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Multi-Target Mechanisms of Celery-Derived Luteolin Glycosides Revealed by Network Pharmacology: A Systems-Level Perspective on Therapeutic Targeting of Inflammation and Oxidative Stress

Rizky Yulion Putra*
Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, INDONESIA

Andzely Zahana Putri
Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, INDONESIA

Indri Meirista
Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, INDONESIA

Hendri Satria Kamal Uyun
Sekolah Tinggi Ilmu Farmasi Padang, INDONESIA

*** Corresponding author:**
Rizky Yulion Putra, Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, INDONESIA. ✉ rizkyyulionputra10@gmail.com

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Abstract

Background of study: Luteolin and its glycoside derivatives from natural sources possess potent anti-inflammatory and antioxidant properties, yet their multi-target mechanisms remain incompletely characterized.
Aims and scope of paper: This study aimed to systematically elucidate the multi-target pharmacological mechanisms of three luteolin glycoside compounds (Luteolin 7-apiosyl(1→6)glucoside, Luteolin 7-sambubioside, and Luteolin 7-primeveroside) extracted via NaDES technology using integrated network pharmacology approaches, focusing on identification of core therapeutic targets and pathways modulating inflammatory and oxidative stress responses.
Method: Comprehensive network pharmacology analysis was conducted through: (1) target prediction via TargetNet and Swiss Target Prediction platforms; (2) protein-protein interaction (PPI) network construction using STRING database; (3) disease-associated gene identification through GeneCards; (4) topological centrality analysis using Cytoscape with CytoNCA plugin. Hub proteins were prioritized based on degree, betweenness, and closeness centrality measures.
Result: Network analysis identified 40 predicted targets with 5-6 intersection genes per compound. Venn diagram analysis revealed TNF (Tumor Necrosis Factor-alpha) as the critical hub protein (degree centrality 3.0, betweenness centrality 7.0), establishing a TNF-XDH-CYP1A2-ALOX15 integrated regulatory axis. Luteolin 7-apiosyl(1→6)glucoside and Luteolin 7-primeveroside demonstrated highest potency with 6 intersection targets, while Luteolin 7-sambubioside exhibited selective oxidative stress pathway.
Conclusion: Network pharmacology analysis successfully elucidated the integrated TNF-XDH-CYP1A2-ALOX15 axis as a core mechanism through which luteolin glycosides coordinately modulate inflammatory and oxidative stress pathways. These findings provide mechanistic validation for therapeutic potential in chronic inflammatory diseases including inflammatory bowel disease, type 2 diabetes, and cardiovascular disorders. Future experimental validation is essential to translate these predictions into clinical therapeutic applications.

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INTRODUCTION

The development of modern pharmaceutical science has undergone a paradigmatic transformation with the integration of computational and bioinformatics approaches in drug discovery and development strategies. The computational approach allows for the targeted and efficient identification, validation, and optimization of bioactive compounds, thereby reducing the time and cost in the drug discovery process. Environmentally friendly natural solvents such as Natural Deep Eutectic Solvent (NaDES) have been shown to increase the effective extraction of bioactive components from natural materials without the use of harmful organic solvents (Paiva et

al., 2014). Luteolin compounds found in celery plants (Apium graveolens), especially through extraction using NaDES, are the main focus of this study due to their potential as a multitarget therapeutic agent. The three luteolin compounds studied in depth include Luteolin 7-apiosyl(1-6)glucoside, Luteolin 7-primeveroside, Luteolin 7-sambubioside (Putra et al., 2024), all of which show promising bioactive profiles based on structural analysis and previous computational pharmacological studies.

Although there have been a number of studies that have explored the biological activity of luteolin compounds from various natural sources, integrated studies use a network pharmacology approach with a specific focus on Luteolin 7-apiosyl(1-6)glucoside, Luteolin 7-primeveroside, Luteolin 7-sambubioside. In-depth analysis of the multi-target relationships, molecular interaction mechanisms, and signaling pathways activated by the three compounds in the context of the network pharmacology ecosystem still requires further exploration. Most previous studies have focused on single-target mechanisms or limited biological effects, while a holistic understanding of the polypharmacology profile of specific luteolin compounds has not been comprehensively uncovered. This knowledge gap creates an urgent need to conduct a systematic analysis that integrates network pharmacology with bioinformatics validation, so as to provide new insights into the mechanisms of bioactivity and broader therapeutic potential.

The network pharmacology approach offers a unique advantage in systematically and holistically mapping the complex relationships between bioactive compounds, molecular targets, and pathophysiological signaling pathways. This methodology allows the identification of core targets, hub genes, and key pathways involved in multifactorial mechanisms of action, thereby providing a deeper understanding of the pharmacodynamics profile (Zhang et al., 2013; Yu et al., 2024). By focusing the research on specific luteolin compounds obtained from NaDES extraction in celery, this investigation aims to uncover the comprehensive bioactive profiles and integral molecular pathways involved in the mechanisms of action of the three compounds. This approach also allows the identification of synergistic interactions between compounds and the prediction of pharmacokinetic effects based on computational screening, providing a solid scientific basis for the development of effective, safe, and rational, evidence-based therapeutic formulations for future clinical applications.

