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Evaluating the anti-cancer activity of myricetin in the management of oral cancer using *in silico* analysis

Shruti Sinnarkar[1], Gauri M Kumbhar[2], Ladke Vaibhav S[3]*, Jitendra Bhawalkar[3] & Janhavi Mahajan[4]

[1]Department of Biotechnology, Dr. D. Y. Patil Biotechnology & Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Tathawade, Pune-411 033, Maharashtra, India

[2]Dr. D. Y. Patil Dental College & Hospital; & [3]Dr. D. Y. Patil Medical College, Hospital and Research Centre, Dr. D. Y. Patil Vidyapeeth, (Deemed to Be University), Sant Tukaram Nagar, Pimpri, Pune: 411 018, Maharashtra, India

[4]Department of Biotechnology, MES Abasaheb Garware College, Pune-411 004, Maharashtra, India

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Myricetin has been examined in various types of human cancer cells. However, there have been very few studies on oral cancer. The aim of this research was to assess Myricetin's anticancer potential in oral cancer using *in silico* network analysis. The *in silico* analysis included the determination of drug-likeness prediction, prediction of common targets between oral cancer and myricetin, Protein-Protein Interactions (PPI), hub genes, top 10 associated pathways by Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO), and molecular docking experiments. 22 common genes were obtained and were seen to be involved in the Ras signaling pathway, PI3K-Akt signaling pathway, chemical carcinogenesis-ROS, Pathways in cancer, and microRNAs in cancer. The most common genes involved in the top 10 pathways were AKT1, EGFR, and MET which were seen associated with the PI3K-Akt signaling pathway which may be the key pathway through which myricetin may aid in treating oral cancer. Molecular docking also indicated its drug-like activity against oral cancer having a high affinity for AKT1.

According to the findings, myricetin possesses anticancer effects and has the potential to be employed as a chemotherapy medication. The *in silico* approach applied in this study can serve as a paradigm for future research in the development of effective cancer treatments.

Keywords: *In silico* analysis, Molecular docking, Myricetin, Oral cancer, PI3K-Akt signaling pathway

Oral cancer has the unfortunate distinction of being India's leading cancer, with an increasing incidence rate of 10.4% and an overall mortality rate of 9.3% for both men and women[1]. It is the eighth most prevalent type of cancer worldwide[1].While surgical resection is still a vital component of cancer treatment, a substantial difficulty arises from the high likelihood of cancer recurrence in many patients, which often manifests within a short timescale[2].

Myricetin [MYR] is a flavonoid compound found in a variety of plants, like bayberry. It was first isolated from the Myrica rubra tree's bark part. It is a polyhydroxy flavonol a compound with the chemical formula C15H10O8, comprised of light yellow crystals, also is readily soluble in polar solvents and methanol[3]. It is abundant in various natural plants and their families, like Leguminosae, Myricaceae, Compositae, and Vitaceae and is usually found in berries, fruits, vegetables, honey, red wine, tea, and other everyday foods as an essential active ingredient and additive in many foods[4–8]. Modern pharmacological research revealed that MYR has a wide biological activities, including anti-inflammatory[9], anticancer[10,11], antibacterial[10], antiviral[12,13], and anti-obesity properties[14]. Because of its antioxidant function as well as cholesterol-lowering effect, European countries created and promoted health products

*Correspondence:
Phone no: +91-9923132047 (Mob)
Fax: +91-020-27805217
E-mail: drvaibhavladke@gmail.com;vaibhav.s.ladke@dpu.edu.in

Abbreviations: DAVID, Database for Annotation, Visualization, and Integrated Discovery; EGFR, Epithelial Growth Factor Receptor; GEPIA, Gene Expression Profiling Interactive Analysis; HPA, Human Protein Atlas; KEGG, Kyoto Encyclopaedia of Genes and Genome; MCC, Maximal Clique Centrality; MET, Mesenchymal Epithelial Transition; MNC, Maximum Neighbourhood Component; mTOR, mechanistic (formerly "mammalian") Target of Rapamycin; MYR, Myricetin; OC, Oral Cancer; OSCC, Oral Squamous Cell Carcinoma; PI3K-AKT, Phosphatidylinositol 3-Kinase/serine/threonine-protein; PPI, Protein-Protein Interaction; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; SwissADME, Swiss Adsorption, Distribution, Metabolism, Excretion

containing MYR, even though it is not yet approved as a new medicine. Nowadays, people are more interested in seeking natural ways to enhance the body rather than taking chemical treatments that have hazardous and negative effects; consequently, this feature pushed scientists to modify their research with MYR. As a result, several studies on MYR's pharmacological effects have already been conducted, however research on MYR's pharmacological activity in oral cancer is still insufficient[3].

MYR is a natural flavanol that shows anti-cancer activity via multiple routes, including apoptosis[15], anti-migratory effects, angiogenesis suppression[16], anti-EMT effects[17,18], DNA repair mechanisms, and anti-proliferative pathways[19], and has shown to be beneficial for the prevention and treatment of cancer[20]. This is accomplished by interacting with several cellular pathways such as the TGF/Smad pathway[21], JAK/STAT pathway[22], and so on. The PI3K/Akt and associated mTOR signaling pathways, for example, are responsible for controlling many cell processes like cell survival, growth, proliferation, as well as differentiation. It stimulates this pathway through elevated Akt phosphorylation, which results in cytoprotection in normal healthy cells and death in cancer cells[23,24]. It is also involved in down-regulating the epidermal growth factor receptor (EGFR) in order to target the PI3K/Akt pathway[25]. MYR regulates numerous cascades that eventually lead to apoptosis by targeting these pathways. It functions as a chemopreventive drug that manages cancer progression and proliferation by influencing main apoptotic pathways as well as DNA repair mechanisms. This has been examined and proven through a variety of cancers, including colon and ovarian cancer[26].

The current study used *in silico* network pharmacology to evaluate the possible anti-cancer properties of MYR, specifically in relation to Oral Cancer. The complete flow work has been summarized in (Fig. 1).

Materials and Methods

In silico analysis

Prediction of Drug-likeness

Lipinski's rule of five (RO5) for assessing oral medicines in humans was used to assess myricetin's drug-likeness. Several parameters were examined. MYR SMILES format, C1=C(C=C(C(=C1O)O)O) C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O, was entered into the "SwissADME server (http://www.swissadme.ch)", an online tool that calculates parameters like absorption, distribution, metabolism, excretion (ADME), oral bioavailability (OB), and drug-likeness (DL)[27].

