424R, Eppendorf), NanoDrop 2000 microplate reader (Thermo Scientific), real-time fluorescent quantitative polymerase chain reaction (PCR) system (QuantStudio 5, Applied Biosystems, USA).

2.3. Cell line

The mouse microglial cell line BV2 was donated by the Cell Resource Center of Guangxi University of Chinese Medicine.

2.4. Network pharmacology analysis of oxyphyllacinol and low back pain-associated targets

The computational workflow was designed as a sequential prioritization strategy rather than as a stand-alone demonstration of mechanism. First, chemical structure-based target prediction was used to generate a candidate oxyphyllacinol target set. Second, disease-associated genes were identified independently and intersected with the compound-related candidates. Third, protein-protein interaction (PPI) topology and functional enrichment were used to identify highly connected genes and biological themes within the overlap. Finally, selected hub proteins were subjected to molecular docking. This order was chosen to reduce the candidate space before experimental testing and to avoid interpreting any single database or algorithm as definitive evidence of target engagement.³¹

2.4.1. Target screening of oxyphyllacinol

In the SwissTargetPrediction database (<https://swisstargetprediction.ch/>, accessed on May 10, 2026), targets of oxyphyllacinol were predicted using the screening criterion of $p \geq 0.1$.³² Additionally, candidate targets of oxyphyllacinol were retrieved from the Bioinformatics Annotation Database for Molecular

Mechanism of Traditional Chinese Medicine (BATMAN-TCM) database (<http://bionet.ncpsb.org.cn/batman-tcm/>, accessed on May 11, 2026) and the SuperPred database (<https://prediction.charite.de/>, accessed on May 11, 2026). All retrieved targets were standardized using the UniProt database (<https://www.uniprot.org/>, accessed on May 10, 2026), and duplicate entries were removed to compile the final target set for oxyphyllacinol.

2.4.2. Target acquisition for low back pain

The following search terms were used: “Low Back Pain,” “Back Pain,” and “Lumbar Pain.” The GeneCards database (<https://www.genecards.org/>, accessed on May 12, 2026) was searched using these terms, and target genes with a relevance score >10 were selected. Additionally, disease-related targets were retrieved from the Online Mendelian Inheritance in Man (OMIM) database (<https://www.omim.org/>, accessed on May 12, 2026). All retrieved target genes were merged, and gene names were standardized through the UniProt database (<https://www.uniprot.org/>, accessed on May 12, 2026), with duplicates removed to construct a consolidated set of target genes associated with LBP.

2.4.3. Construction of the protein-protein interaction network

Topological ranking was used only as a prioritization criterion. Degree reflects the number of network connections of a node, whereas betweenness and closeness centrality describe different aspects of a node's position within the network. Maximal clique centrality (MCC) and Maximum neighborhood component (MNC) provide additional neighborhood-based ranking approaches. Because these metrics can favor well-studied or highly connected proteins, targets were not considered biologically validated solely because they ranked highly. The resulting hub list was instead carried forward to enrichment, docking, and experimental analyses.

The potential target set of oxyphyllacinol and the target set associated with LBP were uploaded to the Bioinformatics online platform (<https://www.bioinformatics.com.cn/>, accessed on May 14, 2026) to plot Venn diagrams and identify overlapping targets, which represent the potential candidate targets for oxyphyllacinol in treating LBP. This list of overlapping targets was then submitted to the STRING database (Version 11.0, <https://string-db.org/>, accessed on May 14, 2026) for PPI analysis. The species was specified as “*Homo sapiens*,” the minimum required interaction score was set to “moderate confidence (0.400),” and disconnected nodes were hidden from the network. The resulting PPI network data were downloaded and saved in TSV format for subsequent topological analyses.³³

2.4.4. Screening and analysis of candidate hub targets

The PPI network data exported from STRING were imported into Cytoscape 3.9.0 (Cytoscape Consortium, USA) for visualization and topological analysis.³⁴ NetworkAnalyzer was used to calculate node degree, betweenness centrality, and closeness centrality. Higher values indicate greater network connectivity or centrality. The cytoHubba plugin was then used to rank candidate hub genes using MCC, MNC, and degree algorithms. Targets consistently ranked highly across the algorithms were prioritized as candidate hub targets.

2.4.5. GO and KEGG enrichment analyses

Enrichment analysis was interpreted as the overrepresentation of annotated functions among the candidate genes. Gene Ontology (GO) terms were used to summarize biological processes, cellular components, and molecular functions represented in the overlap set, while Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was used to identify pathways containing more candidate genes than expected under the enrichment model. Enrichment does not demonstrate that a pathway is activated or inhibited in the experimental system. For this reason, the results were used to generate mechanistic hypotheses and to contextualize the selected targets rather than to claim pathway activity.

The screened list of common targets between oxyphyllacinol and LBP was submitted to the DAVID bioinformatics resource database (<https://david.ncifcrf.gov/home.jsp>, accessed on May 15, 2026) for functional annotation. The species was set as “*Homo sapiens*,” and GO functional enrichment analysis alongside KEGG pathway enrichment analysis were performed. The GO analysis encompassed three functional categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The significance thresholds for enrichment were defined as p (Fisher’s exact test) < 0.01 and false discovery rate (FDR) < 0.05 . Enriched terms were ranked by p -value in ascending order. The top 10 terms for BP, CC, and MF, as well as the top 20 pathways with the smallest p -values in KEGG enrichment analysis, were selected for visualization using the MicroBioInfo online platform (<http://www.microbioinfo.com/>, accessed on May 15, 2026) to generate bubble and bar charts, reflecting the primary biological functions and signaling cascades associated with the candidate targets.

2.4.6. Pharmacokinetic screening and target filtering

To evaluate the drug-likeness of the candidate molecule, the canonical Simplified Molecular Input Line Entry System (SMILES) string of oxyphyllacinol was submitted

to the SwissADME database (<http://www.swissadme.ch/>). Pharmacokinetic and drug-likeness parameters, including GI absorption, oral bioavailability score, and compliance with Lipinski’s rule of five (molecular weight ≤ 500 Da, Moriguchi octanol–water partition coefficient [MLOGP] ≤ 4.15 , hydrogen bond donors ≤ 5 , and hydrogen bond acceptors ≤ 10), were rigorously assessed. After confirming that oxyphyllacinol satisfied these structural and pharmacokinetic criteria, all of its predicted candidate targets were retained for downstream network and topological analyses.

2.4.7. Molecular docking

Molecular docking was performed to evaluate plausible interactions between oxyphyllacinol and selected candidate targets. Crystal structures of prostaglandin-endoperoxide synthase 2 (PTGS2; PDB ID: 5IKR), TRPV1 (7L3W), CB1 (5XR8), and catechol-*O*-methyltransferase (COMT; 3BWM) were obtained from the RCSB Protein Data Bank. Receptor structures were prepared in AutoDock Tools 1.5.7 (The Scripps Research Institute, USA) by removing crystallographic water molecules and non-relevant ligands/ions, adding polar hydrogens and Gasteiger charges, and exporting the structures in PDBQT format. The three-dimensional structure of oxyphyllacinol (PubChem CID: 6321405) was obtained from PubChem, energy-minimized using Open Babel 3.1.1 with the MMFF94 force field, and converted to PDBQT format. Where a co-crystallized ligand was available, redocking was used to assess protocol reproducibility; a root-mean-square deviation (RMSD) < 2.0 Å between the redocked and crystallographic poses was considered acceptable. Docking was performed with AutoDock Vina 1.2.0 (The Scripps Research Institute) using a grid centered on the co-crystallized ligand-binding site and an exhaustiveness value of 16. The top-ranked Vina pose was retained for visualization. Docking scores are reported as computational estimates and were interpreted as hypothesis-generating rather than as measurements of physical binding affinity. PyMOL 2.5 (Schrödinger, Inc., USA) and LigPlot+ 2.2 (EMBL-EBI, United Kingdom) were used to visualize selected ligand–protein interactions.³⁵

2.5. In vitro experimental validation

2.5.1. Cell culture

BV2 microglial cells were cultured in DMEM/F12 supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. Cells were passaged every 2–3 days, and cells in logarithmic growth were used for experiments. Based on preliminary viability screening, 1 µg/mL LPS was selected for subsequent experiments because it produced a measurable reduction

in viability while preserving most cells for downstream molecular analyses.