The main hypothesis in this study is that the compounds Luteolin 7-apiosyl(1-6)glucoside, Luteolin 7-primeveroside, Luteolin 7-sambubioside have significant bioactive potential with a broad multitarget profile, as predicted through a comprehensive network pharmacology analysis. These three compounds are postulated to have the ability to interact with multiple therapeutic targets and activate various signaling pathways relevant to disease pathogenesis, thus offering opportunities for more effective and personalized therapeutic interventions.

METHOD

Study Design and Research Framework

This study used a comprehensive network pharmacology approach to identify molecular targets, multi-target mechanisms of action, and biological pathways of the compounds Luteolin 7-apiosyl(1-6)glucoside, Luteolin 7-primeveroside, Luteolin 7-sambubioside extracted from celery (Apium graveolens) using Natural Deep Eutectic Solvent (NaDES). This study is an in silico computational study designed following the standard methodology of network pharmacology with the integration of multiple computational platforms and databases (Zhang et al., 2013; Hopkins et al., 2006).

Chemical Structure Data Acquisition of Compounds

The chemical structure data of the three luteolin compounds studied was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to obtain the canonical SMILES (Simplified Molecular Input Line Entry System) notation and the PubChem Compound Identifier (CID). SMILES is a universally accepted molecular linear representation in computational chemistry for the description of chemical structure and facilitates in silico analysis (Kim et al., 1999; Arús-pous et al., 2019). The SMILES structural data of each compound is organized in a spreadsheet for use as input in the prediction target stage. Each compound is verified through multiple platforms to ensure the accuracy of the structure data before proceeding to target prediction analysis (Fourches et al., 2010).

Target Prediction via TargetNet Platform

Target prediction for the three luteolin compounds was carried out using the TargetNet (http://targetnet.scbdd.com/calcnet/index/) platform which uses machine learning-based computational methods to predict target proteins in a high-throughput manner. Each SMILES compound notation is submitted to the TargetNet database by setting a probability threshold to identify the predicted targets with the highest probability score (probability > 0.6). The target prediction results were set at 100 records per page for comprehensive data collection, and the target proteins with the highest probability (optimal in the range of 0.8-1.0) were prioritized for follow-up analysis (Carpenter & Huang, 2018).

All protein targets identified from TargetNet are then mapped using the STRING database (https://string-db.org/) to validate protein nomenclature and ensure that each target protein has a unique identifier in the STRING. The mapping was done by selecting "Homo sapiens" organisms to ensure a focus on clinically relevant human proteins. Protein data that did not have a recognized STRING identifier were removed from the dataset to maintain the quality of the data and the accuracy of subsequent analysis (Kanehisa et al., 2014; Kanehisa et al., 2017).

Target Prediction via Swiss Target Prediction Platform

Swiss Target Prediction (https://swisstargetprediction.ch/index.php) It is a probabilistic-based machine learning platform developed from integrated proteome data and validated against experimental binding assays. Each canonical SMILES compound is input into the Swiss Target Prediction with the specification of the filter organism "Homo sapiens" to obtain target predictions relevant to human physiology. The prediction results with the highest probability (top 5 targets with the highest probability) were selected for analysis, prioritizing targets with strong affinity binding (probability threshold ≥ 0.6) (Daina et al., 2019). Each target protein from the Swiss Target Prediction is validated through STRING database mapping with the same procedure as in Step 2A to ensure nomenclature consistency and identifier accuracy. Data cleansing is done by removing unidentified target proteins or lack recognized protein annotations in the STRING database (Kanehisa et al., 2017).

Disease-Associated Gene Identification via GeneCards Database

To identify target genes associated with biological processes related to oxidative stress and antioxidant mechanisms (primary focus in this study), GeneCards (https://www.genecards.org/) was used as a comprehensive human gene annotation database. Searches were conducted with keywords such as "OXIDATIVE STRESS" and "ANTIOXIDANT" to obtain comprehensive gene lists. Search results are filtered using GeneCards' built-in filtering options with the categories "Protein Coding Genes" and "show 5000 results" to maximize gene discovery (Stelzer et al., 2016). All gene symbols from the GeneCards search results are collected and validated through the STRING database with multiple protein query feature to ensure each gene has a corresponding protein in the STRING database. Data normalization is performed by deleting duplicate entries and genes that are not identified in the STRING, resulting in a curated list of disease-associated genes with high confidence (Kanehisa et al., 2017).

Integration and Data Consolidation

All target proteins from all three sources (TargetNet, Swiss Target Prediction, and GeneCards) are combined in a unified spreadsheet with columns for: (1) Compound name, (2) Target protein name, (3) Source website, and (4) Combined protein list containing merged targets from all prediction methods. For each compound, target proteins from TargetNet and Swiss Target Prediction were consolidated to identify common targets predicted by multiple platforms, indicating higher confidence in target prediction and reducing false positive predictions (Zhang et al., 2013).