Prediction of toxicity of Myricetin

ProTox-3 is an online tool designed for toxicity prediction to aid in drug development. Accessible at ProTox-3 Web Server, (https://tox.charite.de/protox3/index.php?site=compound_input) it functions by

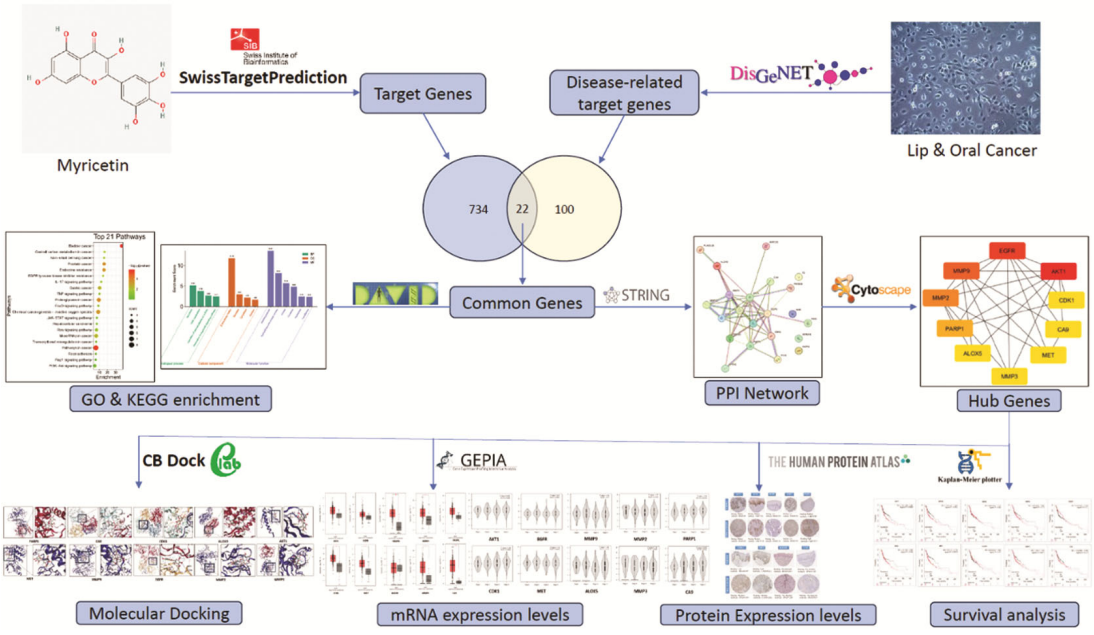

Fig. 1 — The flowchart of the study deciphering the potential mechanisms of Myricetin against oral cancer

analyzing the similarity of compounds with known median lethal doses (LD50) and identifying toxic fragments, offering a novel approach to toxicity prediction. It comprises 33 models for drug development, predicting oral toxicities of chemicals at various toxicity levels. It assesses acute toxicity, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and toxicity (LD50) values in mg/kg body weight. it can predict toxicity classes (I–VI) based on toxic doses. When the SMILES format of MYR, C1=C(C=C(C(=C1O)O)O)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O, was entered into the ProTox-3 software, its toxicity was assessed across different aspects.

Target proteins of myricetin

The Swiss Target Prediction database is a web-based tool designed to predict the most probable protein targets for small molecules, such as drugs or bioactive compounds. It uses a combination of molecular similarity methods to identify potential interactions between compounds and protein targets. The "Swiss Target Prediction database (http://www.swisstargetprediction.ch/)" [28] was utilized to predict the suitable genes related with myricetin in order to predict the targets of MYR.

Potential targets in oral cancer

DisGeNet is a comprehensive database that focuses on the integration and dissemination of information about gene-disease associations. It provides valuable insights into the genetic basis of human diseases by aggregating data from various sources and presenting it in a structured format.This database (https://www.disgenet.org/browser/0/1/0/C0220641/) was used to find genes associated with oral cancer, which were compared to genes associated with MYR. Following that, common genes were found and chosen for future analysis.

Gene Ontology and pathway enrichment

The Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/, ver. 6.8) and ShinyGO database (ShinyGO, http://bioinformatics.sdstate.edu/) were used to determine GO and KEGG pathway enrichment analysis, respectively. DAVID is a tool that aids in the annotation and interpretation of gene lists, whereas the ShinyGO database focuses primarily on GO and pathway enrichment analyses. KEGG, on the other hand, is a comprehensive pathway database that includes graphical representations of biological pathways[29,30]. The GO is a comprehensive repository of functional genomics information, including definitions and classifications of gene functions[31]. Pathways relevant to oral cancer were carefully selected using clinical and pathological data. Bubble charts and histograms were created to show and analyze the data using the online Bioinformatics platform (http://www.bioinformatics.com.cn/), which is specialized to data processing and presentation.

Protein–protein interaction analysis

Protein-protein interaction (PPI) is a crucial component in biological processes which is vital in understanding the complex systems at work within a living cell[32]. The cluster of target genes was analyzed using the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org/; version 11.5) to map the protein-protein interaction (PPI) network. It integrates experimental data, computational predictions, and curated knowledge to facilitate the study of molecular interactions and functional associations among proteins. To ensure high-confidence information, the analysis was limited to "Homo sapiens" as the species, with a threshold of >0.9 used. The resulting PPI network was then built with Cytoscape (https://cytoscape.org/; version 3.10.0), a widely used bioinformatics software for visualization and integration of data[33]. The Cytoscape plugin cytoHubba (https://apps.cytoscape.org/apps/cytohubba; version 0.1) was used to evaluate clusters or highly interconnected regions in the PPI network. Proteins with the highest MNC (Maximum Neighbourhood Component) level rankings were identified as network hub targets.

Molecular docking assessment between hub genes and myricetin

Molecular docking was used to study the interaction between the potential proteins (hub genes) and MYR in greater depth. The docking simulation was designed to assess the interactions among the hub targets and MYR. CB-Dock (http://cao.labshare.cn/cb-dock) was used for the molecular docking simulations, which is capable of automatically identifying active sites within a given protein, estimating their centres and sizes, and modifying the grid box size based on the query ligands[34]. The target protein crystal was obtained from the Protein Data Bank (http://www.rcsb.org).Similarly, the 3D structure of MYR was determined using the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/). These protein and ligand structures were utilized as inputs

for CB-Dock, which performed docking studies to evaluate the binding activities of the myricetin andproteins. The Discovery Studio Visualizer software (Accelrys Software Inc.)was used for visualization and analysis of the docked results[35].