2.5.2. Establishment of the lipopolysaccharide-stimulated BV2 model and oxyphyllacinol treatment

The LPS-stimulated BV2 system was selected as a tractable model of microglial inflammatory activation. LPS activates innate immune signaling in BV2 microglia and is widely used to examine the regulation of pro-inflammatory mediators.^{36–38} The model was not intended to reproduce LBP. Instead, it was used to test whether oxyphyllacinol could modify inflammatory responses in a neural immune cell type relevant to pain sensitization. The concentration-screening step was therefore important for identifying conditions that produced a measurable inflammatory challenge while retaining sufficient cell viability for downstream assays.

BV2 microglia were seeded in 96-well plates for CCK-8 assays and in 6-well plates for RNA-based analyses. After cell attachment, the following groups were established: vehicle control (0.1% DMSO), oxyphyllacinol alone (80 $\mu\text{mol/L}$), LPS model (1 $\mu\text{g/mL}$ LPS), and LPS plus oxyphyllacinol. For the concentration–response viability experiment, oxyphyllacinol was tested at 20, 60, 80, and 120 $\mu\text{mol/L}$ in the presence of 1 $\mu\text{g/mL}$ LPS. Oxyphyllacinol was prepared in DMSO and diluted in culture medium so that the final DMSO concentration did not exceed 0.1%. Cells were incubated under the indicated conditions for 24 h before subsequent assays.

2.5.3. CCK-8 cell viability assay

BV2 microglia were seeded at 5×10^3 cells/well in 96-well plates. For LPS concentration screening, cells were exposed to 0, 0.01, 0.1, 1, 10, 20, or 40 $\mu\text{g/mL}$ LPS for 24 h. For oxyphyllacinol concentration–response testing, cells were treated with 0, 20, 60, 80, or 120 $\mu\text{mol/L}$ oxyphyllacinol under the specified experimental conditions for 24 h. CCK-8 reagent (10 $\mu\text{L/well}$) was then added, and cells were incubated at 37°C for 1–2 h. Absorbance at 450 nm was measured using a microplate reader. Relative cell viability (%) was calculated as:

$$\text{Relative cell viability (\%)} = \frac{OD_{\text{experimental}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100 \quad (1)$$

Based on the concentration–response and viability results, 1 $\mu\text{g/mL}$ LPS and 80 $\mu\text{mol/L}$ oxyphyllacinol were selected for subsequent mechanistic experiments.

2.5.4. Measurement of inflammatory mediators using ELISA

After 24 h of treatment, culture supernatants were collected and centrifuged at $1,000 \times g$ for 10 min at 4°C to remove cellular debris. Supernatants were stored at –80°C until analysis. TNF- α , IL-6, and PGE2 concentrations were measured using the corresponding ELISA kits according to the manufacturers' instructions. Absorbance was measured at 450 nm, and analyte concentrations were calculated from the respective standard curves.³⁹

2.5.5. Quantitative real-time PCR analysis of *PTGS2*, *TRPV1*, *CNR1*, and *COMT* mRNA

After 24 h of treatment, cells were washed twice with pre-cooled phosphate-buffered saline and lysed with TRIzol reagent. Total RNA was extracted according to the manufacturer's protocol, and RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer. One microgram of total RNA was reverse-transcribed using the PrimeScript RT reagent Kit with gDNA Eraser. Quantitative real-time PCR was performed using TB Green Premix Ex Taq II in a 20 μL reaction volume according to the manufacturer's instructions. Cycling conditions were 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30–34 s, followed by melting-curve analysis. *GAPDH* was used as the reference gene, and relative expression of *PTGS2*, *TRPV1*, *CNR1*, and *COMT* was calculated using the $2^{-\Delta\Delta C_t}$ method.^{40,41} Primer sequences are listed in Table 1.

2.6. Statistical analysis

Biochemical and molecular assays were performed in three independent biological experiments ($n = 3$), with independent cell preparations used for each experiment.

Table 1. Primer sequences

Gene	Upstream primer (5'→3')	Downstream primer (5'→3')	Product length (bp)
PTGS2	GCTGGAACATGGACTCACTC	GTGGGTTGCAGCAGTACAGA	120
TRPV1	TTCTTCCTCCTGCTGGTCAT	AGGAAGCGGATGATGAAGGT	145
CNR1	CACCATGCTGGTGGTGTC	GGGCAGGTAACAGGAAGAT	132
COMT	GGTGGTGGATGGCTATGAGA	CAGGAGGTCCTTCAGGTTGG	118
GAPDH	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT	110

Quantitative data are presented as mean $\pm$ standard deviation (SD).

For comparisons involving more than two groups, one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test was used. Two-group comparisons, where applicable, were performed using an unpaired two-tailed Student's *t*-test. Statistical analyses were conducted using SPSS version 26.0 (IBM, USA), and $p < 0.05$ was considered statistically significant.⁴² Because the number of independent biological replicates was small, the statistical findings should be interpreted as exploratory and in the context of effect direction and consistency across experiments. No human participants, human specimens, or live animals were used in this study.

3. Results

3.1. Target screening of oxyphyllacinol

Targets of oxyphyllacinol were retrieved from SwissTargetPrediction, BATMAN-TCM, and SuperPred databases. After UniProt standardization and deduplication, a total of 211 potential targets of oxyphyllacinol were obtained.

3.2. Acquisition of low back pain-related targets and drug-disease common targets

A total of 646 LBP-associated targets were retrieved from GeneCards and OMIM. Intersection of the oxyphyllacinol candidate-target set with the LBP-associated gene set yielded 26 overlapping targets (Figure 1). These overlapping genes were carried forward as candidate targets for subsequent network and enrichment analyses.

3.3. PPI network topology analysis and core target screening

A PPI network was constructed in STRING using the 26 overlapping targets and an interaction score threshold of 0.4. The network contained 26 nodes and 92 edges, with an average node degree of 7.08 and an average local clustering coefficient of 0.624; the STRING PPI enrichment *p*-value was $<1.0 \times 10^{-16}$ (Figure 2). Topological analysis was then used to prioritize highly connected nodes. Seven targets met the prespecified topological criteria, and PTGS2, TRPV1, CB1, and COMT were among the highest-ranked candidate hub targets, as summarized in the candidate hub-target network (Figure 3).