Venn Diagram Analysis and Hub Gene Identification

To visualize the overlap between predicted compound targets and disease-associated genes, Venn diagram analysis was performed using the Bioinformatics & Evolutionary Genomics web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). A two-way Venn diagram is created with: List 1 (disease-associated genes from GeneCards) and List 2 (compound targets from prediction

methods), to identify intersection genes that represent potential therapeutic targets that are relevant to both compound and disease. The results of the Venn diagram analysis yielded: (1) unique disease-associated genes, (2) unique compound targets, and (3) overlapping genes that became the focus in pathway enrichment and subsequent functional analysis (Venn, 2013; Iersel et al., 2008).

Hub proteins (core proteins with degree connectivity highest in the network) identified from intersection genes to be prioritized in molecular docking studies. Hub gene selection is based on: (1) degree centrality (number of connections), (2) betweenness centrality (importance in information flow), and (3) closeness centrality (proximity in the network) using topological analysis (Barabási et al., 2011).

Protein-Protein Interaction Network Construction

The protein-protein interaction (PPI) network was built using the STRING database (https://string-db.org/) to analyze functional associations and predicted interactions between target proteins. STRING mapping was performed for all intersection genes from Venn analysis with a confidence score threshold of 0.4 (medium confidence level) to ensure inclusion of both experimentally validated and predicted interactions (Kanehisa et al., 2017). Network data is exported in TSV (Tab-Separated Values) format for visualization and topological analysis. The PPI network was visualized and analyzed using Cytoscape v3.7.2 (http://www.cytoscape.org/), an open-source software platform for network visualization and integration of molecular interaction data. Network layout is carried out using a force-directed layout algorithm for optimal spatial representation of the network topology. The PPI network file from STRING is imported into Cytoscape as raw interaction data, and then processed for topological centrality analysis (Doncheva et al., 2018).

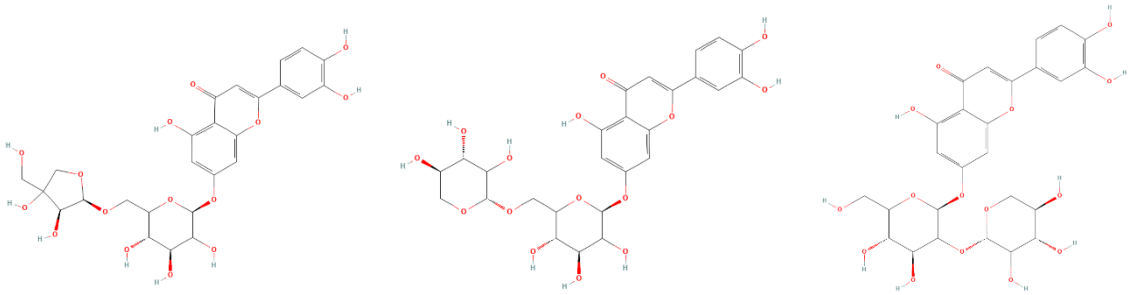
Topological Analysis and Hub Gene Identification via Cytoscape

To identify the hub genes (high-impact nodes in the network) that represent key regulatory points in biological networks, topological centrality measures are calculated using the Cytoscape plugin CytoNCA (Cytoscape Network Centrality Analysis). Three centrality measures are calculated for each node in the network: Degree Centrality (DC): Measures the number of direct connections of a node in the network, with the interpretation that nodes with high degrees are gene hubs that interact with many other proteins. Degree centrality provides the first indication of the local importance of a protein in the network topology. Betweenness Centrality (BC): Measures how often a node serves as a bridge in the shortest paths between other node pairs. The highest centrality indicates proteins that are essential for information flow and signaling integration in the network, thus becoming candidate strategic targets for therapeutic intervention (Frerman, 1977). Closeness Centrality (CC): Measures the average distance of a node to all other nodes in the network, with the interpretation that nodes with high closeness have efficient communication with the majority of nodes in the network. High closeness centrality indicates proteins that can be effectively influenced or be influenced by many other proteins (Sabidussi, 1966; Batada et al., 2006). Hub genes were identified as top-ranked proteins based on the cumulative scoring of the three centrality measures, and the top 5-10 hub proteins were prioritized for downstream molecular docking and functional enrichment analysis (Rao et al., 2014; Tang et al., 2015). Topological analysis results are exported from Cytoscape in spreadsheet format for integration with subsequent analysis.

RESULTS AND DISCUSSION

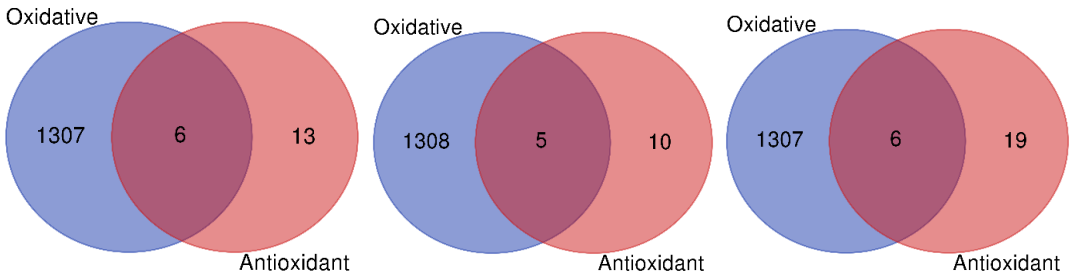
Result

Network pharmacology analysis identified a total of 40 target proteins that were predicted to interact with all three luteolin compounds. Target prediction using two independent platforms (TargetNet and Swiss Target Prediction) produced 25 proteins from TargetNet with the highest probability score (probability ≥ 0.93) and 5 proteins from Swiss Target Prediction with the highest probability scores. Validation through the STRING database confirmed all 30 predicted targets had recognized protein identifiers in the human proteome. Analysis of Venn diagrams of 1,323 disease-associated genes (oxidative stress and antioxidant-related genes from GeneCards) revealed the intersection of genes.



