International Journal of Nutrology
(Official Journal of the ABRAN - Brazilian Association of Nutrology)

ORIGINAL ARTICLE

DOI: 10.54448/ijn25419

Natural flavonoids as ACE2 modulators: computational insights into quercetin's role in oral cancer therapy

Shivani Sivasakthivel[1,*], Pratibha Ramani[2], Pradeep Kumar Yadalam[3]

[1] Research scholar. Saveetha Dental college and Hospitals, and Saveetha institute of Medical and technical sciences, Department of Oral and Maxillofacial pathology, Chennai, Tamil Nadu, India.
[2] Professor and Head. Saveetha Dental college and Hospitals, and Saveetha institute of Medical and technical sciences, Department of Oral and Maxillofacial pathology, Chennai, Tamil Nadu, India.
[3] Assisstant Professor. Saveetha Dental college and Hospitals, and Saveetha institute of Medical and technical sciences, Department of Periodontics, Chennai, Tamil Nadu, India.

*Corresponding author: Shivani Sivasakthivel.
Research scholar. Saveetha Dental college and Hospitals,
and Saveetha institute of Medical and technical sciences,
Department of Oral and Maxillofacial pathology, Chennai,
Tamil Nadu, India.
E-mail: jsshivaninarayanan@gmail.com
DOI: https://doi.org/10.54448/ijn25419
Received: 10-11-2025; Revised: 12-16-2025; Accepted: 12-19-2025; Published: 12-21-2025; IJN-id: 25419

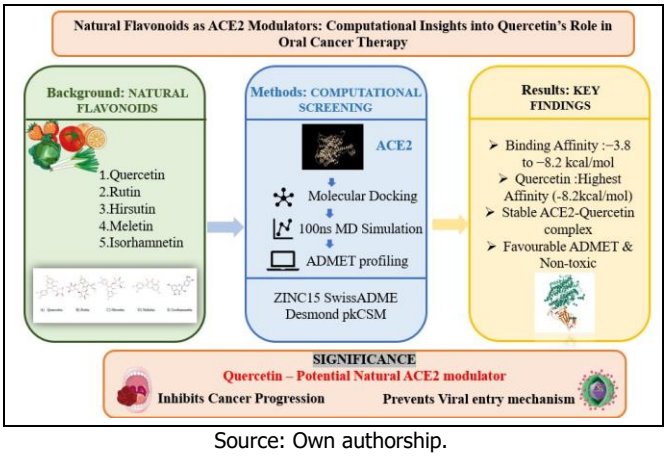
Editor: Dr. Carla Maria Barreto da Silva de Sousa Rego, MD, MsD, Ph.D.

Abstract

Background: Angiotensin-converting enzyme 2 (ACE2) is a key counter-regulatory enzyme in the Renin-Angiotensin System that converts angiotensin II into angiotensin-1–7, which has anti-inflammatory, antifibrotic, and vasodilatory properties. In addition to its cardioprotective function, ACE2 has gained attention in the field of cancer biology, where the dysregulated ACE2/Ang-(1–7)/Mas axis links its downregulation to tumour progression and poor prognosis, including in Oral Squamous Cell Carcinoma (OSCC) the predominant cancers of the head and neck region, which accounts for about 90%, with approximately 270,000 to 300,000 new cases diagnosed world-wide each year, and about 20% occurring in people less than 55 years. Its clinical significance is further emphasized by its role as the SARS-CoV-2 entry receptor; ACE2 expression is influenced by a number of hormones, but little is known about the role of antioxidants. By interacting with signalling pathways, quercetin, a bioflavonoid derived from plants, may alter ACE2. This study analyses the potential effects of quercetin on ACE2 by means of computational methods. **Materials & methods:** Molecular docking, interaction profiling, molecular dynamics (MD) modeling, and in silico ADMET analysis were employed to investigate ligand–protein interactions with ACE2. Top-ranked ZINC library chemicals were docked to ACE2 to identify favorable binding conformations. Binding affinities were assessed using a variety of scoring systems. MD simulations using Desmond were performed for 100 ns in order to evaluate the ACE2-ligand complexes' dynamic behavior and structural stability under physiological settings. Through interaction profiling, strong conformational alterations and binding persistence were found. To evaluate quercetin's pharmacokinetics, drug-likeness, and toxicity, ADMET predictions were also made using the pkCSM program. **Results:** With a docking score of -8.2 kcal/mol, quercetin demonstrated a high binding affinity with ACE2. MD simulations showed complex stability with few fluctuations. Persistent ligand-receptor binding was validated by interaction profiling. **Conclusion:** The research aims to provide insights for future experimental validations and to stimulate the development of bioflavonoid Quercetin as a novel, effective therapeutic agent for modulating the ACE2 activity.

Keywords: ACE2. Angiotensin Converting Enzyme 2. Quercetin. Computational analysis. Flavonoids. Oral Squamous Cell Carcinoma.

Graphical Abstract



Source: Own authorship.

Introduction

Advances in computational research have enabled the pharmaceutical sector to evaluate the safety profile and regulatory requirements with the help of in silico technologies, which have revolutionary advantages. The human diet contains abundant flavonoids. Over a billion years old, flavonoids have a wide range of biological functions that may be able to affect dysregulated mechanisms in disease. They demonstrated potential therapeutic effects for a wide range of diseases. Their physiological effects include lowering oxidative stress, preventing platelet aggregation and oxidation of low-density lipoproteins, and functioning as vasodilators in blood vessels. Antioxidant-rich medicinal plants are essential for producing positive effects and as an alternate source of treatment for illnesses brought on by oxidative stress **[1,2]**. Quercetin, a widely dispersed flavonoid, has been extensively explored. Quercetin also known as Quercetin glycosides ($C_{15}H_{10}O_7$) is a polyphenolic bioflavonoid that is the most abundant flavonoid molecule and is widely spread across the plant kingdom (Figures 1 and 2).

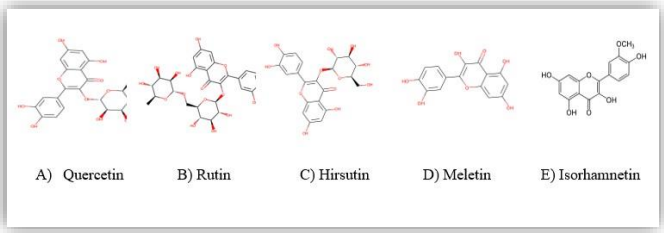


Figure 1. The structures of bioflavonoids Quercetin, Rutin, Hirsutin, Meletin, Isorhamentin. Source: Own authorship.

