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Unraveling the therapeutic mechanisms of myristic acid and luteolin 7-rutinoside in oral cancer: insights from network pharmacology and molecular docking analysis

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All authors have carefully reviewed and given their approval for final version of the manuscript. The all authors contributed equally.
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Abbreviations
NP, Network Pharmacology; OC, Oral Cancer; MD, Molecular Docking; Omim, Online Mendelian Inheritance in Man; PPI, Protein Protein Interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TNF, Tumor necrosis factor; PPARG, Peroxisome Proliferator Activated Receptor Gamma; TP53, Tumor protein.
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Abstract
Background: The compound Luteolin-7-rutinoside (L7R) is a flavone derivative of luteolin, predominantly identified in plant species belonging to the families Asteraceae. Conversely, Myristic acid is characterized by its structure as a 14-carbon, unsaturated fatty acid. In this investigation, we endeavor to elucidate the putative mechanisms underlying the therapeutic effects of Myristic Acid and Luteolin 7-rutinoside in the context of oral cancer treatment, employing network pharmacology coupled with molecular docking methodologies. **Methods:** The protein targets of Myristic Acid and Luteolin 7-rutinoside were identified through a search on the Swiss Target Database. Subsequently, a compound-target network was constructed using Cytoscape 3.9.1. Targets associated with OC were retrieved from the OMIM and GeneCards databases. The overlap between compound targets and OC-related targets was determined, and the resulting shared targets were subjected to protein-protein interaction (PPI) network analysis using the STRING database. Additionally, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted on the identified targets. Molecular docking were performed to investigate the interactions between the core target and the active compound. **Results:** The component target network comprises 103 nodes and 102 edges. Among the proteins in the protein-protein interaction (PPI) network, those with higher degrees are TNF, PPARG, and TP53. Analysis through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways indicates that the treatment of OC with Myristic Acid and Luteolin 7-rutinoside primarily involves the regulation of miRNA transcription and inflammatory response. The identified signaling pathways include Pathways in cancer, PPAR signaling pathway, EGFR signaling pathway, and TNF signaling pathway. Molecular docking studies reveal that Luteolin 7-rutinoside and Myristic acid exhibit higher affinity towards TNF, PPARG, TP53, and EGFR. **Conclusion:** This study reveals the potential molecular mechanism of Myristic Acid and Luteolin 7-rutinoside in the treatment of oral cancer, and provides a reference for subsequent basic research.

Keywords: myristic acid; luteolin 7-rutinoside; network pharmacology; oral cancer; molecular docking

Introduction

Tongue cancer, gum cancer, palate cancer, oropharyngeal cancer, and lip cancer are all forms of oral cancer (OC) [1]. About 2% of all new cancer cases are attributable to OC, making it one of the deadliest malignancies recorded worldwide [2]. There has been a dramatic rise in both the prevalence and death rate of OC in recent decades [3]. Cigarette smoking, alcohol consumption, inadequate dental hygiene, malnutrition, environmental effect, genetic predisposition, and viral disorders have all been identified as possible risk factors for OC [4]. Epigenetic alteration and aberrant activation patterns of proto-and anti-oncogenes lead to tumor growth in OC [5]. Clinical diagnosis of OC at an early stage may be challenging due to its subtle nature and the need for initial therapy [6]. In the clinic, early-stage OC is given priority for surgery due to its higher success rate in treatment [7]. The efficacy of chemotherapy for patients with advanced OC, however, is not yet sufficient [8]. Therefore, early detection markers and further treatment options for oral cancer are required.

Overall patient survival has improved during the 1990s thanks to ongoing efforts in diagnosis and therapy [9]. Multiple genes work together to transform healthy cells into malignant ones in the slow process of cancer. Cancers can become resistant to standard chemotherapy treatments over time [10]. Therefore, identifying novel treatment agents or therapeutic targets is crucial. In modern cancer treatment, newer generations of medications have focused on attacking proteins that are uniquely expressed in various malignancies. Conventional cytotoxic treatments have more severe side effect profiles than those of targeted cancer therapies [11]. The goal of targeted therapy is to develop ligands that selectively interact with their intended pharmacological targets. Combining genomic technology with system biology via computational biological tools, network pharmacology (NP) is a growing field that can aid in the development of new drugs. In order to describe the intricate interplay between biological systems, medications, and diseases, network pharmacology has been developed [12]. It also determines the synergistic effects in cancer treatment and the possible mechanisms of complicated bio-actives using huge data set analysis [13]. For thousands of years, people all over the world have turned to traditional Chinese medicine (TCM) as a means of treating illness and increasing longevity. TCM is based on a complex herbal composition that has been used to treat a wide range of conditions. For the future generation of drug research and development for TCM herbs or herbal formulas, the network-target-based network pharmacology is a potential technique. It offers a fresh methodical framework and technical avenue for discovering and comprehending the workings of TCM pharmaceuticals. In addition to guiding the integrated use of TCM and conventional drugs, network pharmacology also promotes the discovery of effective molecules by highlighting their interconnectedness, elucidating the connection between TCM formulae and diseases or TCM syndrome, and establishing rational TCM drugs [14–16].