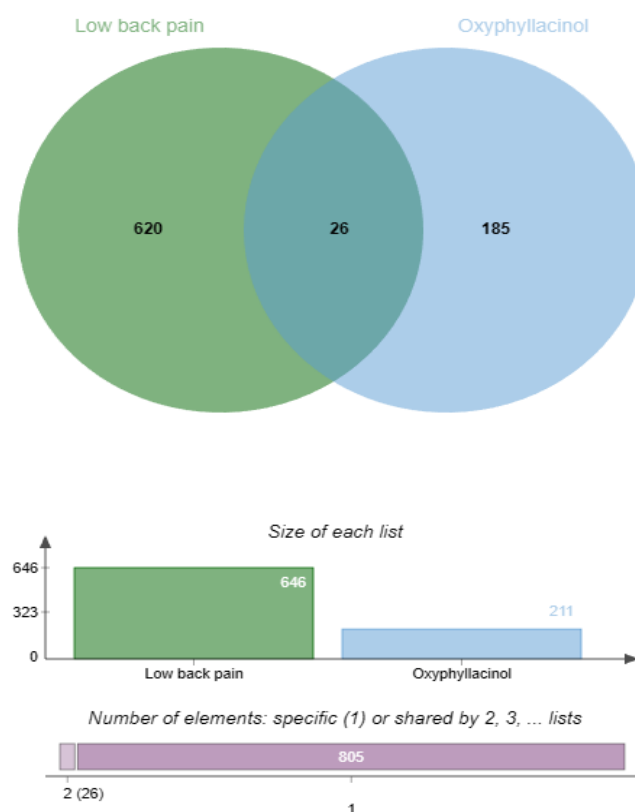


Figure 1. Acquisition of low back pain-related targets and drug-disease common targets

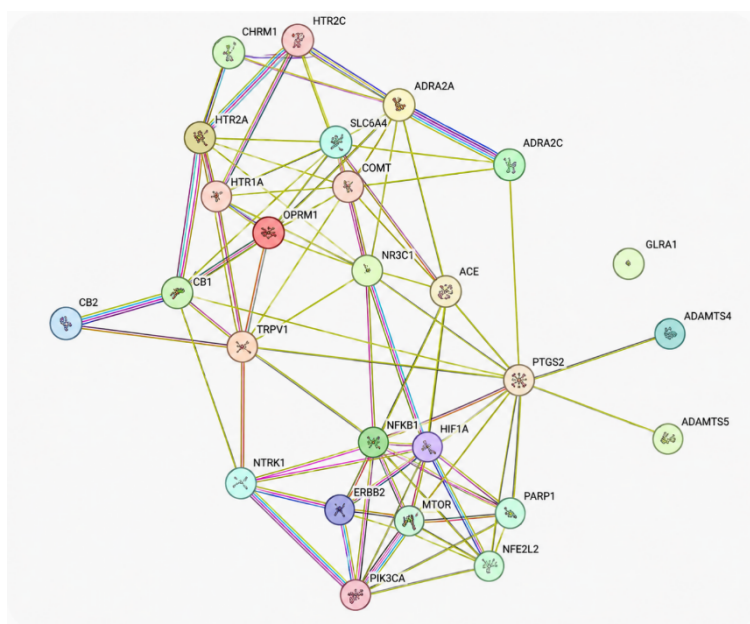


Figure 2. Potential target protein-protein interaction network diagram

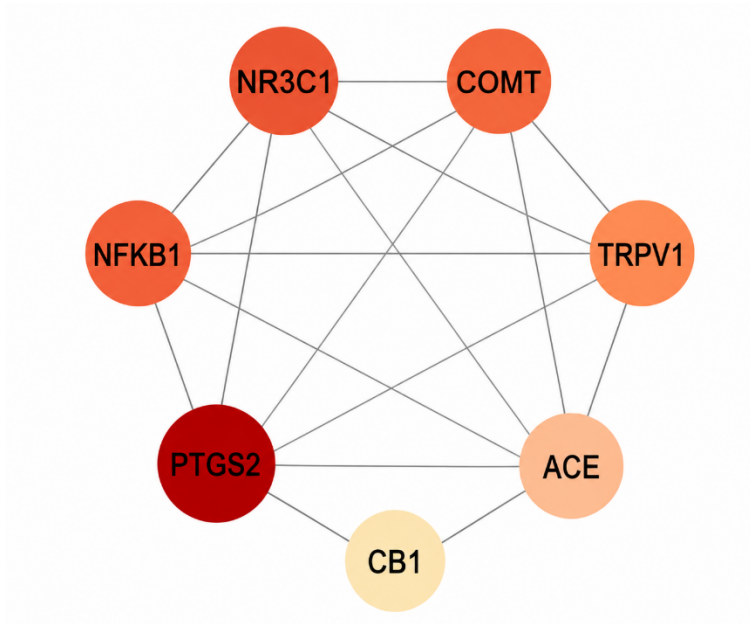


Figure 3. Candidate hub-target network

3.4. Construction of the drug-target-disease network

Oxyphyllacinol, the overlapping LBP-associated targets, and the disease node were imported into Cytoscape 3.9.0 to construct and visualize a drug-target-disease network (Figure 4).

3.5. GO and KEGG enrichment analyses

The 26 overlapping targets were subjected to GO enrichment analysis in DAVID. Using the prespecified significance criteria, enriched terms were identified across BP, CC, and MF categories. The highest-ranked terms included G protein-coupled receptor signaling, monoamine transport,

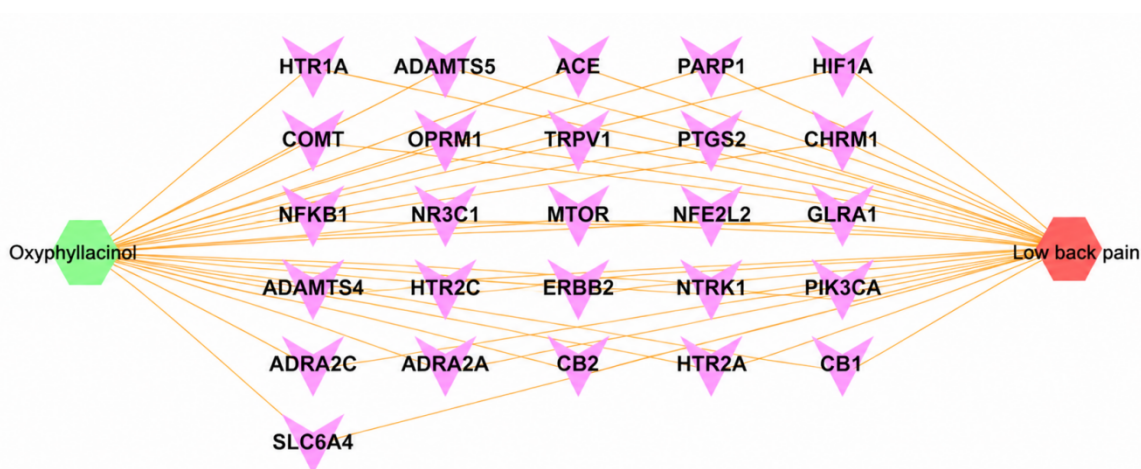


Figure 4. Drug–target–disease network

synaptic membrane components, and neurotransmitter receptor-related molecular functions. The top-ranked terms are shown in Figure 5.

The KEGG enrichment analysis identified pathways including neuroactive ligand–receptor interaction, inflammatory mediator regulation of TRP channels, hypoxia-inducible factor 1 (HIF-1) signaling, serotonergic synapse, phosphoinositide 3-kinase (PI3K)–protein kinase B (AKT) signaling, apoptosis, and calcium signaling (Figure 6). These enrichment results indicate statistical over-representation of the candidate genes in these pathways and do not demonstrate pathway activation or inhibition by oxyphyllacinol.

The simultaneous appearance of neuroactive ligand–receptor interaction and inflammatory mediator regulation of TRP channels was notable because these categories connect neuromodulatory signaling with inflammatory sensory transduction. However, the enrichment analysis was based on the overlapping computational target set and therefore reflects both the annotation structure and biological relationships. The pathway results were consequently used to guide interpretation of *PTGS2*, *TRPV1*, *CNR1*, and *COMT* rather than as independent confirmation that all enriched pathways were altered by oxyphyllacinol.