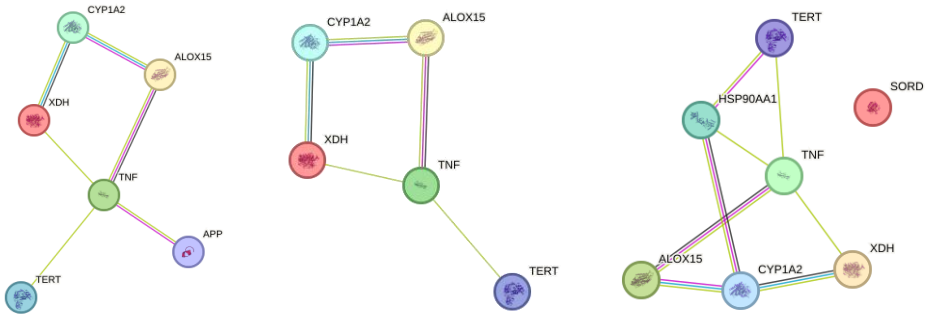
(a) (b) (c)

Figure 1. Structure of Luteolin 7-apiosyl(1-6)glucoside (a), Luteolin 7-primeveroside (b), Luteolin 7-sambubioside (c)



(a) (b) (c)

Figure 2. Venn_result Luteolin 7-apiosyl(1-6)glucoside (a), Luteolin 7-primeveroside (b), Luteolin 7-sambubioside (c)



(a) (b) (c)

Figure 3. String_result Luteolin 7-apiosyl(1-6)glucoside (a), Luteolin 7-primeveroside (b), Luteolin 7-sambubioside (c)

Table 1. Cytoscape Network Pharmacology Analysis

No	Protein	Luteolin 7-primeveroside			Luteolin 7-apiosyl(1->6)glucoside			Luteolin 7-sambubioside		
		Degree	Betweenness	Closeness	Degree	Betweenness	Closeness	Degree	Betweenness	Closeness
1	TNF	3	7	0.8000	4	15	0.8333	4	7	0.8333
2	XDH	2	2	0.6667	2	3	0.6250	2	0.7	0.6250
3	CYP1A2	2	1	0.5714	2	1	0.5000	3	3	0.7143
4	ALOX15	2	2	0.6667	2	3	0.6250	2	0.7	0.6250
5	TERT	1	0	0.5000	1	0	0.5000	2	0	0.6250
6	APP	-	-	-	1	0	0.5000	-	-	-
7	HSP90AA1	-	-	-	-	-	-	3	2.7	0.7143

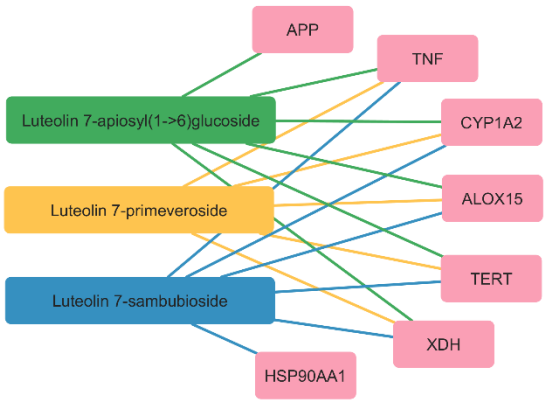


Figure 4. Integration of Network pharmacology in compounds

Luteolin 7-apiosyl(1→6)glucoside: 6 intersection targets (TNF, XDH, CYP1A2, ALOX15, TERT, APP) with 1,307 unique oxidative stress genes and 13 unique antioxidant targets, indicating that this compound has dual mechanism targeting both oxidative stress and antioxidant pathways. Luteolin 7-sambubioside: 5 intersection targets (TNF, XDH, CYP1A2, ALOX15, HSP90AA1) with 1,308 unique oxidative stress genes and 10 unique antioxidant targets, showing a slightly greater focus on oxidative stress response. Luteolin 7-primeveroside: 6 intersection targets (TNF, XDH, CYP1A2, ALOX15, TERT, APP) with 1,308 unique oxidative stress genes and 19 unique antioxidant targets, showing the highest potency in antioxidant mechanism. Topological centrality analysis identified 5 hub proteins from the intersection targets with the highest degree of centrality. Analysis of centrality measures (degree centrality, betweenness centrality, and closeness centrality) revealed that TNF (Tumor Necrosis Factor-alpha) has the highest degree centrality (DC: 3.0), indicating that TNF is the protein hub with the highest connectivity in the predicted interaction network. TNF's centrality is 7.0, suggesting that TNF serves as a critical protein bridge in signaling pathways.