Oral squamous cell carcinoma (OSCC) is the most prevalent tumour in the head and neck region, with an upsurge in incidence in recent decades **[3]**. Epidemiologically the data goes with around 274,000 new cases diagnosed each year, it is the eighth most common cancer in the world **[4]**. Oral cancer is the 16th most common cancer worldwide. It is the 12th most common cancer in men and the 18th most common cancer in women. India, China and the US had the highest number of mouth and oral cancer cases since 2022 **[5]**. While oral cancers predominantly affect older adult, who are around an average diagnostic age of 64 years, approximately 1/5th of cases is observed in people less than 55 years of age.It is a multifactorial disease that is regulated by multiple biomarkers up regulation or down regulation in a genetic or familial susceptibility background **[6,7]**.These alterations are assessed using molecular research, which gives details regarding OSCC and patients' genetic vulnerability to it, predicting overall prognosis and survival.

Various studies shows that Renin Angiotensin System in cancer biology plays a role in a variety of biological processes. The major enzyme of the Renin-Angiotensin System (RAS) is angiotensin-converting enzyme 2, which is a homolog of carboxypeptidase ACE. The angiotensin-converting enzyme (ACE) 2 may counteract the growth-promoting actions of the ACE **[6]**. The RAS converts angiotensin I (AngI) to angiotensin and angiotensin II (AngII) to angiotensin to play a critical role in the cardiovascular system, demonstrating anti-proliferative, anti-fibrotic, and vasodilator effects (Figure 2). Numerous oncogenic pathways and tumour progression characteristics were inversely connected with ACE2, while antitumor immune response and survival prognosis were positively correlated with ACE2 in a variety of malignancies, implying that ACE2 may play a protective role in cancer formation **[7,8]**. ACE2 plays a pivotal role in the renin–angiotensin system and is closely related to coronavirus disease of 2019 **[9]**. In our study, ELISA analysis revealed a significant reduction in ACE2 expression in oral squamous cell carcinoma (OSCC) tissues **[10]**. Whereas PCR analyses reported increased ACE 2 mRNA expression in OSCC tumor samples **[11]**. This observation suggests a potential link between ACE2 expression and the progression of OSCC.Another bioinformatic study study where large-scale cancer transcriptome analyses were carried out. Head and Neck Squamous Cell Carcinoma (HNSC) has been indentified with distinct molecular subgroup of ACE 2 expression patterns when compared with other malignancies **[12]**. Based on these findings, we propose that ACE2 could serve as an important biomarker for OSCC prognosis, warranting further investigation into its role in tumor development and progression However, the role of ACE2 is being explored.

Alongside synthetic compounds, numerous natural products are emerging as viable options for cancer medications, complementing conventional therapies like chemotherapy and radiotherapy **[13]**. The potential for using natural substances to prevent cancer without

noticeable side effects is very promising. Numerous studies have shown that Quercetin, exerts antiproliferative effects by modifying the signal transduction pathways mediated by either EGFR or estrogen receptors. Although the exact mechanism of action is not fully understood, in vitro studies have observed several effects from this substance: reduced expression of mutant p53 protein and p21-ras oncogene, activation of cell cycle arrest in the G1 phase, and inhibition of heat shock protein synthesis **[14]**.

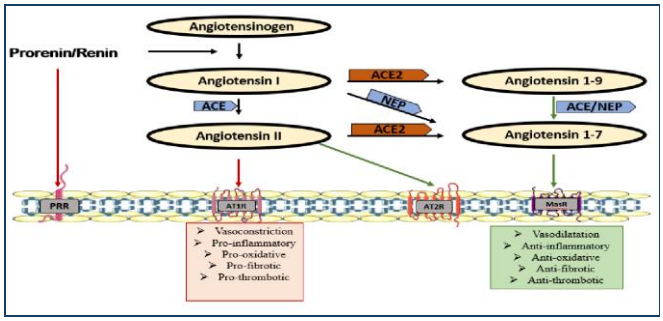


Figure 2. ACE2 - Renin Angiotensin cascade; ACE2- Angiotensin Converting Enzyme 2, ACE- Angiotensin Converting Enzyme; NEP-neprilysin; PRR- pro-renin receptor; AT1R- Angiotensin II receptor type 1; AT2R- Angiotensin II receptor type 2; MasR- Mas receptor. Source: Own authorship.

When combined with chemotherapeutic agents in vitro, quercetin also demonstrates synergy and the ability to reverse the multidrug resistance phenotype. It is a versatile antioxidant known for its capability to protect against tissue damage caused by various drug toxicities. Furthermore, it exhibits anti-inflammatory, anti-hypertensive, vasodilator, anti-obesity, anti-hypercholesterolemic, and anti-atherosclerotic properties **[15]**. It also possesses antiproliferative effects, growth factor suppression, and antioxidant features that can help fight cancer **[16]**.

Molecular docking, a computational technique that samples small molecule conformations in protein binding sites and uses scoring functions to determine which conformation best complements the binding site, is commonly used for lead identification in the drug discovery process. In contrast, molecular docking is crucial for structural molecular biology and drug design using computers. Predicting the most prevalent ligand-protein binding mechanism is the goal of ligand-protein docking.

Oral squamous cell carcinoma treatment and management remain challenging, due to limited efficacy of current conventional therapies. Quercetin, bioflavanoid as natural remedy shows promising anticancer potential, but its molecular interactions and pharmacokinetic properties in OSCC remains unclear.

The purpose of the study is to investigate the effects of the quercetin and its derivatives on OSCC, specifically on their interactions with Angiotensin Converting Enzyme 2 (ACE2). Numerous studies suggests that quercetin may serve as a promising therapeutic agent in OSCC **[17]**. An interventional study by Dai et al. reported that quercetin reversed malignant cellular features and suppressed epithelial-mesenchymal transition (EMT) **[18]**. In addition, to that, in vitro and in vivo models by Liu and colleagues shown that combined treatment with cisplatin and quercetin inhibits tumor growth effectively **[19]**. Recent bioinformatic analyses of active compounds in Chinese medicinal formulations of active phytochemicals SZKJT, including quercetin as a key ingredient, identified as a potential drug for OSCC management **[20]**.