3.6. Molecular docking

Docking protocol validation by redocking yielded RMSD values of 0.85–1.52 Å for the evaluated targets. The top-ranked Vina scores for oxyphyllacinol with *PTGS2*, *TRPV1*, *CB1*, and *COMT* were -7.2 , -7.6 , -9.4 , and -6.9 kcal/mol, respectively. Representative predicted binding

poses are shown in Figure 7. These docking results support the potential of the selected targets for further investigation but do not establish direct physical binding.

Among the four proteins, *CB1* showed the most favorable top-ranked Vina score in this docking analysis, followed by *TRPV1*, *PTGS2*, and *COMT*. Because docking scores from different proteins are affected by binding-site size, receptor preparation, and scoring-function approximations, this ordering should not be interpreted as a quantitative ranking of experimental affinity. The principal value of the docking step was to determine whether structurally plausible poses could be generated within the selected binding sites and to provide target-specific hypotheses for later biochemical testing.

3.7. Cell experiment results

3.7.1. Effect of oxyphyllacinol on lipopolysaccharide-stimulated BV2 cell viability

The effects of LPS and oxyphyllacinol on BV2 cell viability were evaluated using a CCK-8 assay. Compared with vehicle control, LPS at 1, 10, 20, and 40 µg/mL significantly reduced cell viability after 24 h ($p = 0.0065$, 0.0028, 0.0042, and 0.0039, respectively). At 1 µg/mL, LPS reduced viability to approximately 74%; this concentration was therefore selected for subsequent experiments. In the presence of 1 µg/mL LPS, oxyphyllacinol at 20, 60, 80, and 120 µmol/L significantly increased cell viability relative to the LPS model group ($p = 0.032$, 0.017, 0.0092, and 0.0047, respectively). Based on the concentration–response profile and the absence of an evident adverse effect of 80 µmol/L oxyphyllacinol alone on baseline viability, 80 µmol/L was selected for subsequent assays (Table 2).

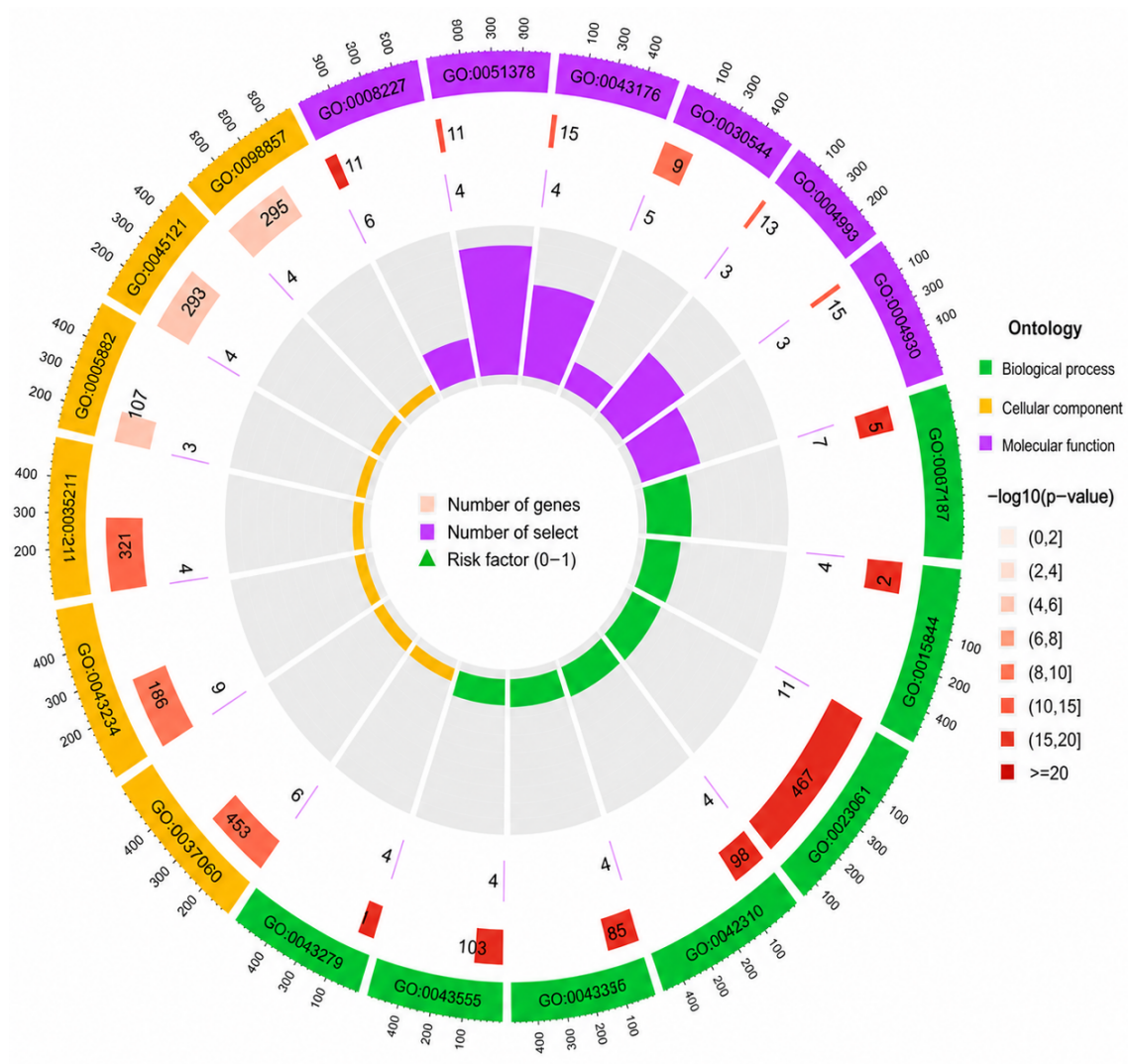


Figure 5. Gene Ontology enrichment analysis

Table 2. Effects of lipopolysaccharide and oxyphyllacinol on BV2 cell viability

Group	Treatment concentration (μmol/L)	Cell viability (%) (with control group as 100%)	p (vs. control group)	p (vs. model group)
Control	0	100.00 ± 3.20	–	–
	1	73.85 ± 4.35	0.0065	–
	10	59.64 ± 3.72	0.0028	–
	20	46.17 ± 3.95	0.0042	–
	40	31.02 ± 3.43	0.0039	–
Lipopolysaccharide model	20	85.22 ± 3.87	–	0.0320
	60	87.45 ± 3.91	–	0.0170
	80	93.76 ± 4.12	–	0.0092
	120	93.58 ± 3.65	–	0.0047

Notes: Data are reported as mean ± SD; n = 3.