Gene expression levels of hub genes

Gene Expression Profiling Interactive Analysis (GEPIA) (GEPIA; http://gepia2.cancer-pku.cn/) was used in this study to confirm the varied expression of the hub genes in Oral Cancer and normal oral tissues. GEPIA is a web-based service that provides interactive and customizable features based on data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases. Furthermore, GEPIA aided in the analysis of these genes based on pathological stages, providing vital insights into their expression patterns at various disease stages[36].

Protein expression intensity of hub genes

The protein expression levels of the hub genes of Oral Cancer tissues were compared to normal oral mucosa tissues in this study,using the Human Protein Atlas (HPA) (Version 23.0)(HPA; https://www.proteinatlas.org/) database. The intensity of the staining and the percentage of stained cells in the tissues were used in this comparison. Additionally, symbolic immunohistochemical [IHC] staining images were retrieved from the HPA database in order to provide visual evidence of the protein expression patterns in the examined tissues[37]. HPA is a comprehensive proteome database that is based on IHC analysis.

Overall survival analysis of hub genes

The Kaplan-Meier [KM] Plotter (http://kmplot.com/analysis/index.php?p=service), a cancer genomics dataset, was used to assess the impact of the hub targets on the overall survival (OS) of patients with Oral Cancer[38]. This dataset enables for the evaluation of genes' prognostic importance on survival outcomes. Patients with Oral Cancer were categorized on the presentation levels of the hub genes into low and high. To compare the survival outcomes of the two groups, a KM survival plot was created. For further statistical significance analysis, hazard ratios (HR) with corresponding 95% confidence intervals and log rank P-values were determined.

Results

Molecular properties of myricetin

Our findings show that MYR follows Lipinski's Rule of Five (RO5). Myricetin's molecular properties met the RO5 criteria, indicating that it has favourable drug-like qualities.

Predicted toxicity of the Myricetin

Through literature screening and the Toxicity Model Report from ProTox-3 (Table 1), it was observed that MYR has a predicted LD50 of 159 mg/kg and falls into Predicted Toxicity Class 3. Additionally, there are indications of potential neurotoxicity, respiratory toxicity, and carcinogenicity, which require further consideration and validation.

Target identification and analysis

The DisGeNet database has been utilized for oral cancer-related targets using the keyword "Lip and Oral Cavity Carcinoma." This screening resulted in the discovery of 734 targets linked to oral cancer. Furthermore, the Swiss Target Prediction database was used to search for MYR targets, resulting in an identification of 100 targets. A comparison between the targets of MYR and the oral cancer-related targets revealed a common set of 22 genes present in both datasets (Fig. 2).

Development of protein–protein interaction network (PPI) and determination of key targets

The STRING Database was used in the study to examine protein-protein interactions among the identified targets. The PPI network was then further analyzed in Cytoscape 3.10.0 using Clusterone to find modules. Clusterone is a popular clustering algorithm available as a Cytoscape plugin. It is designed to detect densely connected regions (clusters or modules) within a PPI network.

The algorithm identifies clusters based on the density of connections (edges) within groups of nodes (proteins). High-density regions suggest functional modules where proteins are likely to interact cohesively to perform specific biological functions. The network of the 22 common genes had a p-value

Table 1 — predicted Toxicity report of Myricetin using ProTox-3.0 based on molecular similarity, pharmacophores, fragment propensities and machine-learning models

Target	Prediction	Probability
Hepatotoxicity	Inactive	0.69
Neurotoxicity	Inactive	0.89
Nephrotoxicity	Active	0.62
Respiratory toxicity	Active	0.83
Cardiotoxicity	Inactive	0.99
Carcinogenicity	Active	0.68
Immunotoxicity	Inactive	0.86
Mutagenicity	Active	0.51
Cytotoxicity	Inactive	0.99

of 1.59e-05, indicating that these proteins have a higher level of interaction among themselves than a randomly selected set of proteins with a similar size and degree distribution from the genome. This enrichment suggests that the proteins are biologically interconnected as a unit, as illustrated in (Fig. 3).

The analysis revealed three distinct clusters. The first cluster had a p-value less than 0.05 (p-value: 7.44e-07), but the second had a p-value of 0.0568. The third cluster had a p-value of 1 and showed no discernible connection. These p-values imply that the target proteins in each cluster are closely related and most likely share common biological processes. Cluster 1 (Fig. 3) was found to be associated with positive control of Cell migration, positive control of Cellular response to reactive oxygen species, and positive cell death regulation. On the other hand, Cluster 2 (Fig. 3) was linked to monitoring of programmed cell death and protein localization to nucleus, and chromosome organization.

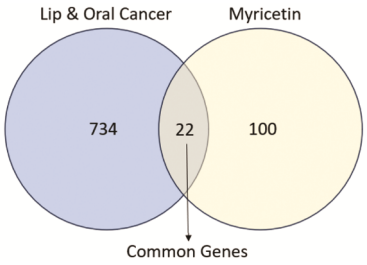

Fig. 2 — Number of genes common between oral cancer and Myricetin

Top Hub genes analysis

The top ten hub genes were found using several algorithms: AKT1, EGFR, MMP9, MMP2, PARP1, CDK1, MET, ALOX5, MMP3, and CA9. AKT1 emerged as the most notably activated gene among these hub genes (Table 2 and Fig. 4).

Table 2 — Various algorithms used to determine top 10 hub genes based on CytoHubba

Algorithms	MCC	MNC	Degree
Genes	EGFR	AKT1	AKT1
	AKT1	EGFR	EGFR
	MMP9	MMP9	MMP9
	MMP2	MMP2	MMP2
	MMP3	PARP1	PARP1
	PARP1	CDK1	ALOX5
	MET	MET	MMP3
	CA9	ALOX5	MET
	CDK1	MMP3	CA9
	ALOX5	CA9	CDK1