Therefore, the purpose of this research is to determine the role of Myristic Acid and Luteolin 7-rutinoside in oral cancer and to forecast its central targets, biological functions, pathways, and mechanism of action using a Network Pharmacology approach. The Network Pharmacology results were verified by doing molecular docking on a subset of Myristic Acid and Luteolin 7-rutinoside and the primary targets.

Materials and methods

Network pharmacology studies

Screening for oral cancer targets of myristic acid and luteolin 7-rutinoside. Protein targets for Myristic Acid and Luteolin 7-rutinoside were acquired from the Swiss Target Prediction database (http://www.swisstargetprediction.ch/). Pertinent data regarding targets associated with oral cancer were sourced from the GeneCards Database (https://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM) Database (https://www.omim.org/). Candidate targets of Myristic Acid and Luteolin 7-rutinoside were identified through the identification of overlapping targets among the gene sets visualized in a Venn diagram plot.

Protein-protein interaction (PPI) data

The identification of potential therapeutic targets for the management of disorders is crucial, as protein-protein interaction (PPI) plays a pivotal role in the regulation of various biological processes [17]. In this investigation, we utilized the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) V11.5 platform (https://string-db.org/) to construct a network of interactions involving multiple proteins associated with oral cancer, with a focus on the compounds Myristic Acid and Luteolin 7-rutinoside, within the context of the human (Homo sapiens) proteome [18].

Gene and KEGG pathway analysis

The signaling pathways, biological processes, molecular functions, and cellular components associated with the shared targets of the drug and disease were investigated utilizing Gene Ontology (GO) functional annotations and the Kyoto Encyclopedia of Genes and Genomes (KEGG) ($P < 0.05$). Enrichment analysis of the pathways was performed using ShinyGO (http://bioinformatics.sdstate.edu/go/) [19].

Compound-target network construction

The compound-target network was constructed by linking Myristic Acid and Luteolin 7-rutinoside. Utilizing Cytoscape 3.9.1 (Cytoscape Consortium, San Diego, CA, USA), a software designed for visualizing interaction networks, the network was generated [20]. In this network, edges signify the interactions between the compounds and their respective targets, while nodes represent the compounds and their associated targets.

Molecular docking analysis

AutoDock Vina was utilized to ascertain the interactions between Myristic Acid and Luteolin 7-rutinoside compounds with the target proteins [21]. The three-dimensional (3D) structures of the target proteins in Protein Data Bank (PDB) format were acquired from (https://www.rcsb.org) for docking analysis [22]. Additionally, the three-dimensional (3D) structures of Myristic Acid and Luteolin 7-rutinoside compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). AutoDock and PyMOL software were employed to automatically discern docking scores (binding affinities) and binding modes between the drugs and target proteins, namely TNF, EGFR, TP53, and PPARG.

Results

Potential targets of myristic acid and luteolin 7-rutinoside network analysis

The Swiss Target Prediction Database (Supplementary Table S1) was queried to identify 102 putative targets of Myristic Acid and Luteolin 7-rutinoside. Candidate genes implicated in Oral Cancer were investigated through gene card annotation and the OMIM database. From the Swiss system predictions, a subset of 102 targets was selected for further analysis. Visualization of the compound-target interactions was conducted using Cytoscape 3.9.1, yielding a network depiction. Figure 1 illustrates Myristic Acid, Luteolin 7-rutinoside, and their respective 102 interacting target proteins, while Figure 1A highlights the shared targets between the compounds and oral cancer. The network analysis revealed several components targeting overlapping entities, resulting in a network comprising 103 nodes and 102 edges. Notably, Myristic Acid and Luteolin 7-rutinoside exhibit therapeutic potential across various diseases and disorders, indicating potential synergistic effects on the identified targets. Table 1 presents topological parameters, including Betweenness centrality, Closeness centrality, and Degree, elucidating the relative importance of each node within the network.

PPI network

Figure 2 illustrates the Protein-Protein Interaction (PPI) network elucidating potential oral cancer targets affected by Myristic Acid and Luteolin 7-rutinoside. The analysis revealed a significant PPI enrichment, with a computed *P*-value of 0.0286. Moreover, the average node degree within the network was determined to be 6.2, indicating a substantial degree of connectivity among the identified targets. Specifically, the node degrees for TNF and PPARG were both measured at 32, while TP53 exhibited a node degree of 24, and EGFR at 20. These findings suggest that TNF, PPARG, TP53, and EGFR may serve as pivotal nodes within the network, potentially playing crucial roles in mediating the effects of Myristic Acid and Luteolin 7-rutinoside on oral cancer.