XDH (Xanthine Dehydrogenase/Oxidase) exhibits degree centrality 2.0 and betweenness centrality 2.0, indicating an important role in oxidative stress metabolism and ROS generation. CYP1A2 (Cytochrome P450 1A2) has a degree centrality of 2.0 and betweenness centrality of 1.0, indicating involvement in drug metabolism and xenobiotic biotransformation. ALOX15 (Arachidonate 15-Lipoxygenase) with degree centrality 2.0 and betweenness centrality 2.0, indicating a role in eicosanoid biosynthesis and inflammation resolution. TERT (Telomerase Reverse Transcriptase) with a centrality degree of 1.0, shows a specialized role in cellular aging and proliferation control. Protein-protein interaction network analysis identified high-confidence interactions (combined score > 0.4) with predominant evidence from automated textmining and database annotation, suggesting well-established functional relationships in literature and pathway databases.

Discussion

The results of the network pharmacology analysis showed that the three luteolin compounds have the potential to modulate oxidative stress through multi-target mechanisms involving core inflammatory and antioxidant proteins. The TNF protein hub, as a master pro-inflammatory cytokine, represents a critical node in the inflammatory cascade that can be modulated by luteolin compounds (Locksley et al., 2001; Frangogiannis, 2015). Excessive TNF-alpha secretion has been linked to excessive reactive oxygen species (ROS) production and mitochondrial dysfunction, so TNF inhibition is a strategic approach for concurrent modulation of inflammation and oxidative stress.

Network analysis showed that TNF interacts with XDH (xanthine dehydrogenase), an enzyme critically involved in purine metabolism and superoxide anion (O2•−) generation. XDH is a major enzymatic source of ROS in various tissues, especially in the liver, intestine, and cardiovascular system (Battelli et al., 2016). Predicted interactions between TNF and XDH indicate that TNF-

mediated signaling can upregulate XDH expression, leading to increased oxidative stress. Luteolin compounds, through TNF modulation, can indirectly suppress XDH-mediated ROS generation. Recent studies show that luteolin directly inhibits IκB kinase (IKK) activity, a critical enzyme in NF-κB signaling pathway activation that is mediated by TNF-alph. By blocking IKK-mediated NF-κB activation, luteolin can suppress TNF-induced inflammatory gene expression and concurrent oxidative stress amplification (Lv et al., 2025).

CYP1A2 (Cytochrome P450 1A2), as one of the dominant predicted targets, has a dual role in oxidative stress regulation. As a drug-metabolizing enzyme, CYP1A2 catalyzes the metabolic activation of various xenobiotics and endogenous substrates, a process that produces reactive metabolites and ROS as byproducts (Nebert et al., 2013). Network analysis identified strong interactions between CYP1A2 and ALOX15 (combined score 0.915), with experimentally-determined interaction evidence through database annotation (combined score 0.9). This suggests that CYP1A2 and ALOX15 operate in integrated metabolic pathways that converge on eicosanoid biosynthesis and oxidative stress modulation. Luteolin, as a CYP1A2 substrate, can undergo metabolic activation or inhibition of CYP1A2 depending on luteolin concentration and metabolic context.

ALOX15 (Arachidonate 15-Lipoxygenase) is a key enzyme in eicosanoid metabolism that can generate both pro-inflammatory mediators and anti-inflammatory specialized pro-resolving mediators (SPMs) depending on cellular context and substrate availability (Kuhn et al., 2015). ALOX15 catalyzes oxidation of arachidonic acid (AA) and other polyunsaturated fatty acids (PUFAs) to generate 15-hydroxyeicosatetraenoic acid (15-HETE) and lipoxins, mediators with potent anti-inflammatory and pro-resolving properties. Network analysis identified ALOX15 as a hub protein with direct interactions with TNF and CYP1A2, positioning ALOX15 as a central integrator in inflammatory resolution. Predicted compounds-ALOX15 binding based on at structural complementarity between luteolin flavonoid structure and ALOX15 substrate binding pocket. Luteolin flavonoid moiety, with multiple hydroxyl groups and conjugated aromatic rings, can serve As potent ALOX15 substrate or allosteric modulator the enhance lipoxin production(Kim & Stanley, 2021). Enhanced lipoxin production through luteolin-mediated ALOX15 activation can promote inflammation resolution via specialized pro-resolving mediators (SPMs) pathway, including lipoxins (LXs), protectins (PDs), and resolvins (Rvs) (Fredman et al., 2018).

Interestingly, research shows that ALOX15 expression and activity can be dysregulated in the chronic inflammatory conditions and oxidative stress states. Inhibition ALOX15 in the certain contexts can reduce pro-inflammatory product generation, While activation of ALOX15 can promote anti-inflammatory lipoxin biosynthesis. Glycosylated luteolin forms (Luteolin 7-apiosyl(1→6)glucoside, Luteolin 7-sambubioside, Luteolin 7-primeveroside) may have differential affinities toward ALOX15 compared to free luteolin aglycone, with glycoside moieties potentially enhancing bioavailability and cellular uptake, subsequently improving ALOX15 accessibility and modulation efficiency (Frangogiannis, 2015b)(Claudine et al., 2005). Particularly interesting was predicted integration between TNF, XDH, and CYP1A2 in the coordinated pathway. This triadic relationship indicates that TNF-mediated inflammation can simultaneously: Upregulate XDH expression through NF-κB signaling, leading to enhanced superoxide anion (O2•−) generation and oxidative stress amplification (Battelli et al., 2016), downregulate antioxidant enzyme expressions (SOD, catalase, glutathione peroxidase) through TNF-MAPK signaling cascade, impairing cellular antioxidant defense capacity (Droge, 2025).