This study addresses the gap by carrying out computational technique to evaluate its pharmacologic qualities against ACE2 as a treatment strategy in OSCC.

Materials and Methods

This study was carried out during December 2024 in Saveetha Dental College and Hospitals, Chennai, using appropriate bioinformatic Insilico methods and databases. The findings were compiled and documented**.**

a) Five bioflavonoids were selected through an extensive literature review. These include:

i) Quercetin – Plant flavonol belonging to flavonoid polyphenols group, with the molecular formula $C_{15}H_{10}O_7$. It is extensively present in various plants as well as foods, encompassing apples, onions, red wine, green tea, as well as berries.

ii) Rutin – Glycoside composed of flavonol quercetin and disaccharide rutinose, with the molecular formula $C_{27}H_{30}O_{16}$. Rutin is glycoside of citrus flavonoid commonly present in plants, including buckwheat, raspberries, and green tea.

iii) Hirsutrin – A flavonol glycoside identified in plants such as Gossypium hirsutum. It is a glycosylated derivative of quercetin.

iv) Meletin – A glycoside derivative of quercetin, also known as quercetagetin. It is a naturally occurring flavonoid with structural similarity to quercetin.

v) Isorhamnetin – An O-methylated flavonol, specifically the 3'-methoxylated derivative of quercetin, with the molecular formula $C_{16}H_{12}O_7$. It is found in food sources such as pears, olive oil, wine, and tomato sauce.

b) Structure Retrieval:

SMILES (Simplified molecular input line entry system) depiction for each compound is listed in Table 1. The SMILES form is a notation system used to represent the chemical structure of a drug in a linear format. The chemical structures of compounds have been recovered from PubChem database in sdf format and changed into three-dimensional (3D) structures in.pdf format utilizing Open Babel software.

Table 1. Simplified Molecular Input Line Entry (SMILE) System form of Quercetin, Rutin, Hirsutin, Meletin, Isorhamnetin.

Flavonoid	SMILE
Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Rutin	(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O
Hirsutin	c(-c2ccc(O)c(O)c2)oc2cc(O)cc(O)c12
Meletin	O=c1c(O)c(-c2ccc(O)c(O)c2)oc2cc(O)cc(O)c12
Isorhamnetin	COc1cc(-c2oc3cc(O)cc(O)c3c(=O)c2O)ccc1O

Source: Own authorship.

Chemical structures of quercetin and its derivatives are given in Figure 1. 3D structure of ACE2 has been retrieved from RCSB Protein Data Bank using the specific identifier 6l6r and is shown in Figure 3. Subsequently, the structure underwent refinement and minimization using Swiss-PdbViewer, a software tool for visualizing and optimizing protein structures. The PubChem database was also used to retrieve the quercetin identifier, a flavonoid compound-5280343 known for its various biological activities. The SMILES form and the structures of quercetin and its derivatives were obtained from ZINC15, and a pharmacophore model was obtained from ZINCPharmer. This process ensured the structure was accurately represented and ready for further compuatational analysis.

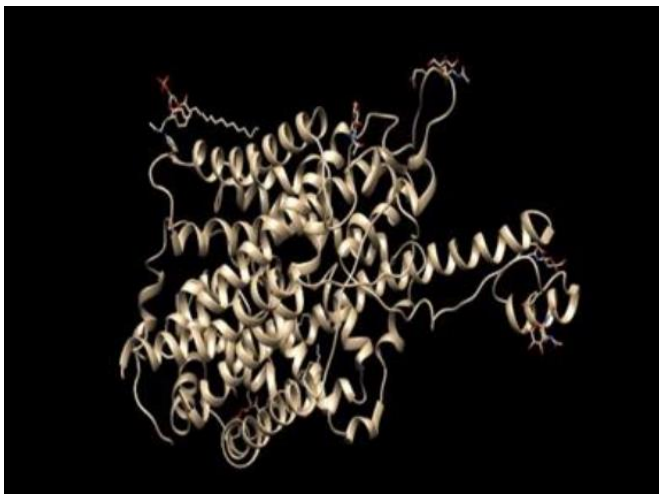


Figure 3. Structure of ACE2 protein. Source: Own authorship.

c) Molecular docking

Schrödinger's Maestro is a molecular modeling software platform that includes docking studies to predict how small molecules interact with biological macromolecules. During the docking process, we prepare the ligands and the receptor, configure the docking experiment, specify the binding site, choose a docking algorithm, and set scoring functions. The software creates various ligand poses within the receptor's binding site and simulates different ligand conformations based on the established scoring function. We analyzed the results, which include docking poses, scoring and ranking, and visualization.

d) Molecular Dynamics Simulation analysis and Interaction Profiling

We used Desmond, a Schrödinger LLC software, to simulate molecular dynamics for hundred nanoseconds. Our initial step in simulating receptor as well as ligand complexes included docking experiments, utilizing molecular dynamics. In static settings, our MD simulations generally simulate atomic movements over time by combining classical equations of motion of Newton. We employed simulations to predict ligand-binding status in physiological environment. To preprocess receptor-ligand complex, we used Maestro's Protein Preparation Wizard, which comprises complex optimisation as well as minimisation. We utilized System Builder tool to create all of systems with a TIP3P, orthorhombic box solvent model (Intermolecular Interaction Potential 3 Points Transferable). We employed OPLS 2005 force field in simulation and added counterions to ensure model neutrality. To simulate physiological circumstances, we added 0.15 M NaCl (sodium chloride). Throughout entire simulation, we used NPT ensemble at 300K temperature as well as 1 atm pressure. Before carrying out simulation, we adjusted models.

e) ADMET analysis

pkCSM online server (http://biosig.unimelb.edu.au/pkcsm/) has been utilized to predict quercetin's "ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity)" profile. Quercetin's standard SMILES representation has been obtained from PubChem database. pkCSM tool, which estimates different pharmacokinetic as well as toxicity parameters utilizing graph-based structural signatures, was then used to receive this SMILES string. Important parameters like intestinal absorption, Caco-2 cell permeability, blood-brain barrier penetration, volume of distribution, cytochrome P450 interactions, total clearance, and a number of toxicity endpoints like AMES mutagenicity, hERG inhibition, hepatotoxicity,

and LD50 values were all included in the prediction. In order to assess quercetin's potential as a therapeutic agent, the results were analysed to determine its drug-likeness and safety profile.