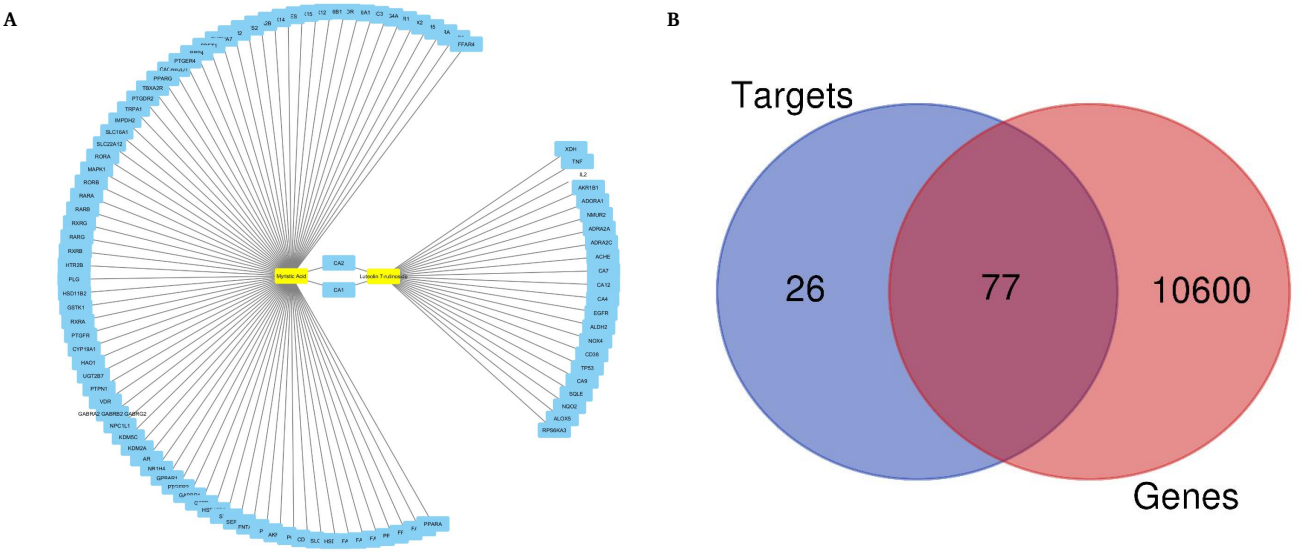

Figure 1 Target analysis. (A) Compound target network. (B) Venn diagram shows the intersection between myristic acid and luteolin 7-rutinoside targets and oral cancer-related genes.

Table 1 Topological analysis of important nodes with network analyzer results

Name	Average shortest path length	Between ness centrality	Closeness centrality	Clustering coefficient	Degree	Topological coefficient
TNF	1.222222	0.033333	0.818182	0.761905	7	0.730159
PPARG	1	0.128241	1	0.611111	9	0.654321
TP53	1.111111	0.063426	0.9	0.678571	8	0.694444
EGFR	1.111111	0.063426	0.9	0.678571	8	0.694444
PPARA	1.444444	0.023148	0.692308	0.7	5	0.8
MAPK1	1.222222	0.050926	0.818182	0.714286	7	0.730159
RARA	1.444444	0.013889	0.692308	0.8	5	0.8
CYP19A1	1.555556	0	0.642857	1	4	0.888889
RXRA	1.555556	0.0125	0.642857	0.666667	4	0.722222
IL2	1.444444	0	0.692308	1	5	0.866667

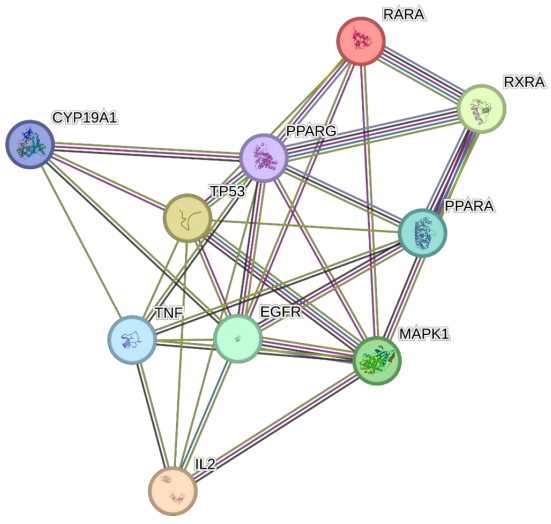

Figure 2 PPI network. Protein–protein interaction network of myristic acid and Luteolin 7-rutinoside in oral cancer.