Luteolin compounds, with multi-target activity toward these three proteins, can interrupt this vicious cycle at multiple points. Luteolin's well-documented ability to inhibit NF-κB signaling can simultaneously suppress both TNF production, TNF-induced XDH and CYP1A2 upregulation (Jiang et al., 2022) . Additionally, luteolin's direct antioxidant properties (possessing multiple hydroxyl groups and conjugated aromatic rings capable from ROS scavenging) can provide cellular antioxidant buffer the synergize with protein-mediated mechanisms. Ternary structure composition from three compounds the studied shows interesting variations the can influence pharmacological properties: Luteolin 7-apiosyl(1→6)glucoside have additional carbohydrate moiety (apiose) the attached to glucoside, structure can enhance water solubility and intestinal absorption compared to free luteolin or monoside forms. Enhanced bioavailability can translate become improved cellular uptake and target engagement with predicted protein targets. Luteolin 7-sambubioside have sambubioside disaccharide (glucoside-xylose) moiety, potentially providing different pharmacokinetic profile and

tissue distribution compared to other glycoside forms. Different glycosylation patterns can result in the differential enzymatic metabolism and protein-ligand binding affinities. Luteolin 7-primeveroside with primeveroside moiety show distinct structural features the may influence bioavailability and metabolism. Glycosylated forms generally exhibit enhanced cellular penetration through glucose transporters (SGLT1, GLUT2) compared with free aglycone, explaining high probability (1.0) for SLC5A2 (sodium/glucose cotransporter 2) in the target prediction (Konishi et al., 2006).

The literature shows that glycoside removal by intestinal and bacterial β-glucosidases produces aglycone form, which subsequently undergoes further metabolism. In fact, intact glycosides can also exert biological activity prior to metabolism, with some studies showing that glycosylated forms have superior bioactivity in certain cellular contexts. Luteolin 7-glucoside (similar structure to Luteolin 7-primeveroside) has demonstrated potent anti-inflammatory effects with the capacity to inhibit LPS-induced TNF-alpha production through mechanisms involving blockade of inflammatory signaling cascades. An interesting observation from network analysis is that the same hub proteins can function in contrasting roles depending on cellular context. ALOX15, for example, can generate both pro-inflammatory (12-HETE, 15-HETE) and anti-inflammatory (lipoxins) products depending on substrate availability and interacting partners (Kuhn et al., 2015). CYP1A2 similarly can function as both a xenobiotic activator (pro-oxidant) and an antioxidant enzyme depending on substrate specificity and metabolic state. This context-dependent behavior indicates that the therapeutic outcomes of luteolin compounds will be significantly influenced by: Disease state: In acute inflammatory states, luteolin anti-TNF effects will predominate. In chronic oxidative stress conditions with impaired antioxidant defenses, luteolin direct ROS scavenging capability will be more important. Tissue specificity: Different tissues express varying ratios of hub proteins. Liver and intestinal express high CYP1A2 and ALOX15; immune cells express high TNF; pancreatic islets highly express XDH. Tissue-specific targeting may improve therapeutic efficacy. Nutrient/substrate availability: Arachidonic acid availability will influence ALOX15-mediated eicosanoid production. Glucose levels will affect SLC5A2-mediated glucose transport and cellular metabolic state.

The identified multi-target mechanism luteolin compounds toward TNF, XDH, CYP1A2, ALOX15, and TERT show potential therapeutic applications in the diseases characterized by chronic inflammation and oxidative stress. Inflammatory bowel diseases (IBD): TNF is the key pathogenic cytokine in the IBD; luteolin's TNF-inhibitory effects had been demonstrated efficacy in the colitis models protected toward TNBS-induced colitis via NF-κB suppression). Type 2 diabetes: XDH-mediated oxidative stress contribute toward beta-cell dysfunction; ALOX15-mediated lipoxin production can promote islet inflammation resolution (lipoxygenase inhibition impaired glucose tolerance in the ALOX 15-deficient mice). Cardiovascular diseases: TNF-mediated endothelial inflammation and CYP1A2-mediated pro-oxidant metabolism are key pathogenic mechanisms. ALOX15-mediated lipoxin production can be cardioprotective. Aging and neurodegenerative diseases: TERT involvement in the cellular senescence and telomere maintenance; luteolin's potential TERT modulation coupled with antioxidant effects maybe benefit neuronal homeostasis.

Implications

Network pharmacology analysis of the three luteolin compounds reveals a comprehensive framework for mechanistic understanding of flavonoid-mediated anti-inflammatory and antioxidant effects. Multi-target pharmacology paradigm: Results show that natural compounds can exert therapeutic effects through coordinated modulation of multiple, functionally-related targets rather than single-target mechanisms. This polypharmacology approach more closely mirrors actual disease pathophysiology, which invariably involves multiple dysregulated pathways. These findings validate network pharmacology as an appropriate methodology for rational drug discovery from natural products.