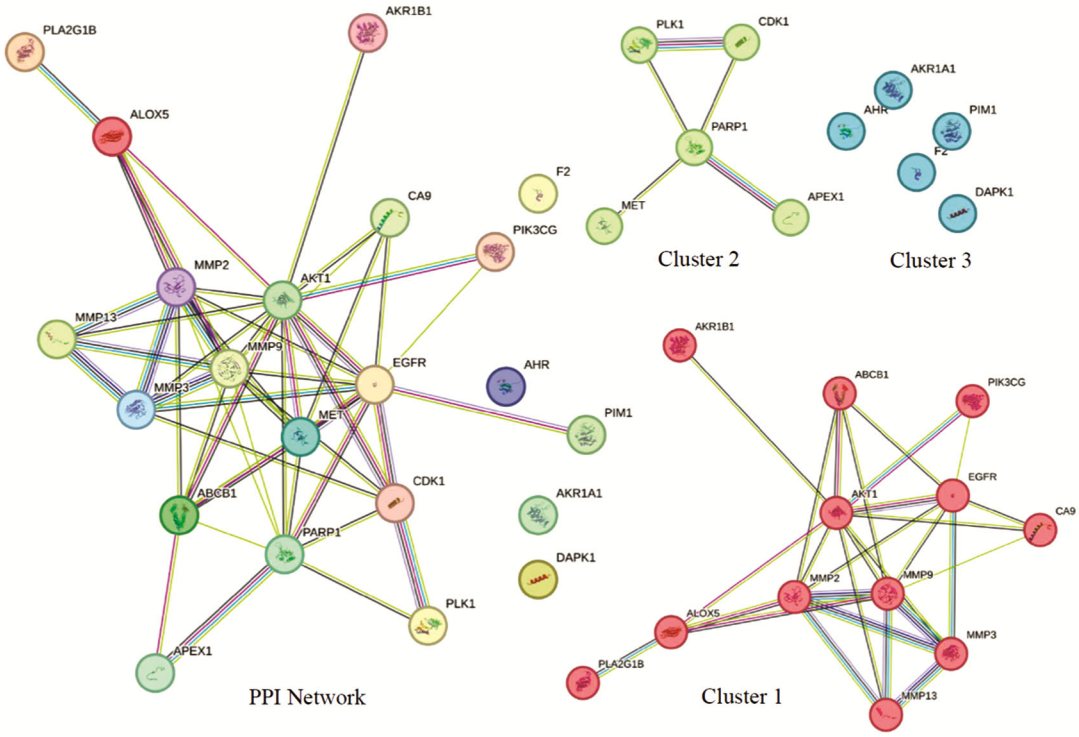

Fig. 3 — Protein-Protein Interaction network of 22 genes: Cluster 1: 12 proteins [nodes] and their association with each other [28 edges], (PPI enrichment p-value: 7.44e-07). Cluster 2: 5 proteins [nodes] and their association with each other [5 edges], (PPI enrichment p-value: 0.0568). Cluster 3: 5 proteins [nodes] and their association with each other [0 edges], (PPI enrichment p-value: 1)

GO enrichment analysis

The GO enrichment study of the ten hub genes identified around 14 GO keywords. The investigation revealed that these targets are implicated in apoptosis, lipid metabolism, activation of phospholipase A2 activity, and the Interleukin-18-mediated signaling pathway. The extracellular matrix, secretions, cytoplasm, and nucleus were among the cellular component (CC) results. In terms of molecular function (MF), the targets mostly play a role in metalloprotease activity and cyclin-dependent protein serine/threonine kinase activity (Fig. 5).

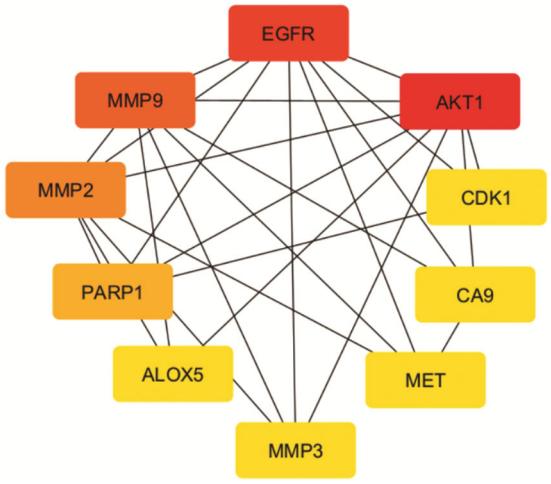

Fig. 4 — Figure showing Top 10 hub genes from common 22 genes

KEGG enrichment analysis

In total 34 pathways were discovered using KEGG pathway analysis. The top 21 paths were chosen for further study from among these. The top ten hub genes were discovered to have close relationships with many pathways, most notably the PI3K-Akt signaling system, the Ras signaling pathway, chemical carcinogenesis-ROS, Pathways in cancer, and MicroRNAs in cancer (Fig. 6).

The genes MET, AKT1, and EGFR were consistently found to be involved in the top ten pathways. These genes were significantly associated with the PI3K-Akt signaling pathway, which is having significant role in regulating a wide range of cellular functions that lead to carcinogenesis, including metabolic pathways, cell survival, cell proliferation, gene expression, and protein synthesis[38]. This finding, as shown in Table 3, shows that MYR could be used to treat oral cancer, with the PI3K-Akt signaling pathway being of particular interest having significant presence of AKT1 gene.

Validation of Hub target through molecular docking analysis

Ten hub genes were chosen as target proteins for molecular docking studies in order to assess the credibility of drug-target interactions. CB-DOCK was used to study MYR's docking capability with AKT1, EGFR, MMP9, MMP2, PARP1, CDK1, MET,

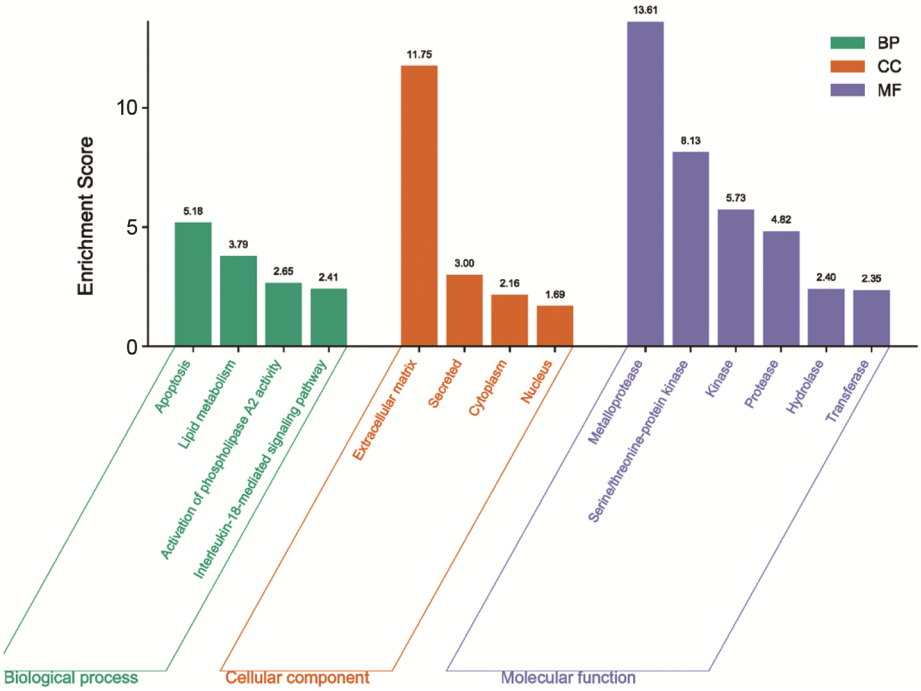

Fig. 5 — GO enrichment analysis showing 4 biological processes (BP), 4 Molecular functions (MF) and 6 Cellular components (CC) from DAVID Database for the targets in myricetin treating oral cancer