Results

This study evaluated the docking simulation of Quercetin and its derivatives with ACE2 (Figure 3).

a) Docking

The docking results indicate that the interactions between the receptor and ligand are significant and highly stable. Findings indicated that binding affinities varied from -3.8 to -8.2 kcal/mol, where Quercetin showed the greatest binding affinity and Isorhamnetin the least binding affinity, -3.8 kcal per mol. Docking score of Quercetin -8.2 kcal/mol further supports this idea, as lower scores typically reflect stronger binding affinities between the involved molecules. This score suggests that the ligand fits effectively into receptor's binding site, facilitating necessary interactions for biological activity (Figure 4).

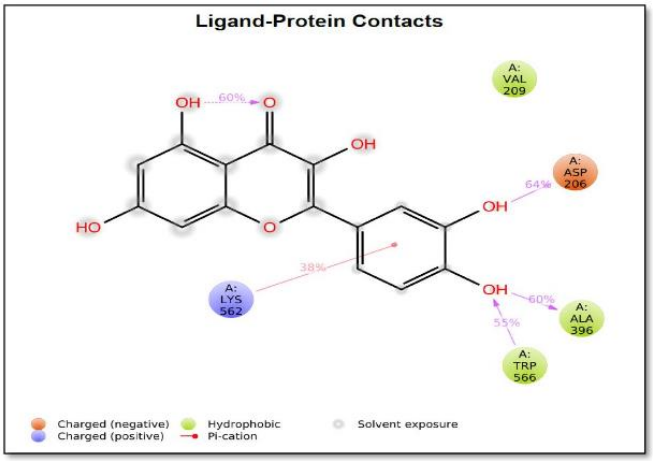


Figure 4. A schematic of detailed ligand protein interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 100.00 nsec), are shown. Source: Own authorship.

b) Molecular Dynamic Simulation and Interaction Profiling:

❖**Root mean square deviation:** "RMSD (Root Mean Square Deviation)" is utilized to calculate average change in displacement of atoms set for given frame about reference frame. It is computed for every trajectory frame. Tracking RMSD of protein gives information about its structural conformation during simulation (Figure 5).

❖**Protein RMSD:** Preceding graph shows RMSD progression of protein (left Y-axis). All protein structures are initially aligned to reference frame backbone, after which RMSD is computed as per selected atoms.

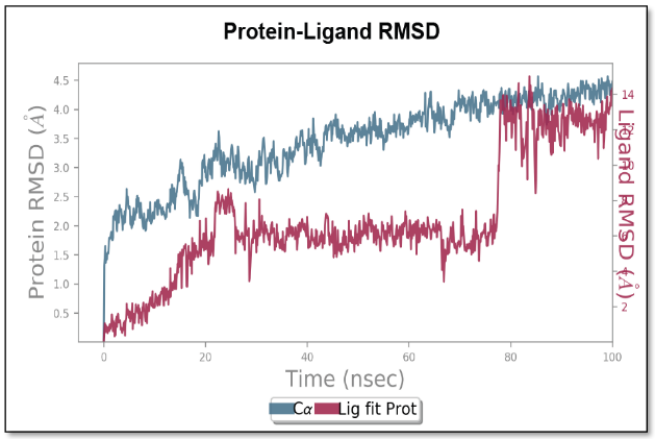


Figure 5 shows the RMSD of ACE2 and Quercetin interactions simulated via Schrödinger software. Source: Own authorship.

❖**Ligand RMSD:** Ligand RMSD (right Y-axis) signifies stability of ligand to protein along with its binding pocket. Above graph titled 'Lig fit Prot' illustrates RMSD of ligand after aligning protein-ligand complex to reference protein backbone, followed by measurement of RMSD of ligand's heavy atoms.

Results of molecular dynamics simulation showed that protein and ligand fluctuations are quite low during the initial 0 to 80 nanoseconds, ranging from 0.5 to 4.0 Angstroms. This stability indicates a robust interaction between the protein and ligand during this period. After 80 nanoseconds, slight fluctuations emerge, reflecting some variability, but these changes are still deemed acceptable within the simulation's context. Overall, the findings emphasize that the system retains a stable configuration, underscoring the dependability of protein-ligand interactions throughout most of simulation duration.

❖**Protein Secondary structure:** Protein SSE (secondary structure elements), including alpha-helices as well as beta-strands, are observed during simulation. Graph below illustrates SSE distribution by residue index across protein structure **(**Figure 5).

MD simulations have been carried out to assess conformational stability as well as binding interactions of quercetin with target protein. "RMSF (root mean square fluctuation)" analysis exhibited variable flexibility across protein backbone, with increased fluctuations observed in loop areas around residues 100–120 and 280–300, indicative of their inherent mobility. Notably, residues implicated in ligand binding, including Glu109, Glu110, Tyr200, and Ser546, showed lower RMSF values, suggesting stable interaction interface during simulation period. RMSF analysis of the ligand atoms showed moderate flexibility, particularly at terminal atoms, while the central core of the flavonoid scaffold remained relatively rigid, consistent with a well-seated binding conformation. Protein–ligand contact analysis further substantiated

these findings, highlighting a combination of hydrogen bonds (notably with Glu109, Glu110, and Ser546), hydrophobic interactions (with Leu89, Val94, and Ala179), and water-mediated bridges (especially with Tyr200 and Glu208) as key contributors to complex stability. Ionic interactions were negligible. These results collectively indicate that the quercetin–protein complex maintains dynamic stability and specific intermolecular contacts, supporting its potential as a viable ligand for further optimization and development.

❖**ACE2-QUERCETIN Interactions**: Protein-ligand interactions are observed continuously during simulation. Such interactions are categorised by type and summarized (Figure 6).

Significance of polar as well as solvent-accessible residues in ligand stabilization is highlighted by the interaction fingerprint of the ligand-binding site, which shows a high prevalence of hydrogen bonds and water bridges. While hydrophobic residues like PHE-245 and LEU-98 improve spatial stabilization, GLU-209, GLU-99, and ASN-208 are found to be essential for ligand recognition. A useful framework for structure-based drug design that targets this binding site is provided by this multifaceted interaction landscape.