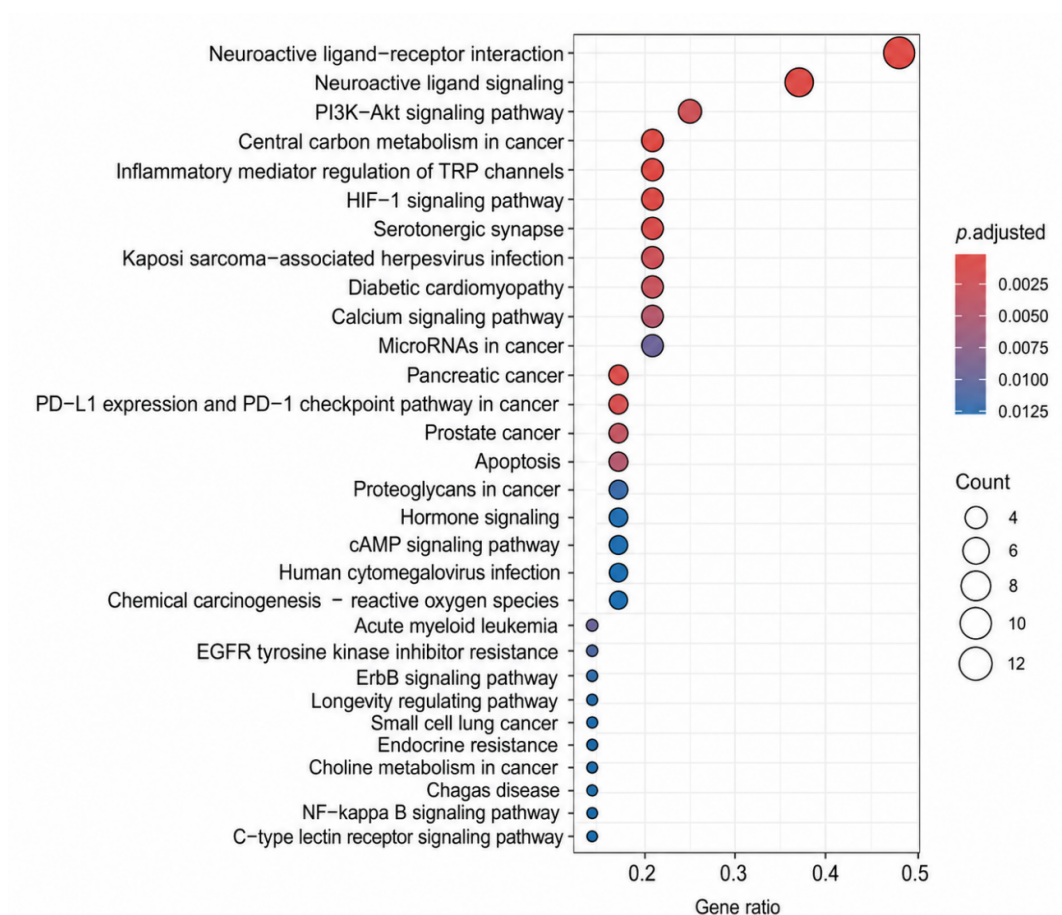


Figure 6. Kyoto Encyclopedia of Genes and Genomes enrichment analysis

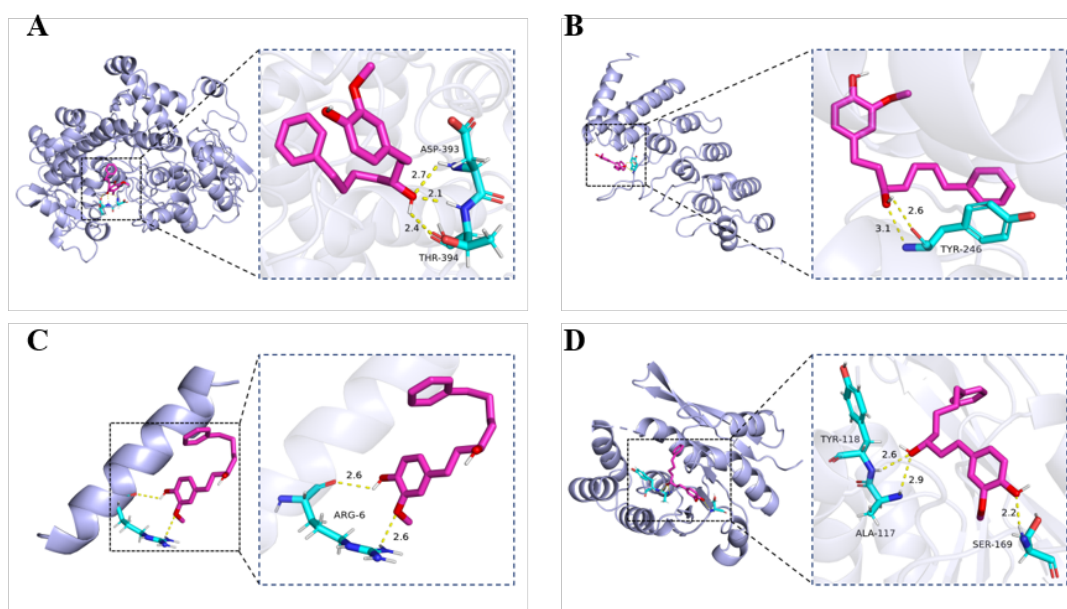


Figure 7. Predicted docking poses of oxyphyllacinol with (A) prostaglandin-endoperoxide synthase 2 (PTGS2), (B) transient receptor potential vanilloid 1 (TRPV1), (C) cannabinoid receptor 1 (CB1), and (D) catechol-O-methyltransferase (COMT)

3.7.2. Effect of oxyphyllacinol on inflammatory mediators in lipopolysaccharide-stimulated BV2 cells

The ELISA results showed that PGE2, TNF- α , and IL-6 concentrations were higher in the LPS model group than in the vehicle control group ($p = 0.0070$, 0.0042 , and 0.0095 , respectively). Treatment with $80 \mu\text{mol/L}$ oxyphyllacinol reduced PGE2, TNF- α , and IL-6 concentrations relative to the LPS model group ($P = 0.0065$, 0.0036 , and 0.0012 , respectively). These results indicate that oxyphyllacinol has an anti-inflammatory effect in LPS-stimulated BV2 cells (Table 3).

3.7.3. Effects of oxyphyllacinol on PTGS2, TRPV1, CNR1, and COMT mRNA expression in lipopolysaccharide-stimulated BV2 cells

Quantitative reverse transcription PCR was used to quantify mRNA expression of *PTGS2*, *TRPV1*, *CNR1*, and *COMT* (Table 4). Relative to the vehicle control, LPS significantly increased *PTGS2* and *TRPV1* mRNA expression ($p = 0.0075$ and 0.0042 , respectively) and decreased *CNR1* expression ($p = 0.0094$). The reduction in *COMT* expression after LPS exposure did not reach statistical significance ($p = 0.071$). Compared with the LPS model group, treatment with $80 \mu\text{mol/L}$ oxyphyllacinol decreased *PTGS2* and *TRPV1* expression ($p = 0.0064$ and

0.0023 , respectively) and increased *CNR1* and *COMT* expression ($p = 0.0038$ and 0.0019 , respectively). These transcriptional changes are consistent with modulation of candidate inflammatory and pain sensitization-related pathways in this cell model.

4. Discussion

This study combined network pharmacology, molecular docking, and an LPS-stimulated BV2 microglia model to investigate the anti-neuroinflammatory activity of oxyphyllacinol and its potential relationship to LBP-associated molecular pathways. Computational analyses prioritized *PTGS2*, *TRPV1*, *CB1*, and *COMT* as candidate hub targets and identified enrichment in neuroactive ligand–receptor interaction and inflammatory mediator regulation of TRP channels. Docking generated plausible predicted poses for oxyphyllacinol with the selected proteins, while cellular experiments showed reduced inflammatory mediator release and corresponding changes in mRNA expression of the four candidate genes. These findings provide a hypothesis-generating mechanistic framework; they do not establish direct target binding or therapeutic efficacy in LBP.

The *PTGS2* protein contributes to prostaglandin synthesis, and PGE2 is an important mediator of

Table 3. Expression of inflammatory factors in normalized cell supernatants

Groups	Concentration (ng/L)		
	PGE2	TNF- α	IL-6
Control	65.38 $\pm$ 10.24	10.85 $\pm$ 1.97	16.42 $\pm$ 3.58
LPS model	1,583.76 $\pm$ 135.42	305.47 $\pm$ 19.86	568.31 $\pm$ 48.22
Oxyphyllacinol	1,129.54 $\pm$ 98.65	235.18 $\pm$ 15.73	274.89 $\pm$ 41.67
LPS model vs. Control	$p < 0.0070$	$p < 0.0042$	$p < 0.0095$
Oxyphyllacinol vs. LPS model	$p < 0.0065$	$p < 0.0036$	$p < 0.0012$

Notes: Data are reported as mean $\pm$ SD; $n = 3$.