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GO and KEGG enrichment analysis

Candidates for oral cancer treatment were identified through GO enrichment analysis of genes related to Myristic Acid and Luteolin 7-rutinoside. Several biological processes Table 2 emerged as significant, including response to oxygen-containing compounds, negative regulation of cell differentiation, regulation of miRNA transcription, response to organic substances, regulation of multicellular organismal processes, regulation of DNA metabolic processes, regulation of inflammatory responses, and positive regulation of MAP kinase activity as shown in Figure 3A. Among the most common molecular functions Table 3 were nuclear receptor activity, phosphatase binding, transcription co-regulator binding, DNA

Table 2 Go biological process

Description	Count in gene set	False discovery rate
Response to oxygen-containing compound	10	1.45E-07
Negative regulation of cell differentiation	8	7.45E-07
Regulation of miRNA transcription	5	7.45E-07
Response to organic substance	10	4.03E-06
Regulation of multicellular organismal process	10	4.47E-06
Regulation of DNA metabolic process	6	4.04E-05
Regulation of inflammatory response	5	0.00014
Positive regulation of MAP kinase activity	2	0.0476

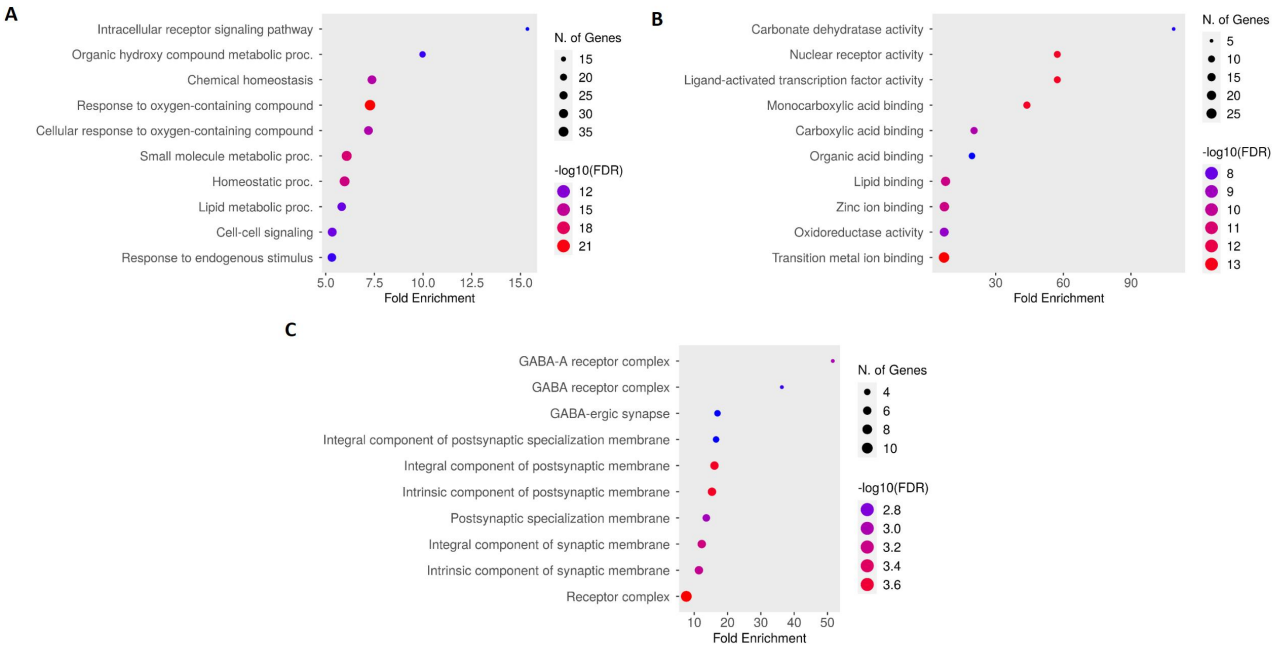

Figure 3 Enrichment analysis for gene ontology. (A) Biological process. (B) Molecular function. (C) Cellular component.

Table 3 Go molecular process

Description	Count in gene set	False discovery rate
Nuclear receptor activity	4	6.43E-05
Phosphatase binding	5	6.43E-05
Transcription co regulator binding	4	0.00031
DNA binding	8	0.002
Protein domain specific binding	5	0.0043
Signaling receptor activity	5	0.0497
Enzyme binding	8	0.00058
Transcription factor binding	5	0.0029

binding, protein domain-specific binding, signaling receptor activity, enzymatic activity, transcription factor binding, and enzyme binding shown in Figure 3B. Regarding cellular components Table 4 , the analysis highlighted terms such as transcription regulator complex, host cell nucleus, RNA polymerase II transcription regulator complex, and nuclear chromatin shown in Figure 3C. KEGG analysis Table 5 further revealed multiple pathways associated with the potential for oral cancer, including pathways in cancer, MAPK signaling pathway, PPAR signaling pathway, EGFR tyrosine kinase inhibitor resistance, IL-17 signaling pathway, apoptosis, Th1 and Th2 cell differentiation, TNF signaling pathway, and cytokine-cytokine receptor interaction shown in Figure 4A–4C. Additionally, cancer signaling pathways related to epidermal growth factor receptor, mitogen-activated protein kinase, p53, and peroxisome proliferator-activated receptor were identified as shown in Figure 5A–5E.