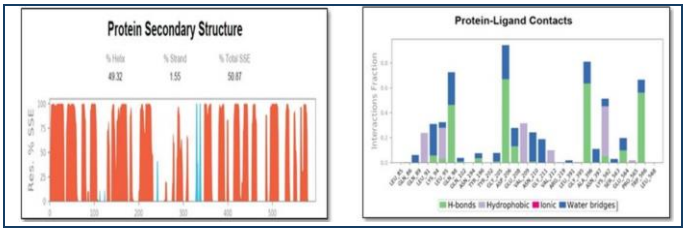


Figure 6. SSE and Interaction profiling. Source: Own authorship.

c) MD Trajectory Analysis

✓ Root mean square frequency

❖**ACE 2:** RMSF analysis of ACE2 protein along molecular dynamic simulation trajectory is depicted in this Figure 7. The flexibility or rigidity of ACE 2 protein regions is indicated by RMSF, which calculates the average deviation of each residue (usually Cα atoms) from its mean position. The elevated peaks in the RMSF plot indicate that some regions, especially those surrounding residues ~80–120 and ~300, exhibit noticeably higher flexibility. Loops or terminal regions, which are typically more dynamic, are represented by these areas. Structural rigidity is indicated by lower fluctuations in residues embedded within secondary structural elements (highlighted in pink). The comparatively moderate fluctuation of the green bars indicating functionally relevant sites suggests stable binding interactions, which is advantageous for complex stability and ligand binding.

❖**Quercetin:** Atomic flexibility varies throughout the ligand, as indicated by the RMSF plot (Figure 7). The majority of atoms show moderate fluctuations (~2–4 Å), suggesting that the ligand keeps its conformation comparatively stable while binding. Certain atoms, particularly those close to the end (atom 22), exhibit greater fluctuations, though, which may indicate that they are situated in areas that are less tightly bound or more exposed to solvent. Atoms with lower RMSF values, on the other hand (such as atoms 6–10), are probably more buried in binding pocket and have stronger interactions with protein.

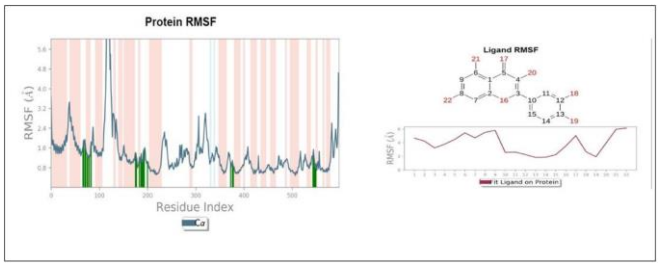


Figure 7. shows the RMSF of ACE2 protein and Ligand Quercetin. Source: Own authorship.

The RMSF analysis offers thorough examination of fluctuations of every atom in ligand, which is depicted in a 2D structural format in the upper panel. By analyzing the RMSF of the ligand, investigators could gain valuable insights into how various fragments within the ligand interact with protein, as well as understand the entropic contributions these fragments make during the binding process. In the lower panel, a line labeled 'Fit Ligand on Protein' (Figures 7 and 8) illustrates ligand's fluctuations around protein structure. Before measuring the RMSF, protein-ligand complex is aligned as per protein backbone, facilitating accurate assessments of ligand's fluctuations, particularly focusing on heavy atoms of ligand. This comprehensive analysis not only highlights the dynamic behavior of the ligand but also aids in elucidating the mechanisms by which ligands influence protein activity.

d) ADMET Analysis: Pharmacokinetic as well as toxicity profile of quercetin was evaluated utilizing pkCSM tool to predict its ADMET properties (Table 2). Quercetin demonstrated moderate water solubility (log S=–2.925) and low Caco-2 cell permeability (–0.229 log Papp), though it exhibited a high predicted human intestinal absorption of 77.2%. Skin permeability has been low (–2.735 log Kp), alongside it was identified as substrate of P-glycoprotein (P-gp) but not inhibitor of either P-gp I or II. Distribution analysis revealed a high distribution volume (log VDss = 1.559) and moderate fraction unbound in plasma (Fu = 0.206). However, blood-brain barrier (log BB = –1.098) as well as CNS (log PS=–3.065) permeability were low, indicating

limited central nervous system penetration. Metabolic predictions indicated that quercetin is neither a substrate for CYP2D6 nor CYP3A4 but acts as an inhibitor of CYP1A2. It does not inhibit CYP2C19, CYP2C9, CYP2D6, or CYP3A4. The predicted total clearance was 0.407 log ml per min per kg, as it is not substrate for renal OCT2 transporters. Toxicity predictions suggested a favorable safety profile, with no AMES toxicity, hERG inhibition, hepatotoxicity, or skin sensitisation. Predicted maximum tolerated dose in humans was 0.499 log mg per kg per day. Acute oral toxicity in rats (LD50) was 2.471 mol per kg, and chronic toxicity (LOAEL) was 2.612 log mg per kg_bw per day. Environmental toxicity, assessed through Tetrahymena pyriformis and minnow models, yielded values of 0.288 log µg/L and 3.721 log mM, respectively. These findings support the drug-like properties of quercetin while highlighting the need for further experimental validation.

Table 2. ADMET properties table of quercetin.