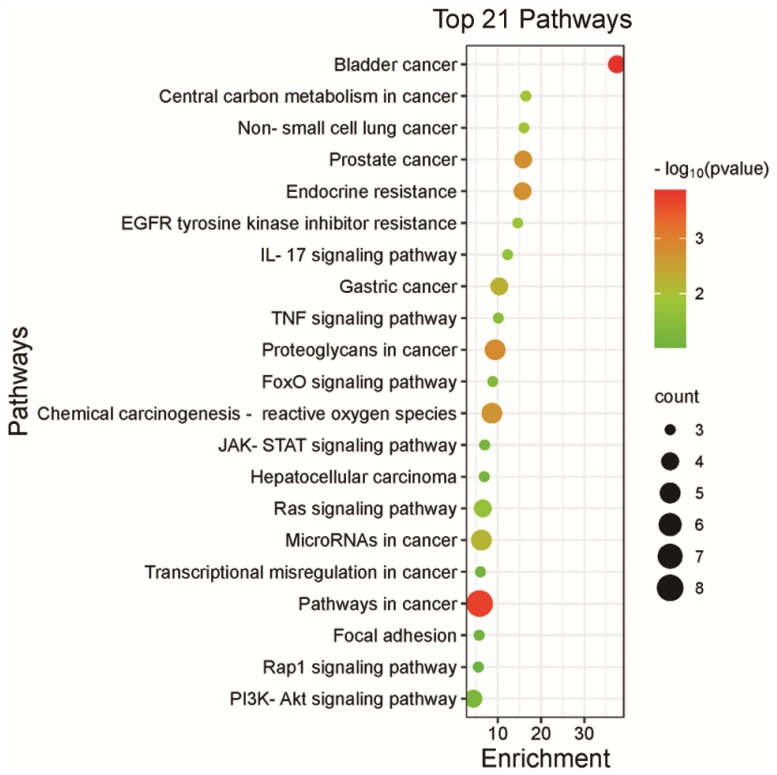

Fig. 6 — A Bubble plot depicting the enrichment of the top 21 signaling pathways associated with Oral Cancer was generated. The X-axis represents the enrichment factor of the genes, while the Y-axis represents the different pathways. The circles in the plot are color-coded and sized based on the Log10 [p-value], where red signifies pathways with the highest number of genes and light green indicates pathways with fewer genes

Table 3 — Top 10 pathways showing the genes involved

Pathway Term Id	Fold Enrichment	P-Value	Gene Count	Genes
Pathways in cancer	5.796952577	1.97E-04	8	DAPK1, MMP2, PIM1, AKT1, F2, MET, MMP9, EGFR
MicroRNAs in cancer	6.20601173	0.00644465	5	ABCB1, PIM1, MET, MMP9, EGFR
Chemical carcinogenesis - reactive oxygen species	8.627191194	0.001971784	5	AKR1A1, AKT1, AHR, MET, EGFR
Proteoglycans in cancer	9.384700665	0.001446323	5	MMP2, AKT1, MET, MMP9, EGFR
Bladder cancer	37.53880266	1.32E-04	4	DAPK1, MMP2, MMP9, EGFR
Prostate cancer	15.86691659	0.001670143	4	MMP3, AKT1, MMP9, EGFR
Endocrine resistance	15.70500928	0.001720139	4	MMP2, AKT1, MMP9, EGFR
Gastric cancer	10.32946919	0.005633331	4	ABCB1, AKT1, MET, EGFR
Ras signaling pathway	6.521571649	0.019637465	4	PLA2G1B, AKT1, MET, EGFR
PI3K-Akt signaling pathway	4.347714432	0.055199643	4	AKT1, MET, EGFR, PIK3CG

ALOX5, MMP3, and CA9. A lower energy value indicates the ligand is in a more stable conformation while binding to the receptor, implying a higher likelihood of contact. The binding energies between MYR and the core target proteins were found to be less than -5.0 in this investigation, showing that myricetin and the core targets had a significant binding activity. The specific binding energies are presented in (Table 4).The docking sketch maps illustrating the interactions between the target proteins and myricetin are depicted in (Fig. 7). Based on the

Table 4 — Molecular docking scores of myricetin and hub target proteins

Receptor	PDB ID	Binding energy kcal/Mol
AKT1	1UNP	-6.6
EGFR	1IVO	-8.8
MMP9	1L6J	-8.8
MMP2	1CK7	-8.9
PARP1	1UK1	-9.7
CDK1	4YC6	-8.1
MET	1R1W	-7.6
ALOX5	3O8Y	-8.4
MMP3	1B3D	-9.7
CA9	6FE2	-9.2