Table 4 Go cellular components

Description	Count in gene set	False discovery rate
Transcription regulator complex	5	0.0011
Host cell nucleus	4	0.0011
RNA polymerase II transcription regulator complex	4	0.0011
Nuclear chromatin	5	0.0072

Table 5 KEGG pathways

Description	Count in gene set	False discovery rate
Pathways in cancer	7	1.65E-07
MAPK signaling pathway	4	0.00014
PPAR signaling pathway	3	0.00012
EGFR tyrosine kinase inhibitor resistance	2	0.0044
IL-17 signaling pathway	2	0.0054
Apoptosis	3	0.00035
Th1 and Th2 cell differentiation	2	0.0052
TNF signaling pathway	2	0.0068
Cytokine-cytokine receptor interaction	2	0.0296

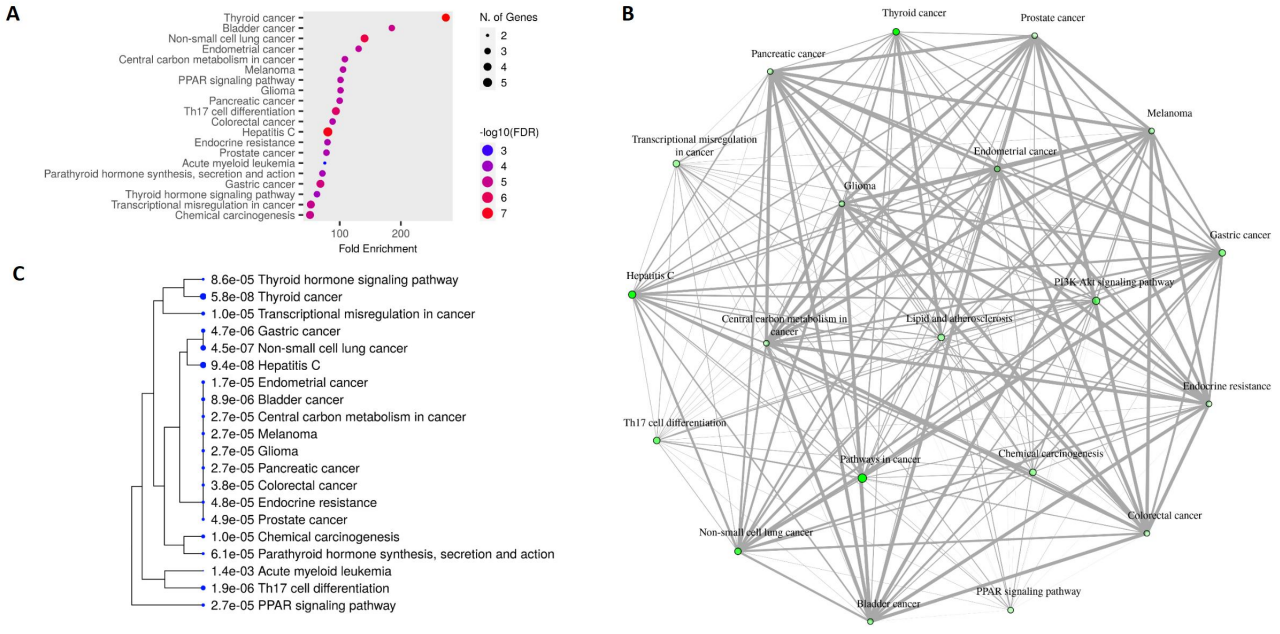

Figure 4 KEGG pathways. (A) Top 20 pathways. (B) Pathway network. (C) Tree plot.