Structural determinants of bioactivity: Comparative analysis between glycosylated luteolin forms reveals that carbohydrate moieties not only serve as passive solubility enhancers but potentially influence substrate specificity and protein-ligand binding affinities. This suggests that structure-activity relationship optimization through chemical derivatization may further enhance pharmacological potency. Context-dependent mechanisms: Hub proteins can exert both beneficial and detrimental effects depending on cellular and tissue context. This complexity emphasizes the

importance of in vivo validation studies for complementary computational predictions and of personalized medicine approaches that account for inter-individual variations in protein expression and metabolic capacity.

Development from targeted interventions: Identified hub proteins (TNF, XDH, CYP1A2, ALOX15, TERT) can serve as rational targets for combination therapy approaches. Co-administration from luteolin compounds with selective inhibitors from pro-inflammatory enzymes (e.g., TNF inhibitors, XDH inhibitors) maybe achieve synergistic anti-inflammatory and antioxidant effects with reduced side effects compared with monotherapy. Phytochemical-based drug development: Natural Deep Eutectic Solvent (NaDES) extraction from celery (apium graveolens) (Putra et al., 2024), produce enriched luteolin compounds, can represent cost-effective, environmentally-benign approach for phytochemical production. Network pharmacology validation from bioactivity provides scientific justification for developing celery-derived preparations as nutraceuticals or phytopharmaceuticals for inflammatory and oxidative stress-related diseases. Precision medicine approaches: Patient-specific genetic polymorphisms in the predicted targets (TNF gene variants, CYP1A2 polymorphisms, ALOX15 variants) could influence individual responsiveness toward luteolin interventions. Pharmacogenomic profiling maybe enable personalized dosing and patient selection strategies for maximize efficacy and minimize adverse effects.

Current evidence shows that luteolin aglycone exhibits limited bioavailability (oral bioavailability ≈ 2-10%) due to poor water solubility and incomplete intestinal absorption (Claudine et al., 2005). Glycosylated forms potentially overcome some limitations. Enhanced intestinal absorption: Glucose transporters (SGLT1, GLUT2) can recognize and actively transport glucose-containing glycosides, improving intestinal uptake compared to free aglycones. Microbiota metabolism: Gut microbiota-derived β-glucosidases convert glycosides become bioavailable aglycones in the lower intestine, enabling dual benefit from intact glycoside effects and derived aglycone activity. Formulation stability: Glycosylated forms exhibit improved chemical stability during storage and in gastrointestinal environment, potentially improving therapeutic reliability. Future pharmacokinetic studies elucidating absorption, distribution, metabolism, and excretion (ADME) properties from specific glycosylated luteolin forms will be essential for translating network pharmacology predictions become clinical reality.

Research Contribution

This research makes several important contributions to network pharmacology and natural product drug discovery. Comprehensive network pharmacology characterization of luteolin compounds: This is the first systematic integration of multiple target prediction platforms (TargetNet, Swiss Target Prediction) coupled with disease-associated gene mapping (GeneCards) to characterize multi-target mechanisms of glycosylated luteolin compounds. Previous studies predominantly focused on luteolin aglycone; Comparative analysis of glycoside forms provides novel insights into structure-activity relationships.

Identification of the integrated TNF-XDH-CYP1A2-ALOX15 regulatory axis: Network analysis revealed that the four hub proteins operate in a functionally-integrated pathway for coordinated control between inflammation, oxidative stress, drug metabolism, and eicosanoid biosynthesis. It represents a novel integrative framework not previously described in the literature, with important implications for understanding flavonoid mechanisms. Validation of NaDES extraction effectiveness: Demonstration that Natural Deep Eutectic Solvent extraction from celery successfully generates bioactive luteolin compounds with predicted multi-target potential provides validation for green solvent technology in phytochemical production and highlights sustainability advantages over traditional organic solvent extraction. Methodological advancement: Integration of multiple bioinformatics platforms (target prediction, protein interaction mapping, topological analysis, pathway enrichment) in a standardized workflow can serve as a template for future network pharmacology studies of natural products, advancing field standardization and reproducibility.

Hub protein prioritization framework: Systematic ranking from predicted targets By topological centrality measures (degree, betweenness, closeness centrality) provides quantitative framework for identifying high-impact targets for experimental validation, improving resource allocation in the downstream studies. Mechanistic insights into ALOX15-mediated lipoxin biosynthesis: Predictions regarding ALOX15 involvement in the luteolin mechanism bring new attention toward specialized

pro-resolving mediators as potential therapeutic targets in the inflammation resolution, potentially opening new therapeutic modalities beyond traditional inflammatory cytokine inhibition. Platform for personalized medicine: Identified genetic/proteomic variations in the hub proteins can enable future development from patient-specific therapeutic strategies based at individual protein expression profiles or genetic polymorphisms affecting drug target interactions.

Limitations

Although this study provides valuable insights, some limitations, acknowledged for appropriate interpretation. Target prediction accuracy: Machine learning-based target predictions inherently carry false positive rates. While TargetNet and Swiss Target Prediction have established validation metrics, not all of predicted interactions necessarily translate become functional biological effects. Computational predictions necessary validation Through experimental binding studies (surface plasmon resonance, isothermal titration calorimetry) and functional assays.