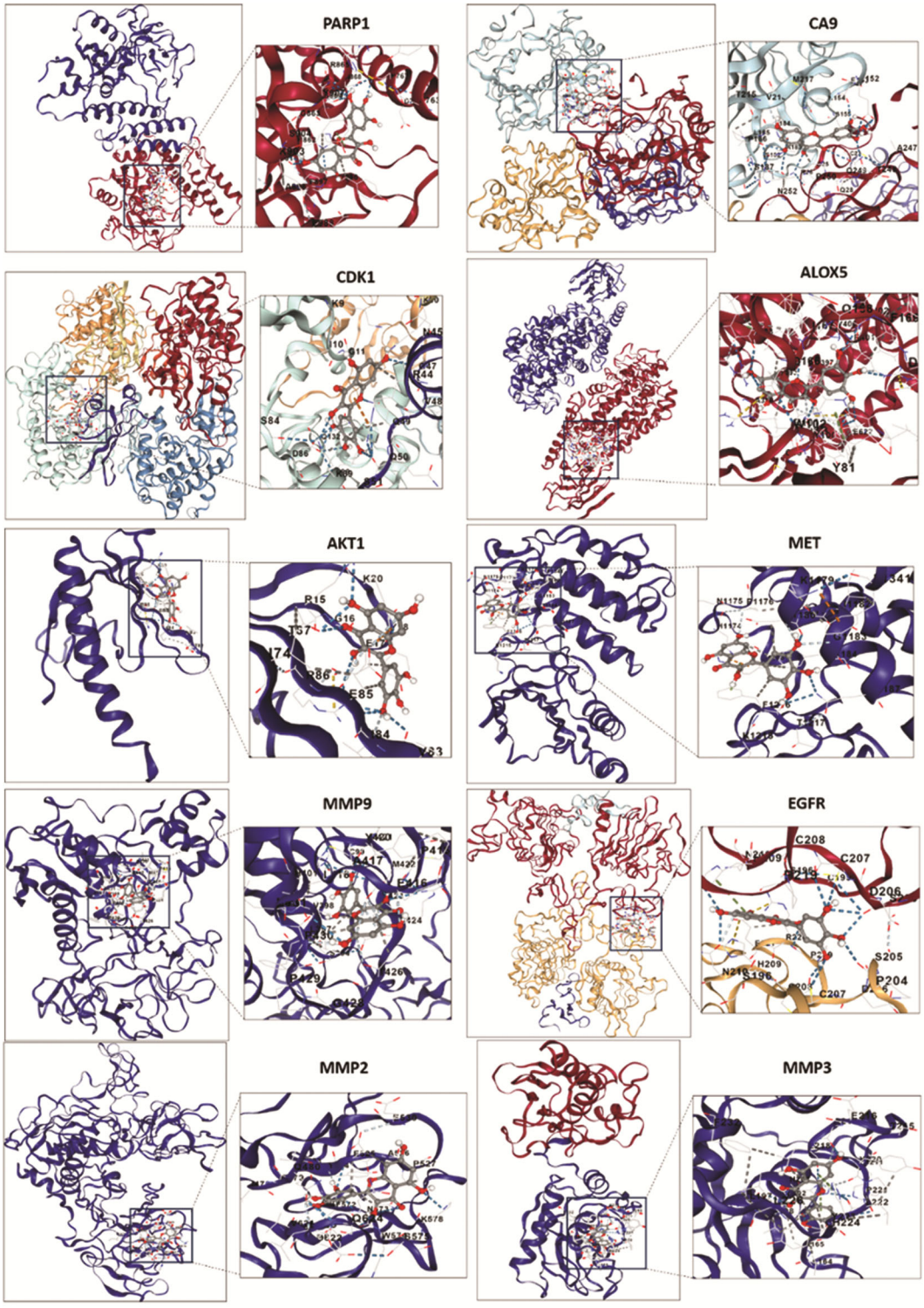

Fig. 7 — Sketch and matching diagrams of molecular docking of Myricetin and top 10 hub genes [target proteins]

binding energy and sketch maps, AKT1[-6.6] and MET [-7.6] were showing a strong bond and activity of MYR. This suggests a role of MYR having activity through the AKT1 gene in the PI3K-Akt pathway.

External validation of hub genes

mRNA expression levels of hub genes

We used the GEPIA database to compare the hub genes expression in oral cancer tissues and normal

tissues. AKT1, MMP2, MMP3, PARP1, MET CA9 and MMP9 mRNA levels were substantially higher in oral cancer specimens compared to normal oral mucosa samples ($P< 0.01$) (Fig. 8A).

Furthermore, we studied the association among mRNA levels of the hub genes and the pathological stages of oral cancer. The results indicated that the expression levels of MMP9, ALOX5, CA9, MMP3 and CDK1 changed significantly across different clinical phases. ALOX5, MMP3 and CA9 showed significant increase in stage I and II. Mild increase was seen in stage II and IV with MMP2 and MMP9 (Fig. 8B). These findings imply that the levels of expression of these five genes may be linked to the progression of oral cancer.

Protein expression levels of hub genes

We analyzed IHC staining photos from the HPA database to measure the protein expression levels of the hub genes of oral cancer. ALOX5 and CA9 were the only hub genes that did not show any expression in normal as well as oral cancer tissues. The remaining eight hub genes, on the other hand, showed varying levels of expression in normal oral mucosa tissues.

PARP1, CDK1, MET expression levels in oral cancer tissues were slightly higher than in normal oral mucosa tissues. AKT1, EGFR, MMP2 and MMP9 expression levels, on the other hand, were comparable in both oral cancer and normal oral mucosa tissues. Notably, MMP3 expression in normal as well as oral