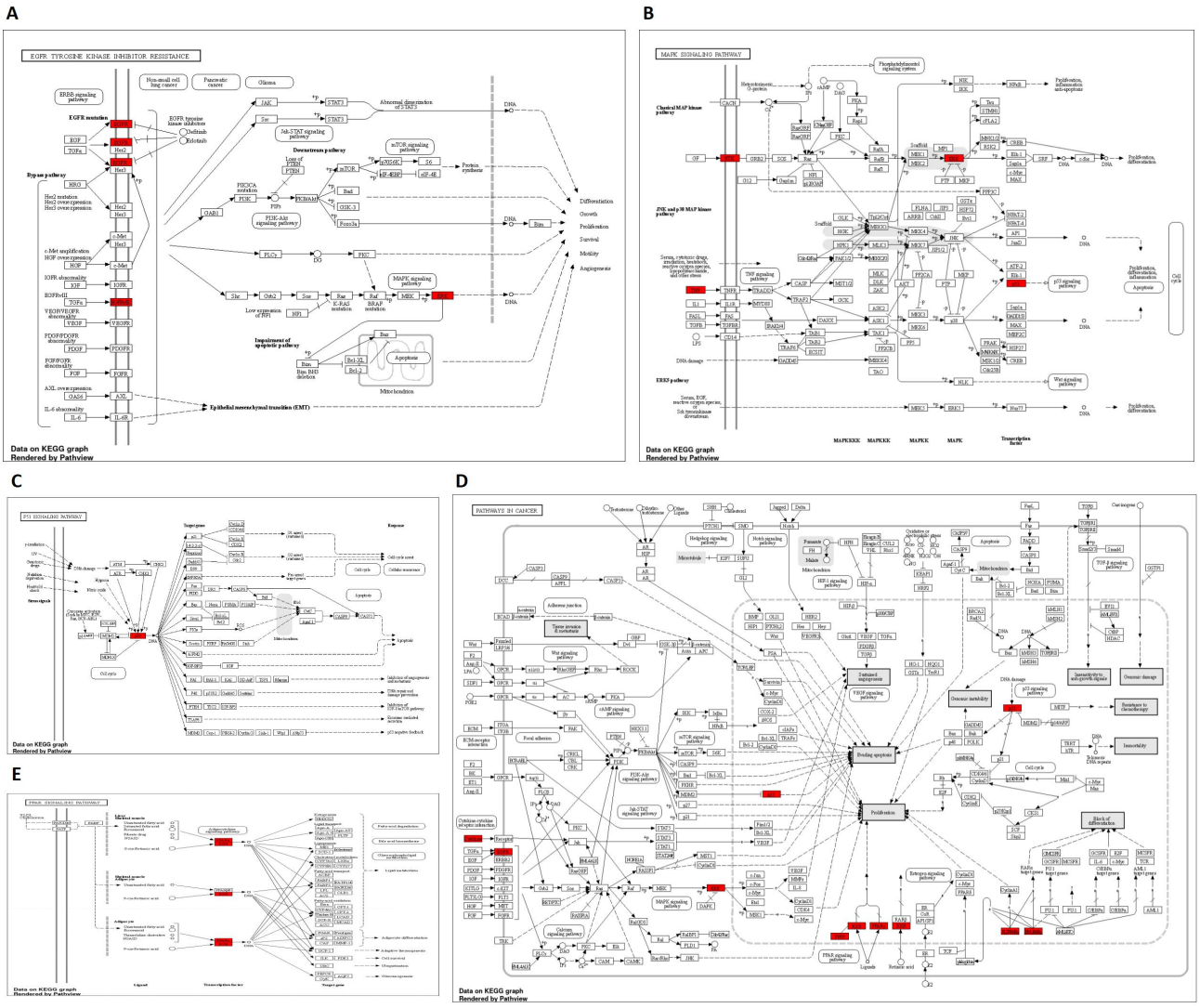

Figure 5 Signalling pathway. (A) EGFR tyrosine kinase inhibitor resistance. (B) MAPK signaling pathway. (C) P53 signaling pathway. (D) Pathways in cancer. (E) PPAR signaling pathway.

Molecular docking analysis

The compounds Myristic Acid and Luteolin 7-rutinoside, along with their interactions with protein targets TNF, PPARG, TP53, and EGFR, were assessed using molecular docking techniques to ascertain their binding affinities. More negative docking scores are indicative of stronger and more stable interactions between the chemicals and proteins. Table 6 presents the docking scores for the compounds present in Myristic Acid and Luteolin 7-rutinoside, alongside their corresponding protein targets. The modes of binding between the compounds and proteins are illustrated in Figures 5A–5F. Protein Data Bank (PDB) coordinates for TNF (PDB: 1TNF), EGFR (PDB: 2JIT), TP53 (PDB: 3DCY), and PPARG (PDB: 8B8X) crystal structures were acquired from the rcsb.org website. Auto Dock Vina was utilized to dock the suggested ligands into the receptors to predict potential binding interactions, with the amino acid residues surrounding the active sites serving as the focal point of the grid. The results were expressed in terms of free binding energies (in kcal/mol). Molecular docking studies revealed that the estimated free energy of binding of the docked ligands ranged between −5 to −11.1 kcal/mol for TNF (PDB: 1TNF), −4.7 to −9.2 kcal/mol for EGFR (PDB: 2JIT), −5.2 to −9.9 kcal/mol for TP53 (PDB: 3DCY), and −5.3 to −7.8 kcal/mol for PPARG (PDB: 8B8X). Further analysis of the results indicated that Luteolin 7-rutinoside exhibited the most potent activity, displaying the highest affinity with binding energies of −11.1 kcal/mol towards TNF (PDB: 1TNF), −9.2 kcal/mol towards EGFR (PDB: 2JIT), −9.9 kcal/mol for TP53 (PDB: 3DCY), and −7.8 kcal/mol for PPARG (PDB: 8B8X) compared to the standard Doxorubicin as shown in Figure 6A–6D and the amino acid interaction residue was shown in the Table 7.