PROPERTY	MODEL NAME	PREDICTED VALUE	UNIT
ABSORPTION	Water solubility	-2.925	Numeric (log mol/L)
	Caco2 permeability	-0.229	Numeric (log Papp in 10-6 cm/s)
	Intestinal absorption (human)	77.207	Numeric (% Absorbed)
	Skin Permeability	-2.735	Numeric (log Kp)
	P-glycoprotein substrate	Yes	Categorical (Yes/No)
	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
DISTRIBUTION	VDss (human)	1.559	Numeric (log L/kg)
	Fraction unbound (human)	0.206	Numeric (Fu)
	BBB permeability	-1.098	Numeric (log BB)
	CNS permeability	-3.065	Numeric (log PS)
METABOLISM	CYP2D6 substrate	No	Categorical (Yes/No)
	CYP3A4 substrate	No	Categorical (Yes/No)
	CYP1A2 inhibitor	Yes	Categorical (Yes/No)
	CYP2C19 inhibitor	No	Categorical (Yes/No)
	CYP2C9 inhibitor	No	Categorical (Yes/No)
	CYP2D6 inhibitor	No	Categorical (Yes/No)
	CYP3A4 inhibitor	No	Categorical (Yes/No)
EXCRETION	Total Clearance	0.407	Numeric (log ml/min/kg)
	Renal OCT2 substrate	No	Categorical (Yes/No)
TOXICITY	AMES toxicity	No	Categorical (Yes/No)
	Max. tolerated dose (human)	0.499	Numeric (log mg/kg/day)
	hERG I inhibitor	No	Categorical (Yes/No)
	hERG II inhibitor	No	Categorical (Yes/No)
	Oral Rat Acute Toxicity (LD50)	2.471	Numeric (mol/kg)
	Oral Rat Chronic Toxicity (LOAEL)	2.612	Numeric (log mg/kg_bw/day)
	Hepatotoxicity	No	Categorical (Yes/No)
	Skin Sensitisation	No	Categorical (Yes/No)
	T. Pyriformis toxicity	0.288	Numeric (log ug/L)
	Minnow toxicity	3.721	Numeric (log mM)

Source: Own authorship.

Discussion

The discovery of ACE2 has increased our understanding of the Renin Angiotensin System role in health and illness, despite the fact that it was previously only known for its role in cardiovascular physiology and pathophysiology. ACE 2 being a membrane protein, is a zinccontaining metalloenzyme that is found in the kidneys, gut, lungs, and heart, among other organs. Together with serving as an anchoring mechanism for SARS CoVs to adhere to host cell membranes for fusion, ACE 2 also regulates the RAS system to preserve homeostasis. Since the COVID-19 epidemic, ACE2's function has attracted a lot of interest and investigation. ACE 2 is the main cellular receptor for the SARS-CoV-2 virus that causes SARS CoV infections **[21]**.

ACE 2 levels are inversely correlated with primary carcinogenic factors, such as the cell cycle process, vascular endothelial growth factor, and transforming Wnt and Notch signalling, growth factor signalling, etc. However, it also discovered that ACE 2 expression levels in cancer were positively connected with several antitumor immune signatures, such as Jak-STAT signalling, T cell and B cell receptor signalling, cytokine-cytokine receptor interaction, chemokine signalling, natural killer cell-mediated cytotoxicity **[22]**. ACE 2 processing and presentation, and nucleotide binding and oligomerization domain receptor signalling. These results finally suggest that ACE 2 may have a preventive function in carcinogenesis **[13]**.

On the other hand, by preventing the formation of new blood vessels, quercetin can also have anti-tumor effects. Quercetin inhibits the expression of the downstream regulatory factor AKT, targeting the VEGFR-2-mediated angiogenesis pathway, and limits tumor growth in array of malignancies **[23]**. Several in vitro and in vivo studies have proven quercetin's potent role in apoptosis promotion, metastasis inhibition, and cell cycle and tumor angiogenesis regulation.

Protein-ligand interactions, often called 'contacts', fall into four categories: Hydrogen Bonds, Hydrophobic interactions, Ionic bonds, and Water Bridges. Interactions can exceed 100% because certain residues may form multiple interactions of the same type with a single ligand atom. Ligand-protein docking aims to predict the most probable binding mode(s) of a ligand using the known three-dimensional structure of a protein. Understanding the pharmacological action of natural compounds under therapeutic settings and discovering new compounds with greater potency and selectivity can both be accomplished with the use of the information from in silico molecular docking studies. Thus, we have carried out molecular docking investigation to analyse Quercetin's mechanism of action against Oral Squamous Cell Carcinoma by analysing the structural interactions between ACE2 and Quercetin.

Docking indicates that the stability and the interactions between the receptor and ligand are quite significant, with a docking score of -8.2 kcal/mol. No similar studies have been performed earlier to analyse the structural alterations of ACE2 for drug design against OSCC. However, ACE2 receptors have been targeted in various cancers for drug design.

Previous literature has analysed the complex relationship between ACE2 and SARSCoV-2. Researchers have experimented with molecular binding sites on the spike proteinbound structure with its receptor and found that hesperidin (−8.99 kcal/mol), emodin (−6.19 kcal/mol), and chrysin (−6.87 kcal/mol) produced alterations in the structure, particularly hesperidin, which destabilizes the ACE2-bound structure. Molecular docking confirms that ligand-protein interactions promote a conformational shift in the three-dimensional structure of protein ACE2 **[24]**.

Another study evaluated the efficacy of chloroquine, hydroxychloroquine, and quinine in their interaction with ACE2 in COVID-19, revealing that quinine exhibits the highest affinity for the ACE2 receptor (-4.89 kcal/mol), followed by hydroxychloroquine (-3.87 kcal/mol) and chloroquine (-3.17 kcal/mol) **[25]**. Invitro studies have demonstrated quercetin when treating prostate tumor, blocks VEGF-induced activation of VEGF receptor 2 along with its related protein kinases, AKT, mTOR, and ribosomal protein S6 kinase. It was shown to inhibit tumorigenesis by targeting angiogenesis, reducing cell viability and triggering apoptosis **[26]**.

After quercetin treatment, apoptosis, Mcl-1 expression, and Bax activation and translocation were assessed in leukemia cells in one study, revealing significant apoptosis not in normal peripheral blood mononuclear cells but in both transformed and primary leukemia cells. This effect was induced through various mechanisms, including Mcl-1 down-regulation, Bax conformational change, and mitochondrial translocation, which triggered cytochrome c release **[27]**. In addition to its effects on cancer patients, quercetin has been shown to benefit individuals with hypertension, familial disorders, and kidney diseases **[28]**. ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis shows that, the toxicity value of quercetin was 3.020 is within the permissible limit **[26]** which is in accordance with our study.

Root mean square deviation (RMSD) is a metric that measures the average difference between corresponding atoms in two structures and is used to evaluate how well a docked molecule aligns with a known reference structure. In this study, RMSD OF ACE2 and quercetin shows that during the first 0 to 80 nanoseconds, the simulation revealed that protein and ligand variations are quite small, ranging from 0.5 to 4.0 Angstroms. This stability suggests that there was a strong contact between the ligand and protein throughout this time. The flexibility of various regions of a protein or ligand within a potential binding site is essentially indicated by Root Mean Square Fluctuation (RMSF), which measures how much individual atoms or residues in a molecule move or fluctuate over time during a simulation; a higher RMSF value indicates greater flexibility in that region.