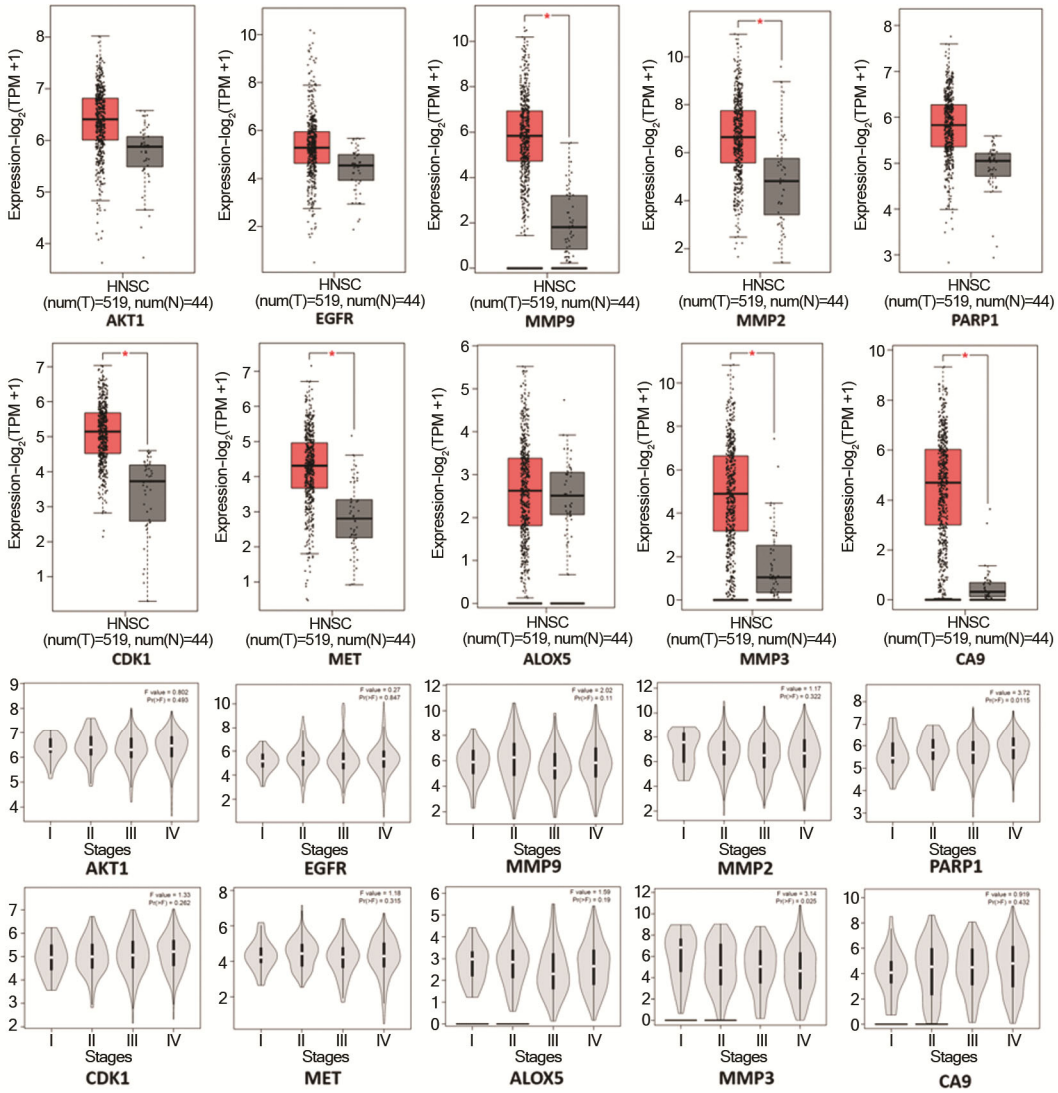

Fig. 8 — The mRNA expression levels of hub genes in The Cancer Genome Atlas (TCGA) and Genotype–Tissue Expression (GTEx) databases. (A) mRNA expression levels in the GEPIA database [Boxplot of hub genes]. Red depicts Oral Cancer tissue, and grey depicts normal oral mucosa; and (B) mRNA expression level and pathological stage in the GEPIA database. [Stage plot of hub genes]

cancer tissues was not available in HPA database (Fig. 9).

Survival analysis of the hub genes

Survival analysis was done on the following ten hub genes: AKT1, EGFR, MMP9, MMP2, PARP1, CDK1, MET, ALOX5, MMP3, and CA9. The study was performed on a cohort of 500 oral cancer patients from the TCGA database. The findings revealed that all of the hub genes exhibited a significant association with poor prognosis ($P< 0.05$, Fig. 10).

Discussion

Despite continued research efforts and therapeutic advances, HNSCC clinical outcomes and overall survival rates have shown little improvement in recent decades, with a depressing 5-year survival rate as low as 50%[39,40]. Current research efforts are concentrated on examining alternative medicines that have lower toxicity profiles due to the dismal clinical outcomes and considerable toxicity associated with current treatment modalities for head and neck cancer. The goal is to improve clinical results while reducing side effects from the medication. In recent years, there has been more emphasis placed on the investigation of complementary and alternative medicine as an attractive option for cancer management.

In this study, an *in silico* approach was employed to identify the anti-cancer properties of MYR against oral cancer. Analysis revealed 22 common genes associated with MYR and oral cancer, participating in key biological processes such as Ras and PI3K-Akt signaling pathways, chemical carcinogenesis-ROS,

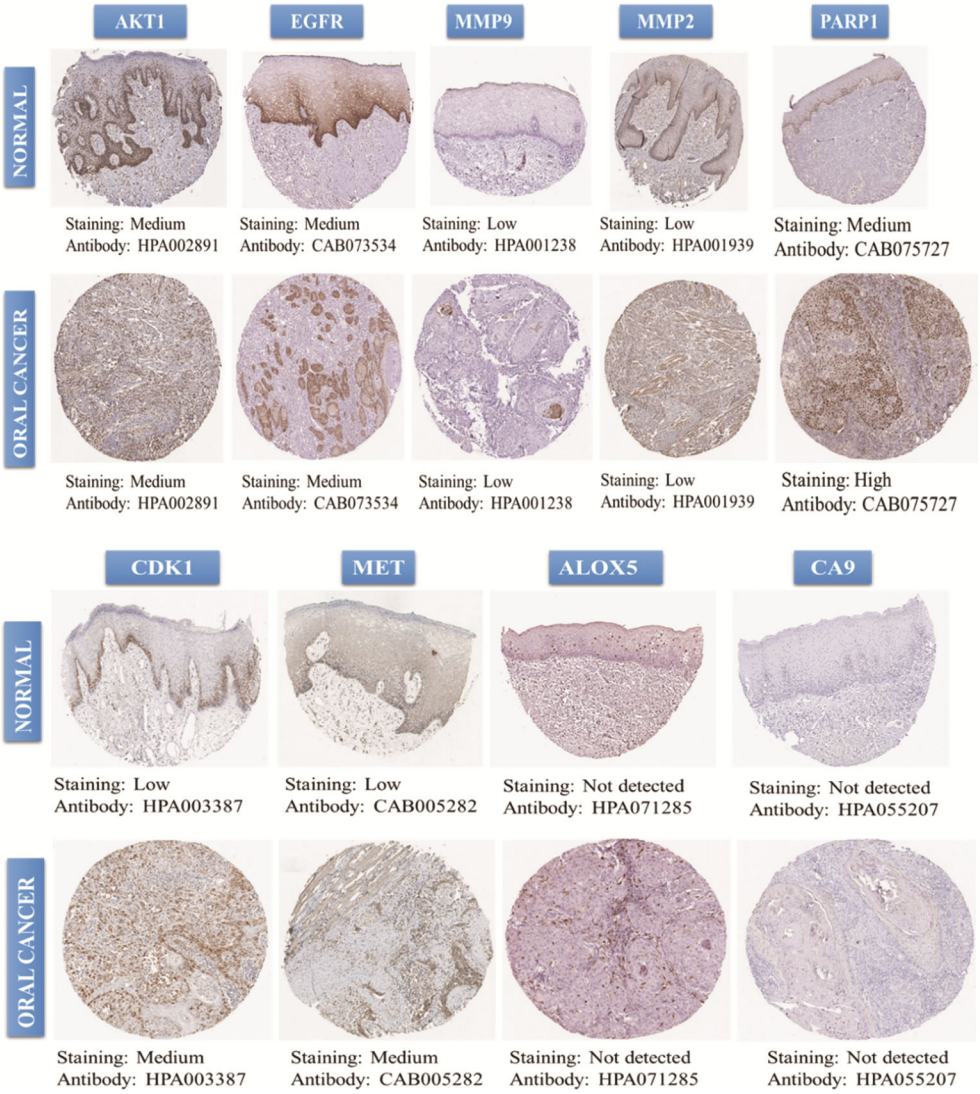

Fig. 9 — Protein expression levels of hub genes in the HPA database [Immunohistochemical images]