Discussion

Network pharmacology endeavors to explore the relationship between pharmaceutical agents and diseases through the paradigm of multi-targeted therapy, diverging from the traditional "one drug, one target" doctrine of drug development [23]. Leveraging principles of systems biology, network analysis, connectedness, and redundancy exemplify the innovative nature of this approach. To elucidate how uncharted signaling pathways interface with pharmaceutical agents, investigators have turned to network pharmacology inquiries [24, 25]. A robust and auspicious tool for elucidating disease etiologies at a systemic level and uncovering potential bioactive compounds is the Network Pharmacology methodology, which offers fresh insights into the systemic interplay between therapeutic targets and the entirety of a disease [26]. The present investigation has established a distinctive network that furnishes a comprehensive depiction of the molecular mechanisms underlying the actions of Myristic Acid and Luteolin 7-rutinoside. The identification of a plant bioactive target within the oral cancer pathway and the subsequent construction of a disease network represent significant advancements in our understanding of

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Table 6 Docking scores of compounds myristic acid and luteolin 7-rutinoside and potential targets

Compounds	Binding energy (kcal/mol) targets			
	1TNF	2JIT	3DCY	8B8X
Luteolin-7-rutinoside	−11.1	−9.2	−9.9	−7.8
Myristic acid	−5	−4.7	−5.2	−5.3
Doxorubicin	−7.2	−9	−8.5	−7

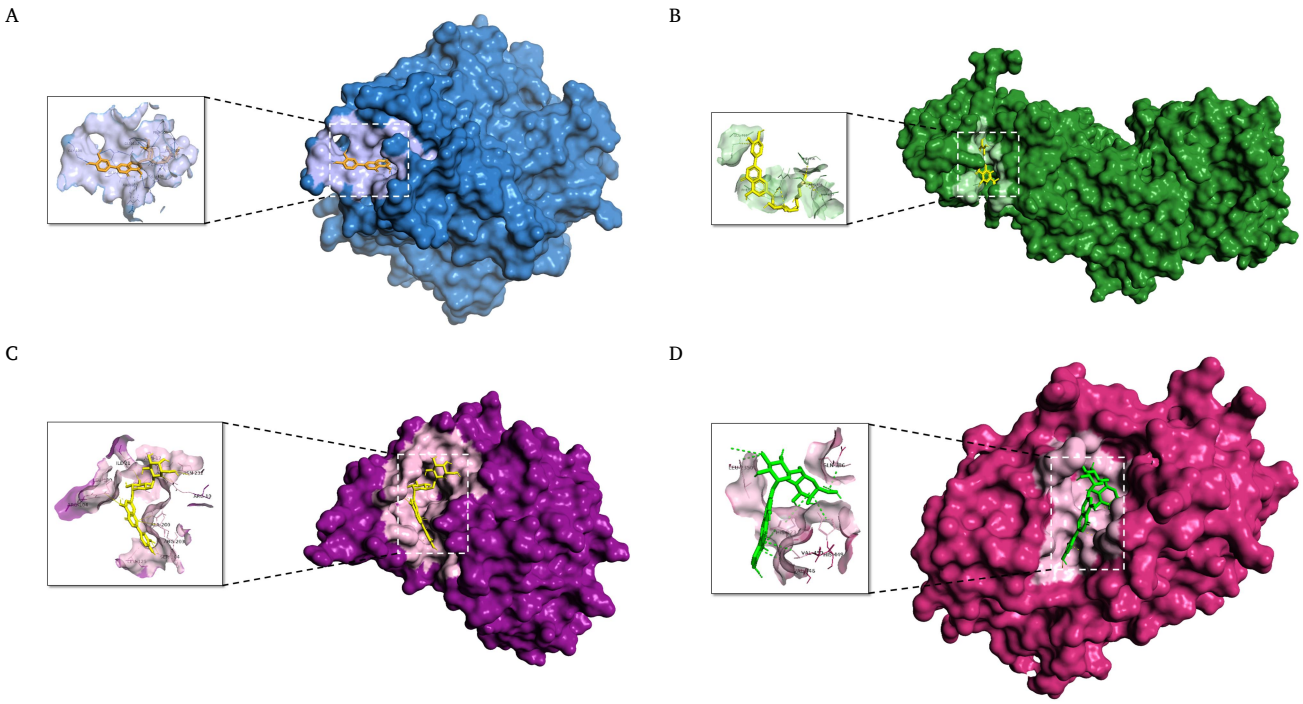

Figure 6 3D poses of interactions. (A) 3D docking pose of luteolin-7-rutinoside with TNF (PDB: 1TNF). (B) 3D docking pose of luteolin-7-rutinoside with EGFR (PDB: 2JIT). (C) Figure 8 3D docking pose of luteolin-7-rutinoside with TP53 (PDB: 3DCY). (D) 3D docking pose of luteolin-7-rutinoside with PPARG (PDB: 8B8X).