Abbreviations: IL-6: Interleukin-6; LPS: Lipopolysaccharide; PGE2: Prostaglandin E2; TNF- α : Tumor necrosis factor- α .

Table 4. Relative messenger RNA expression of PTGS2, TRPV1, CNR1, and COMT

Group	Fold change			
	<i>PTGS2</i>	<i>TRPV1</i>	<i>CNR1</i>	<i>COMT</i>
Control	1.00 $\pm$ 0.09	1.00 $\pm$ 0.08	1.00 $\pm$ 0.07	1.00 $\pm$ 0.19
LPS model	1.52 $\pm$ 0.12	2.24 $\pm$ 0.35	0.28 $\pm$ 0.05	0.47 $\pm$ 0.11
Oxyphyllacinol	1.08 $\pm$ 0.15	1.21 $\pm$ 0.22	1.33 $\pm$ 0.21	1.35 $\pm$ 0.24
LPS model vs. Control	$p < 0.0075$	$p < 0.0042$	$p < 0.0094$	$p < 0.0710$
Oxyphyllacinol vs. LPS model	$p < 0.0064$	$p < 0.0023$	$p < 0.0038$	$p < 0.0019$

Notes: Data are reported as mean $\pm$ SD; $n = 3$.

Abbreviation: LPS: Lipopolysaccharide.

inflammatory nociceptive sensitization. In the present BV2 microglia model, oxyphyllacinol reduced both LPS-stimulated *PTGS2* mRNA expression and PGE2 release, consistent with the reported anti-inflammatory activity of diarylheptanoids from *A. oxyphylla*.²¹ TRPV1 is a major transducer of inflammatory and noxious stimuli, and increased TRPV1 signaling activity is associated with pain sensitization.²⁷ Oxyphyllacinol reduced *TRPV1* mRNA expression in LPS-stimulated BV2 microglia. CB1 is a principal cannabinoid receptor involved in endogenous pain modulation,²⁹ whereas COMT regulates catecholamine metabolism and has been linked to pain sensitivity.³⁰ Oxyphyllacinol increased *CNR1* and *COMT* mRNA expression relative to the LPS model group. However, mRNA changes alone do not demonstrate corresponding changes in protein abundance, enzymatic activity, receptor signaling, or analgesic function; these relationships require direct experimental confirmation.

The *PTGS2*/PGE2 observation is particularly coherent across the computational and cellular components of the study. *PTGS2* was prioritized in the network analysis, and oxyphyllacinol treatment was associated with lower *PTGS2* mRNA and lower extracellular PGE2 in LPS-stimulated cells. This parallel change is biologically plausible because *PTGS2* catalyzes a rate-limiting step in inducible prostaglandin synthesis. Nevertheless, the present data do not establish that *PTGS2* is the direct molecular target responsible for the reduction in PGE2. Reduced transcription could arise secondarily from upstream changes in inflammatory signaling, and enzyme activity was not measured. Future experiments using *PTGS2* protein quantification, selective inhibition, and target-rescue approaches would be required to distinguish direct from indirect regulation.

The TRPV1 protein provides a complementary connection to sensory sensitization. Although TRPV1 is best known for its role in nociceptive neurons, TRP-channel-related signaling also intersects with inflammatory processes. Experimental evidence has linked increased TRPV1 expression in dorsal root ganglia to LBP-related phenotypes in rats,²⁷ and broader reviews identify TRP channels as key transducers of nociception.²⁸ In the present study, the decrease in *TRPV1* mRNA after oxyphyllacinol treatment is therefore consistent with a potential effect on a sensitization-related pathway. However, BV2 transcriptional changes cannot be extrapolated directly to neuronal TRPV1 function. Electrophysiological assays, calcium imaging, protein measurements, and co-culture or neuronal models would be necessary to determine whether the transcriptional effect translates into altered TRPV1-dependent sensory signaling.

The *CNR1* and *COMT* findings point toward neuromodulatory mechanisms that differ from the *PTGS2*/PGE2 inflammatory axis. Endocannabinoid signaling is an established regulator of pain processing, with CB1 acting at multiple peripheral and central sites. COMT, in turn, influences catecholamine metabolism and has been implicated in individual differences in pain sensitivity and in interactions between catecholaminergic signaling and inflammation. In this experiment, *CNR1* and *COMT* mRNA were higher after oxyphyllacinol treatment than in the LPS model group. The observation is compatible with restoration or enhancement of endogenous modulatory pathways, but it remains descriptive. Receptor abundance, ligand levels, COMT enzymatic activity, and downstream signaling were not measured, and the docking predictions do not resolve these gaps.

The computational components of this study should be interpreted as target-prioritization tools rather than as definitive evidence of drug-target interactions or pathway activation.¹⁶ Accordingly, the enrichment of neuroactive ligand-receptor and TRP-channel-related pathways should be viewed as a biologically coherent hypothesis that is supported by the direction of the cellular findings but still requires direct target-engagement and functional experiments.

The relationship between these cellular findings and clinical LBP should therefore be framed cautiously. Human LBP comprises multiple phenotypes, and the relative contribution of inflammation, peripheral sensitization, central sensitization, structural pathology, and psychosocial factors differs among individuals. A compound acting on microglial inflammatory responses could theoretically be relevant to a subset of mechanisms involved in persistent pain, but the present study does not identify such a patient subgroup and does not model mechanical loading, disc degeneration, facet-joint pathology, muscle injury, and psychological contributors. The value of the current results lies in mechanistic prioritization rather than direct clinical translation.

This distinction also has implications for drug development. Current LBP guidelines emphasize non-pharmacological management and the selective use of pharmacological therapies according to clinical context. The present data should not be interpreted as evidence that oxyphyllacinol can replace established management or that it has demonstrated analgesic efficacy. Instead, the results identify a reproducible direction of anti-inflammatory activity in a defined experimental system and a set of candidate molecular pathways that can be tested more rigorously. Demonstrating translational potential would require pharmacokinetic characterization, dose-exposure

relationship assessment, safety evaluation, target-engagement evidence, efficacy study in appropriate pain models, and ultimately controlled clinical investigation.

The cellular data provide partial experimental support for this computational prioritization. Oxyphyllacinol reduced PGE₂, TNF- α , and IL-6 release and altered *PTGS2*, *TRPV1*, *CNR1*, and *COMT* transcription in LPS-stimulated BV2 microglia. These observations are compatible with an anti-neuroinflammatory effect and with modulation of pathways relevant to pain sensitization. However, the KEGG enrichment of PI3K-AKT, HIF-1, and other pathways was not directly tested experimentally in this study. Accordingly, no conclusion can be drawn regarding activation, inhibition, or causal involvement of those pathways, and they should be considered candidates for future validation.

An additional strength of the integrated design is that the computational and experimental components address different levels of evidence. Database intersection and PPI analysis identify genes that are simultaneously associated with the compound prediction space and the disease literature; enrichment analysis organizes these genes into functional themes; docking evaluates whether selected protein structures can accommodate plausible ligand poses; and the BV2 experiments determine whether treatment changes measurable inflammatory and transcriptional outcomes. Agreement across these levels increases the coherence of a mechanistic hypothesis, but it does not eliminate the need for causal experiments. In particular, the current study cannot determine whether *PTGS2*, *TRPV1*, *CB1*, and *COMT* act independently, sequentially, or as parts of a broader upstream regulatory response.