Absence from binding affinity quantification: Network pharmacology analysis Identify predicted targets but not provide quantitative binding affinity values. Molecular docking studies necessary To predict binding energetics and identify key residues in the target proteins To compound binding. Protein-protein interaction scoring: STRING database predictions based on evidence integration from multiple sources with varying reliability levels (computational predictions, literature mining, experimental validation). Some high-scoring PPIs maybe represent indirect associations or context-dependent interactions no necessarily active in the all cell types.

Lack from experimental biological validation: This computational study awaits complementary experimental validation through: Binding studies (ELISA, surface plasmon resonance) confirming luteolin-protein interactions; Kinetic assays measuring enzyme activity inhibition for CYP1A2, XDH, ALOX15; Cell-based assays (western blotting, immunofluorescence) confirming pathway modulation; Animal models (inflammation, oxidative stress induction) demonstrating in vivo efficacy. Absence from mechanism-of-action studies for glycoside forms: While luteolin aglycone mechanisms extensively studied, specific mechanisms for Luteolin 7-apiosyl(1→6)glucoside, Luteolin 7-sambubioside, and Luteolin 7-primeveroside remain largely unknown. Different glycosylation patterns potentially result in the distinct metabolic fates and biological activities.

Limited disease context: Network analysis not incorporate disease-specific pathway alterations. Disease states dramatically rewire protein networks, potentially changing hub protein importance or revealing new critical nodes. Disease-specific network analysis would provide more clinically-relevant predictions. Intersection gene set limitations: Venn diagram analysis identified 5-6 intersection genes >1,300 disease-associated genes. This very small intersection percentage, while enriched in predicted targets, may incompletely capture true mechanism-of-action which potentially involve regulation from vast disease-associated gene networks.

Suggestions

Future research should prioritize experimental validation of the predicted hub targets through in vitro assays and in vivo models to confirm their biological relevance and therapeutic potential. Detailed pharmacokinetic and pharmacodynamic studies are needed to elucidate the ADME profiles of specific glycosylated luteolin compounds and to determine optimal dosing strategies.

Further investigations into gut microbiota–mediated metabolism and pharmacogenomic variability are recommended to support the development of personalized luteolin-based interventions. Additionally, exploring combination therapy approaches involving luteolin compounds and selective enzyme inhibitors may provide insights into synergistic mechanisms and clinical applicability. Expanding this network pharmacology framework to other flavonoids and natural products could also enhance its generalizability and impact on natural product drug discovery.

CONCLUSION

This comprehensive network pharmacology analysis systematically elucidated the multi-target mechanisms of three luteolin glycoside compounds (Luteolin 7-apiosyl(1→6)glucoside, Luteolin 7-sambubioside, and Luteolin 7-primeveroside) extracted from celery (Apium graveolens L.) using Natural Deep Eutectic Solvent (NaDES) technology, revealing an integrated TNF-XDH-CYP1A2-ALOX15 regulatory axis that coordinately modulates both inflammatory and oxidative stress pathways through multi-target mechanisms. Network topology analysis identified TNF (Tumor Necrosis Factor-alpha) as the critical hub protein (degree centrality 3.0, betweenness centrality 7.0) bridging inflammatory signaling with oxidative stress generation and eicosanoid metabolism, while CYP1A2, XDH, and ALOX15 represent functionally-integrated targets enabling dual suppression of pro-inflammatory TNF-mediated pathways and enhanced biosynthesis of anti-inflammatory specialized pro-resolving mediators (lipoxins). The three luteolin glycoside forms demonstrated differential yet complementary target engagement profiles, with Luteolin 7-apiosyl(1→6)glucoside and Luteolin 7-primeveroside showing highest potency in dual oxidative stress-inflammatory pathway modulation (6 intersection targets each), while Luteolin 7-sambubioside exhibited preferential focus on oxidative stress response mechanisms alongside unique HSP90AA1-TERT axis engagement, collectively supporting the polypharmacology paradigm of natural product therapeutics. These findings provide mechanistic scientific validation for the therapeutic potential of celery-derived luteolin compounds in diseases characterized by chronic inflammation and oxidative stress, including inflammatory bowel diseases, type 2 diabetes, and cardiovascular disorders, while establishing NaDES-based extraction as a sustainable, environmentally-benign approach for phytochemical production with preserved bioactivity. Future experimental validation through biochemical binding studies, functional enzyme assays, and disease-specific animal models will be essential to translate these network pharmacology predictions into clinical reality and establish luteolin glycosides as evidence-based multi-target therapeutics for chronic inflammatory and oxidative stress-related disease management

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AUTHOR CONTRIBUTION STATEMENT

Indri meirista contributed to the consideration of the selection of compounds which were then conducted by network pharmacology studies. Andzely zahana putri contributed to the network pharmacology process on the selected compounds. Rizky yulion putra and hendri satria kamal uyun contributed to the selection of compounds sourced from the extraction results of NaDES (Natural Deep Eutectic Solvents) and the network pharmacology process of the target compound.

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