Table 7 Estimated free energy of binding, H-Bond interactions, hydrophobic interactions and other interactions between compounds and receptors

Targets	Compounds	Interacting residues			
		H-bonding interactions	Hydrophobic interactions	π-Stacking	Salt bridges
1TNF	luteolin-7-rutinoside	SER289, GLN102, GLU104, GLY108, GLU116	GLU104		ARG103
	Myristic acid	GLN102	GLN102	--	
	Doxorubicin	ASN30, ARG31, ARG44, ASN46	LEU37, LEU43		
2JIT	luteolin-7-rutinoside	SER289, ARG836, ASP837, PHE856	SER289, GLU758, ARG836, ASP837		
	Myristic acid	ASN842, THR854	SER289, PHE723, VAL726, ALA743, LEU844	--	--
	Doxorubicin	LYS745, ASP837, THR854, LYS875	LEU718, VAL726, LEU792, LEU844, PHE856		
3DCY	luteolin-7-rutinoside	ARG10, GLN23, ARG104, ARG203, SER204, ASN232	LEU100, LEU125, ALA200		
	Myristic acid	SER289, GLY199, ALA200, ARG203	SER289, TYR92, LEU100, LEU103		
	Doxorubicin	SER289, ASN17, GLN23, ARG203, VAL229, THR230, ASN232	LYS18, TYR92	ARG10	ARG10
8B8X	luteolin-7-rutinoside	SER289, HIS323, GLU324, GLY2349, LEU2350	GLN286, VAL446, VAL450		HIS323, HIS449
	Myristic acid	SER289, GLU343, GLY344	SER289, ARG288, ALA292, ILE296, ILE326, LEU330	--	ARG288
	Doxorubicin	THR349, ARG350, GLU351	VAL248, TYR250, GLU351		

oral cancer pathogenesis. Through an analysis of protein-protein interactions and the exploration of various pathways, it has been revealed that Myristic Acid and Luteolin 7-rutinoside possess the potential to modulate oral cancer progression. Protein-protein interaction (PPI) analysis elucidated a network comprising 77 nodes and 279 edges, with key genes including TNF, PPARG, TP53, EGFR, PPARA, MAPK1, RARA, CYP19A1, RXRA, and IL2. Further examination via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses uncovered multiple pathways associated with these genes, underscoring the significance of bioactive compounds in the regulation of oral cancer.

GO enrichment analysis underscored the critical role of bioactive compounds in oral cancer control, while KEGG pathway analysis provided additional support for the potential therapeutic utility of Myristic Acid and Luteolin 7-rutinoside. Specifically, pathways such as the MAPK signaling pathway, the PPAR signaling pathway, EGFR tyrosine kinase inhibitor resistance pathway, the IL-17 signaling pathway, apoptosis, th1 and th2 cell differentiation, the TNF signaling pathway, and cytokine-cytokine receptor interaction emerged as crucial nodes within the network. Moreover, the exploration of multiple signaling pathways including PI3K-Akt, transcriptional dysregulation in cancer, adipocytokine signaling, chemokine receptor signaling, T cell receptor signaling, sphingolipid signaling, thyroid hormone signaling, and estrogen signaling further suggested the broad therapeutic potential of Myristic Acid and Luteolin 7-rutinoside beyond oral cancer.

Additionally, experimental evidence has demonstrated the efficacy of Myristic Acid and Luteolin 7-rutinoside against various cancer types, encompassing those affecting the colon, pancreas, endometrium, prostate, melanoma, bladder, lungs (both small and large cell), liver, and stomach. Furthermore, the validation of targets through docking investigations has provided further credence to the therapeutic potential of these compounds. The elucidation of structure-activity relationships through affinity testing offers immediate insights into the mechanisms underlying their therapeutic effects.

Conclusions

This in silico investigation employs network pharmacology and docking analysis to comprehensively examine the pharmacological mechanism underlying the therapeutic efficacy of Myristic Acid and Luteolin 7-rutinoside in oral cancer management. Furthermore, it underscores the utility of network pharmacology in elucidating the mechanism of action of Myristic Acid and Luteolin 7-rutinoside, emphasizing its pivotal role in advancing our understanding of their therapeutic effects.

Limitations and future work

In this research, we introduced a computational methodology aimed at identifying potential compounds for treating oral cancer, coupled with a predictive mechanism. We acknowledge various limitations inherent in our study. Despite our considerable efforts to compile extensive drug-target networks sourced from publicly available databases, it's possible that the network data is incomplete, and certain drug-target interactions might have biological relevance. A more thorough identification of cancer cells, along with their specific biological effects via functional genomics assays, would notably enhance the accuracy of the proposed network-based methodologies. Preclinical studies are essential to assess the in vivo efficacy and potential side effects through cell validation studies before progressing to clinical trials. Consequently, further experimental validation regarding the prediction of network pharmacology is imperative to substantiate the presented hypothesis in future research endeavors.

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