The concentration-response findings also deserve careful interpretation. Oxyphyllacinol improved viability across the tested concentrations in the presence of LPS, with smaller *p*-values at 80 and 120 μ mol/L. The choice of 80 μ mol/L for mechanistic experiments was based on the overall concentration-response profile and the absence of an evident adverse effect of the compound alone on baseline viability. This design supports evaluation of an anti-inflammatory effect under the selected conditions, but it does not establish an optimal pharmacological concentration. Future work should characterize a broader concentration range, determine concentration-response parameters, and relate *in vitro* concentrations to achievable exposure in pharmacokinetic studies.

The inflammatory mediator data are internally consistent in direction: LPS increased PGE₂, TNF- α , and IL-6, whereas oxyphyllacinol reduced each mediator relative to the LPS model group. Because these mediators represent partially distinct branches of inflammatory

signaling, their coordinated reduction suggests that the effect is not limited to a single measured analyte. Nonetheless, the study did not examine upstream signaling events such as NF- κ B and mitogen-activated protein kinase (MAPK) activation, despite prior evidence that *A. oxyphylla* extracts can influence these pathways in LPS-stimulated BV2 microglia. Measuring phosphorylation, nuclear translocation, or pathway-specific reporter activity would help determine whether oxyphyllacinol acts through similar upstream mechanisms or through a distinct regulatory process.

This study has several limitations. First, the LPS-stimulated BV2 cell line is a reductionist model of microglial inflammation and does not reproduce the structural, biomechanical, peripheral neuronal, and systemic components of LBP. Therefore, the present experiments cannot demonstrate analgesic efficacy or disease modification in LBP. Second, network pharmacology and molecular docking provide computational predictions; direct binding of oxyphyllacinol to *PTGS2*, *TRPV1*, *CB1*, or *COMT* was not measured. Biophysical binding assays and target perturbation experiments will be required to establish target engagement and causality. Third, validation was limited primarily to mRNA expression and secreted inflammatory mediators; protein expression, phosphorylation, receptor activity, and enzyme activity were not examined. Fourth, only three independent biological experiments were performed, limiting statistical precision. Future studies should include larger replicate numbers, protein-level and functional validation, target knockdown or overexpression, and appropriate *in vivo* models relevant to LBP-associated pain mechanisms.

Several additional methodological considerations should guide subsequent studies. Target-prediction databases differ in training data, chemical-space coverage, and annotation depth, while disease databases are influenced by publication intensity. Consequently, highly studied genes may be preferentially represented in overlap and network analyses. Docking further depends on the selected protein conformation and scoring function. Experimental validation should therefore include orthogonal methods, such as surface plasmon resonance, microscale thermophoresis, cellular thermal shift assays, or other appropriate target-engagement approaches where technically feasible. For functional causality, pharmacological antagonists, gene silencing, or gene editing could be used to determine whether blocking an individual candidate target attenuates the observed effect of oxyphyllacinol.

The biological model could also be expanded in a stepwise manner. Primary microglia or human-derived

microglial models would help assess whether the findings are specific to the BV2 cell line. Neuron–microglia co-culture systems could test whether changes in inflammatory mediator release alter neuronal excitability or sensitization. Appropriate *in vivo* models could then evaluate behavioral pain outcomes together with spinal or peripheral neuroimmune markers. Only after such studies demonstrate consistent efficacy, target engagement, and acceptable exposure and safety would clinical investigation in well-characterized LBP populations be justified. This progression is especially important because LBP is heterogeneous and because a mechanistic effect on neuroinflammation may be relevant only to a subset of patients.

5. Conclusion

Oxyphyllacinol attenuated LPS-stimulated inflammatory mediator release in BV2 microglia and modulated the mRNA expression of *PTGS2*, *TRPV1*, *CNR1*, and *COMT*. Integrated network and docking analyses identified these genes and related pathways as candidates for pain sensitization and LBP-associated neuroinflammatory mechanisms. The findings support further mechanistic investigation of oxyphyllacinol as an anti-neuroinflammatory compound but do not establish direct target binding or therapeutic efficacy in LBP. Protein-level validation, target-engagement studies, and *in vivo* experiments are required before translational conclusions can be drawn.

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Conflict of interest

The authors declare no competing interests.

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Investigation: Shangzhi Wu

Methodology: Shangzhi Wu

Writing–original draft: Kok Hong Tang

Writing–review & editing: Kok Hong Tang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

Further disclosure

OpenAI ChatGPT was used under author supervision for English-language polishing, readability, coherence, reference-style checking, and manuscript-formatting assistance. The authors reviewed and approved the final text and remain responsible for the scientific content, data, interpretation, and conclusions.

References

1. Hartvigsen J, Hancock MJ, Kongsted A, *et al.* What low back pain is and why we need to pay attention. *Lancet*. 2018;391(10137):2356–2367.
doi: 10.1016/S0140-6736(18)30480-X
2. GBD 2021 Low Back Pain Collaborators. Global, regional, and national burden of low back pain, 1990–2020, its attributable risk factors, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. *Lancet Rheumatol*. 2023;5(6):e316–e329.
doi: 10.1016/S2665-9913(23)00098-X
3. Maher C, Underwood M, Buchbinder R. Non-specific low back pain. *Lancet*. 2017;389(10070):736–747.
doi: 10.1016/S0140-6736(16)30970-9
4. Morris P, Ali K, Merritt M, Pelletier J, Macedo LG. A systematic review of the role of inflammatory biomarkers in acute, subacute and chronic non-specific low back pain. *BMC Musculoskelet Disord*. 2020;21(1):142.
doi: 10.1186/s12891-020-3154-3
5. Licciardone JC, Kearns CM, Hodge LM, Bergamini MV. Associations of cytokine concentrations with key osteopathic lesions and clinical outcomes in patients with nonspecific chronic low back pain: results from the OSTEOPATHIC Trial. *J Am Osteopath Assoc*. 2012;112(9):596–605.
doi: 10.7556/jaoa.2012.112.9.596
6. Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and Central Sensitization in Chronic and Widespread Pain. *Anesthesiology*. 2018;129(2):343–366.
doi: 10.1097/ALN.0000000000002130
7. Inoue K, Tsuda M. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. *Nat Rev Neurosci*. 2018;19(3):138–152.
doi: 10.1038/nrn.2018.2