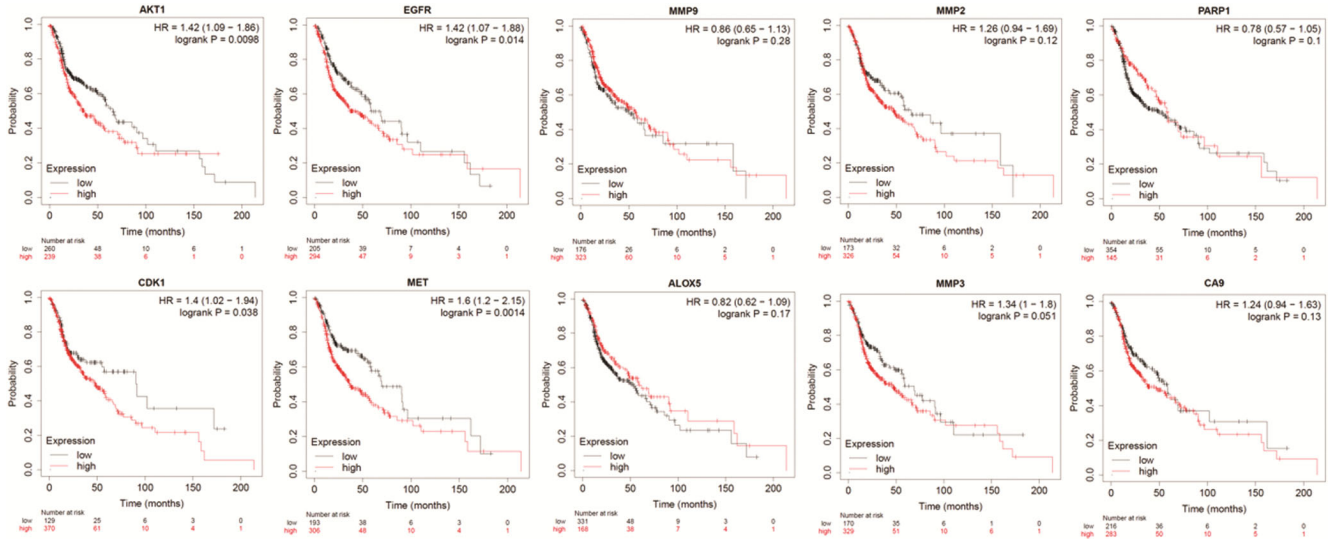

Fig. 10 — Kaplan–Meier overall survival analyses of patients with Oral cancer based on expression of the ten hub genes. HR, hazard ratio ("http://kmplot.com/analysis/index.php?p=service&cancer=pancancer_rnaseq")

pathways in cancer, and microRNAs in cancer. Notably, AKT1, EGFR, and MET emerged as significant hub genes, consistently implicated in the top 10 pathways. EGFR signaling, particularly in conjunction with AKT1, appeared to be a pivotal pathway for the potential therapeutic impact of MYR on oral cancer. Elevated mRNA levels of AKT1, MMP2, PARP1, MET, and CA9 were observed in oral cancer tissues, with immunohistochemistry revealing a slight increase in protein expression of PARP1, CDK1, and MET compared to normal oral tissues.

Based on the results of molecular docking, the binding energies and sketch maps of AKT1[-6.6] and MET [-7.6] showed a strong bond and activity of myricetin. This suggests a role of MYR having activity through the AKT1 gene in the PI3K-Akt pathway.

No *in silico* research on MYR's capability to treat oral cancer has been done prior to this work. Nevertheless, there are two *in vitro* studies available. In an earlier *in vitro* study, Maggioni et al.discovered that MYR inhibits cell growth and proliferation in an oral cancer cell line by impairing the cell cycle. Despite not inducing apoptosis, MYR had a selective cytostatic effect on cancer cells. However, a plateau effect was seen and there was no increase in the inhibitory effect with time. In SCC-25 cells, a G0/G1 to S phase blockage was observed after 24 hours of treatment, demonstrating the inhibition of cell growth by MYR. It reduced Cyclin D1 levels in SCC-25 cells. MYR inhibits Cyclin D1 expression and inactivates AKT, which is crucial for pathways including cell migration and proliferation. According to results from the anti-migratory and Boyden chamber experiments, it also decreased cell motility and invasiveness. In conclusion, myricetin demonstrated cytostatic action through the inhibition of cell cycle progression and its anti-migratory potential, indicating its application as an antimetastatic medication[41].Yuan et al. found that MYR decreased HPV E6's ability to bind *in vitro* to both caspase 8 and E6AP, the protein that promotes p53 degradation, in another study on HPV-mediated malignancies. It sensitized HPV+ oral cancer cells, but not HPV- oral cancer cells, to apoptosis brought on by the cancer-specific ligand TRAIL also the chemotherapeutic drugs doxorubicin as well as cisplatin, at a higher dosage of 100 μM[42].

Limitations of the study

The study is confined to a specific pivotal gene, intentionally excluding a comprehensive analysis of the entire dataset for the present. The authors deliberately focused on particular genes, aiming to streamline the study and keep the scope manageable. While *in silico* approaches provide powerful tools for biological research, their limitations underscore the necessity of combining them with experimental methods and continuously refining models to enhance accuracy and applicability.

Conclusion

The study highlights myricetin's potential efficacy against oral cancer by targeting multiple pathways

and crucial genes. Molecular docking techniques provide evidence of favourable interactions with key targets and hub genes. *In silico* analysis enriches our understanding of myricetin's mechanisms, allowing a thorough evaluation across different parameters, as detailed in this methodology. However, it's important to note that *in silico* results are initial predictions requiring subsequent experimental validation to confirm their accuracy and significance. Emphasizing the exploratory nature of these findings would provide a more balanced and cautious interpretation of the study's outcomes.

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Conflict of interest

All authors declare no conflict of interest.

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