The earlier studies reported docking scores significantly lower than those in this study involving ACE2-Quercetin **[29,30]**. Thus, these receptor-ligand interactions can be leveraged to develop a drug for OSCC. Future directions for developing a ligand-receptor complex include improved structural studies utilizing X-ray crystallography or cryo-electron microscopy, longer molecular dynamics simulations to observe stability over time, in vitro and in vivo testing to assess the ligand's efficacy and toxicity, analogue development to investigate structure-activity relationships, mechanical studies to comprehend the ligand's role in the viral life cycle, and evaluating the effectiveness of combining the ligand with existing antiviral agents to enhance its efficacy against SARS-CoV-2.

Limitations

The study has its own limitations, including constraints related to binding sites, simulation parameters, biological complexity, and a short evaluation period. Predefined binding sites may not accurately depict the dynamic nature of receptors, and MD simulations may not encompass all relevant conformational states or solvation effects. Factors such as cellular uptake, metabolism, and potential off-target effects should be considered.

Conclusion

This study used molecular docking, molecular dynamics simulations, interaction profiling, and ADMET analysis to investigate the binding mechanism, interactions of ACE2 and the bioflavonoid Quercetin. Quercetin has shown favorable docking scores, and molecular dynamics simulations validated the ligand-receptor complex's stability with low variations. Persistent interactions were confirmed using interaction profiling. These data demonstrate that quercetin has the capacity to regulate ACE2 activity, laying the groundwork for future experimental validation. The study delivers a new paradigm that advocates bioflavonoids such as Quercetin as natural, feasible and potent therapeutic agents for oral cancer treatment as well as ACE2-related pathways linked to viral vulnerability and chronic illnesses.

CRediT

Author contributions: Conceptualization - SS, PR, PKY.; Methodology - SS and PKY.; Software - PKY.; Validation - SS and PKY.; Formal analysis - PR and PKY.; Investigation - SS and PKY.; Resources - SS and PKY.; Data curation - PKY.; Writing-original draft preparation - SS.; Writing - review and editing - SS, PR, PKY; Visualization - PKY.; Supervision - PR and PKY. All authors have read and agreed to the published version of the manuscript.

Acknowledgment

Illustrations used in the graphical abstract were adapted from Servier Medical Art under Creative commons Attribution 3.0 license.The authors gratefully acknowledge Servier Medical Art and Protein Data Bank for access to protein structure which were utilized in the study.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Funding

This study received no particular grants from public, commercial, or non-profit funding entities.

Data Sharing Statement

All the relevant data is provided in the article.

Conflict of Interest

The authors declare that they have no known competing interests.

Similarity Check

It was applied by Ithenticate@.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

References

1. Lomada D, Gulla S, Reddy MC. Anti-Inflammatory and Antioxidant Activity of Titanium Dioxide Nanotubes Conjugated with Quercetin. Chemistry & Biodiversity [Internet]. 2023 Oct 12;20(11):e202301188. Available from: https://doi.org/10.1002/cbdv.202301188
2. National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 5280343, Quercetin. Retrieved February 26, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Quercetin.
3. Pavani NPm, Srinivas P, Kothia NR, Chandu VC. Recent Advances in the Early diagnosis of Oral Cancer: A Systematic review. International Journal of Medical Reviews [Internet]. 2018 Nov 1;4(4):119–25. Available from: https://doi.org/10.29252/ijmr-040406
4. Shin D, Vigneswaran N, Gillenwater A, Richards-Kortum R. Advances in fluorescence imaging techniques to detect oral cancer and its precursors. Future Oncology [Internet]. 2010 Jul 13;6(7):1143–54. Available from: https://doi.org/10.2217/fon.10.79
5. World Cancer Research Fund. Available on: https://www.wcrf.org/preventing-cancer/cancer-

statistics/mouth-and-oral-cancer-statistics/. Accessed on: 12/12/2025.