8. Sessler K, Blechschmidt V, Hoheisel U, Mense S, Schirmer L, Treede RD. Spinal cord fractalkine (CX3CL1) signaling is critical for neuronal sensitization in experimental nonspecific, myofascial low back pain. *J Neurophysiol.* 2021;125(5):1598-1611.
doi: 10.1152/jn.00348.2020
9. Foster NE, Anema JR, Cherkin D, *et al.* Prevention and treatment of low back pain: evidence, challenges, and promising directions. *Lancet.* 2018;391(10137):2368-2383.
doi: 10.1016/S0140-6736(18)30489-6
10. Parisien M, Lima LV, Dagostino C, *et al.* Acute inflammatory response via neutrophil activation protects against the development of chronic pain. *Sci Transl Med.* 2022;14(644):eabj9954.
doi: 10.1126/scitranslmed.abj9954
11. Qaseem A, Wilt TJ, McLean RM, Forciea MA. Noninvasive Treatments for Acute, Subacute, and Chronic Low Back Pain: A Clinical Practice Guideline From the American College of Physicians. *Ann Intern Med.* 2017;166(7):514-530.
doi: 10.7326/M16-2367
12. Schreijenberg M, Koes BW, Lin CWC. Guideline recommendations on the pharmacological management of non-specific low back pain in primary care—is there a need to change? *Expert Rev Clin Pharmacol.* 2019;12(2):145-157.
doi: 10.1080/17512433.2019.1565992
13. George SZ, Fritz JM, Silfies SP, *et al.* Interventions for the Management of Acute and Chronic Low Back Pain: Revision 2021. *J Orthop Sports Phys Ther.* 2021;51(11):CPG1-CPG60.
doi: 10.2519/jospt.2021.0304
14. Zhai Y, Liu L, Zhang F, *et al.* Network pharmacology: a crucial approach in traditional Chinese medicine research. *Chin Med.* 2025;20:8.
doi: 10.1186/s13020-024-01056-z
15. Hopkins AL. Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol.* 2008;4(11):682-690.
doi: 10.1038/nchembio.118
16. Li S. Network pharmacology evaluation method guidance-draft. *World J Tradit Chin Med.* 2021;7(1):146-154.
doi: 10.4103/wjtc.wjtc_11_21
17. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J Chem Inf Model.* 2021;61(8):3891-3898.
doi: 10.1021/acs.jcim.1c00203
18. Liao J, Zhao X. Recent Research Progress on the Chemical Constituents, Pharmacology, and Pharmacokinetics of *Alpinia oxyphylla* Fructus. *Molecules.* 2024;29(16):3905.
doi: 10.3390/molecules29163905
19. Qiu C, Mu L, Wang J, *et al.* Sesquiterpenoids from the fruits of *Alpinia oxyphylla* Miq. and their neuroprotective effect. *Phytochemistry.* 2023;211:113680.
doi: 10.1016/j.phytochem.2023.113680
20. Shi W, Zhong J, Zhang Q, Yan C. Structural characterization and antineuroinflammatory activity of a novel heteropolysaccharide obtained from the fruits of *Alpinia oxyphylla*. *Carbohydr Polym.* 2020;229:115405.
doi: 10.1016/j.carbpol.2019.115405
21. Dong J, Li H, Zhou M, Yao X, Geng J, Yu Y. Four new diarylheptanoids and two new terpenoids from the fruits of *Alpinia oxyphylla* and their anti-inflammatory activities. *Chin J Nat Med.* 2024;22(10):929-936.
doi: 10.1016/S1875-5364(24)60723-5
22. Xu M, Yang Y, Peng J, *et al.* Effects of *Alpinia oxyphylla* Fructus on microglial polarization in a LPS-induced BV2 cells model of neuroinflammation via TREM2. *J Ethnopharmacol.* 2023;302:115914.
doi: 10.1016/j.jep.2022.115914
23. Chang X, Zhang D, Shi W, *et al.* An arabinoxylan (AOP70-1) isolated from *Alpinia oxyphylla* alleviates neuroinflammation and neurotoxicity by TLR4/MyD88/NF- κ B pathway. *Int J Biol Macromol.* 2024;277:134339.
doi: 10.1016/j.ijbiomac.2024.134339
24. Wu B, Gan A, Wang R, Lin F, Yan T, Jia Y. *Alpinia oxyphylla* Miq. volatile oil ameliorates depressive behaviors and inhibits neuroinflammation in CUMS-exposed mice by inhibiting the TLR4-mediated MyD88/NF- κ B signaling pathway. *J Chem Neuroanat.* 2023;130:102270.
doi: 10.1016/j.jchemneu.2023.102270
25. Lin Y, Wongkrajang K, Shen X, *et al.* Discovery of diarylheptanoids that activate $\alpha 7$ nAChR-JAK2-STAT3 signaling in macrophages with anti-inflammatory activity in vitro and in vivo. *Bioorg Med Chem.* 2022;66:116811.
doi: 10.1016/j.bmc.2022.116811
26. Mlost J, Kostrzewa M, Malek N, Starowicz K. Molecular Understanding of the Activation of CB1 and Blockade of TRPV1 Receptors: Implications for Novel Treatment Strategies in Osteoarthritis. *Int J Mol Sci.* 2018;19(2):342.
doi: 10.3390/ijms19020342
27. Chen X, Chen Z, Ma G, *et al.* Reactive Oxygen Species Induced Upregulation of TRPV1 in Dorsal Root Ganglia Results in Low Back Pain in Rats. *J Inflamm Res.* 2024;17:2245-2256.
doi: 10.2147/JIR.S446841
28. Mickle AD, Shepherd AJ, Mohapatra DP. Sensory TRP Channels: The Key Transducers of Nociception and Pain.

- In: *Progress in Molecular Biology and Translational Science*. Elsevier; 2015;131:73-118.
doi: 10.1016/bs.pmbts.2015.01.002
29. Starowicz K, Di Marzo V. Non-psychotropic analgesic drugs from the endocannabinoid system: “magic bullet” or “multiple-target” strategies? *Eur J Pharmacol*. 2013;716(1-3):41-53.
doi: 10.1016/j.ejphar.2013.01.075
 30. Vetterlein A, Monzel M, Reuter M. Are catechol-O-methyltransferase gene polymorphisms genetic markers for pain sensitivity after all? A review and meta-analysis. *Neurosci Biobehav Rev*. 2023;148:105112.
doi: 10.1016/j.neubiorev.2023.105112
 31. Li X, Wei S, Niu S, *et al*. Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. *Comput Biol Med*. 2022;144:105389.
doi: 10.1016/j.compbiomed.2022.105389
 32. Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res*. 2014;42(W1):W32-W38.
doi: 10.1093/nar/gku293
 33. Szklarczyk D, Gable AL, Lyon D, *et al*. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. 2019;47(D1):D607-D613.
doi: 10.1093/nar/gky1131
 34. Shannon P, Markiel A, Ozier O, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498-2504.
doi: 10.1101/gr.1239303
 35. Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model*. 2011;51(10):2778-2786.
doi: 10.1021/ci200227u
 36. Huang P, Zhang Z, Zhang P, *et al*. TREM2 deficiency aggravates NLRP3 inflammasome activation and pyroptosis in MPTP-induced Parkinson's disease mice and LPS-induced BV2 cells. *Mol Neurobiol*. 2024;61(5):2590-2605.
doi: 10.1007/s12035-023-03713-0
 37. Jin CY, Lee JD, Park C, Choi YH, Kim GY. Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. *Acta Pharmacol Sin*. 2007;28(10):1645-1651.
doi: 10.1111/j.1745-7254.2007.00651.x
 38. Bao Y, Zhu Y, He G, *et al*. Dexmedetomidine attenuates neuroinflammation in LPS-stimulated BV2 microglia cells through upregulation of miR-340. *Drug Des Devel Ther*. 2019;13:3465-3475.
doi: 10.2147/DDDT.S210511
 39. Munoz OB. Determination of Proinflammatory and Antiinflammatory Cytokines by ELISA Technique. In: Matson RS, eds. *ELISA. Humana*; 2023:101-108. Walker JM, ed. Methods in Molecular Biology; vol 2612.
doi: 10.1007/978-1-0716-2903-1_8
 40. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*. 2001;25(4):402-408.
doi: 10.1006/meth.2001.1262
 41. Spinsanti G, Zannolli R, Panti C, *et al*. Quantitative Real-Time PCR detection of TRPV1-4 gene expression in human leukocytes from healthy and hyposensitive subjects. *Mol Pain*. 2008;4:51.
doi: 10.1186/1744-8069-4-51
 42. Kim J, Kim DH, Kwak SG. Comprehensive guidelines for appropriate statistical analysis methods in research. *Korean J Anesthesiol*. 2024;77(5):503-517.
doi: 10.4097/kja.24016