6. Muthusamy M, Ramani P, Arumugam P, Rudrapathy P, Kangusamy B, Veeraraghavan VP, et al. Assessment of various etiological factors for oral squamous cell carcinoma in non-habit patients- a cross sectional case control study. BMC Oral Health [Internet]. 2025 Jan 13;25(1):62. Available from: https://doi.org/10.1186/s12903-024-05406-z
7. Sundar, S., Paneerseelvam, S., Ramani, P., Anandapadmanabhan, L. T., & Ramadoss, R. (2025). Decoding OSCC prognosis: Insights into age-linked patterns and survival dynamics using AJCC 8th edition and CAP protocols. Journal of stomatology, oral and maxillofacial surgery, 126(6S), 102527. https://doi.org/10.1016/j.jormas.2025.102527
8. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel Angiotensin-Converting Enzyme–Related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circulation Research [Internet]. 2000 Sep 1;87(5):E1-9. Available from: https://doi.org/10.1161/01.res.87.5.e1
9. Dai YJ, Hu F, Li H, Huang HY, Wang DW, Liang Y. A profiling analysis on the receptor ACE2 expression reveals the potential risk of different type of cancers vulnerable to SARS-CoV-2 infection. Annals of Translational Medicine [Internet]. 2020 Apr 1;8(7):481. Available from: https://doi.org/10.21037/atm.2020.03.61
10. Sivasakthivel S, Ramani P, Jayaraman S. Expression of angiotensin converting enzyme 2 in patients with oral squamous cell carcinoma. Oral Oncology Reports [Internet]. 2024 May 14;10:100476. Available from: https://doi.org/10.1016/j.oor.2024.100476
11. Sivasakthivel S, Ramani P. Angiotensin-converting Enzyme 2 Expression in Oral Squamous Cell Carcinoma: Correlation with p53 and Vascular Endothelial Growth Factor Using Reverse Transcriptase Polymerase Chain Reaction - An Evaluative Study. Annals of Maxillofacial Surgery [Internet]. 2025 Sep 23; Available from: https://doi.org/10.4103/ams.ams_26_25
12. Sivasakthivel S, Ramani P, Yadalam P. ACE2 EXPRESSION AS a PREDICTIVE BIOMARKER IN ORAL SQUAMOUS CELL CARCINOMA: a NON-LINEAR DIMENSIONALITY REDUCTION APPROACH. BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY [Internet]. 2025 Oct 4;423–33. Available from: https://doi.org/10.58240/1829006x-2025.21.7-423
13. Wang XS. Angiotensin-converting enzyme 2 connects COVID-19 with cancer and cancer immunotherapy. World Journal of Gastrointestinal Oncology [Internet]. 2021 Mar 9;13(3):157–60. Available from: https://doi.org/10.4251/wjgo.v13.i3.157
14. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. AngiotensinConverting enzyme 2: SARS-COV-2 receptor and regulator of the Renin-Angiotensin system. Circulation Research [Internet]. 2020 Apr 8;126(10):1456–74. Available from: https://doi.org/10.1161/circresaha.120.317015
15. Wang J. Natural compounds as anticancer agents: Experimental evidence. World Journal of Experimental Medicine [Internet]. 2012 Jan 1;2(3):45. Available from: https://doi.org/10.5493/wjem.v2.i3.45
16. Asgharian P, Tazekand AP, Hosseini K, Forouhandeh H, Ghasemnejad T, Ranjbar M, et al. Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets. Cancer Cell International [Internet]. 2022 Aug 15;22(1):257. Available from: https://doi.org/10.1186/s12935-022-02677-w
17. David AVA, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy Reviews/Bioinformatics Trends/Pharmacognosy Review [Internet]. 2016 Jan 1;10(20):84. Available from: https://doi.org/10.4103/0973-7847.194044
18. Lotfi N, Yousefi Z, Golabi M, Khalilian P, Ghezelbash B, Montazeri M, et al. The potential anti-cancer effects of quercetin on blood, prostate and lung cancers: An update. Frontiers in Immunology [Internet]. 2023 Feb 28;14:1077531. Available from: https://doi.org/10.3389/fimmu.2023.1077531
19. Wang J, Yang JH, Xiong D, Chen L. Activation of SIRT3/AMPK/mTOR-mediated autophagy promotes quercetin-induced ferroptosis in oral squamous cell carcinoma. Human & Experimental Toxicology [Internet]. 2025 Feb 26;44:9603271251323753. Available from: https://doi.org/10.1177/09603271251323753
20. Dai Y, Wang C, Xiao Y, Tan Y, Ye Y, Liu Y, et al. Jiawei Danxuan Koukang and its key component Quercetin intervened in OSF carcinogenesis by inhibiting the AR/eIF5A2 signaling pathway-mediated EMT. Archives of Oral Biology [Internet]. 2025 Feb 12;173:106194. Available

from: https://doi.org/10.1016/j.archoralbio.2025.106194

21. Li X, Guo S, Xiong XK, Peng BY, Huang JM, Chen MF, et al. Combination of quercetin and cisplatin enhances apoptosis in OSCC cells by downregulating xIAP through the NF-κB pathway. Journal of Cancer [Internet]. 2019 Jan 1;10(19):4509–21. Available from: https://doi.org/10.7150/jca.31045
22. Tan CH, Sivakumar H, Luo DG, Cen YX. Integrative Network Pharmacology and Molecular docking analyses on the mechanisms of San-Zhong-Kui-Jian-Tang in treating oral squamous cell carcinoma. Current Medical Science [Internet]. 2025 Jun 17;45(4):755–74. Available from: https://doi.org/10.1007/s11596-025-00067-7
23. Beyerstedt S, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. European Journal of Clinical Microbiology & Infectious Diseases [Internet]. 2021 Jan 3;40(5):905–19. Available from: https://doi.org/10.1007/s10096-020-04138-6
24. Koka V, Huang XR, Chung ACK, Wang W, Truong LD, Lan HY. Angiotensin II Up-Regulates Angiotensin I-Converting Enzyme (ACE), but Down-Regulates ACE2 via the AT1-ERK/p38 MAP Kinase Pathway. American Journal of Pathology [Internet]. 2008 Apr 11;172(5):1174–83. Available from: https://doi.org/10.2353/ajpath.2008.070762
25. Pratheeshkumar P, Budhraja A, Son YO, Wang X, Zhang Z, Ding S, et al. Quercetin inhibits angiogenesis mediated human prostate tumor growth by targeting VEGFR- 2 regulated AKT/MTOR/P70S6K signaling pathways. PLoS ONE [Internet]. 2012 Oct 18;7(10):e47516. Available from: https://doi.org/10.1371/journal.pone.0047516
26. Basu A, Sarkar A, Maulik U. Molecular docking study of potential phytochemicals and their effects on the complex of SARS-CoV2 spike protein and human ACE2. Scientific Reports [Internet]. 2020 Oct 19;10(1):17699. Available from: https://doi.org/10.1038/s41598-020-74715-4
27. Lestari K, Sitorus T, Instiaty I, Megantara S, Levita J. Molecular Docking of Quinine, Chloroquine and Hydroxychloroquine to Angiotensin Converting Enzyme 2 (ACE2) Receptor for Discovering New Potential COVID-19 Antidote. J Adv Pharm Educ Res. 2020;10(2):1-4.
28. Cheng S, Gao N, Zhang Z, Chen G, Budhraja A, Ke Z, et al. Quercetin Induces Tumor-Selective Apoptosis through Downregulation of Mcl-1 and Activation of Bax. Clinical Cancer Research [Internet]. 2010 Dec 1;16(23):5679–91. Available from: https://doi.org/10.1158/1078-0432.ccr-10-1565
29. Brüll V, Burak C, Stoffel-Wagner B, Wolffram S, Nickenig G, Müller C, et al. Effects of a quercetin-rich onion skin extract on 24 h ambulatory blood pressure and endothelial function in overweight-to-obese patients with (pre-)hypertension: a randomised double-blinded placebo-controlled cross-over trial. British Journal of Nutrition [Internet]. 2015 Sep 2;114(8):1263–77. Available from: https://doi.org/10.1017/s0007114515002950
30. Hasan MM, Khan Z, Chowdhury MS, Khan MA, Moni MA, Rahman MH. In silico molecular docking and ADME/T analysis of Quercetin compound with its evaluation of broad-spectrum therapeutic potential against particular diseases. Informatics in Medicine Unlocked [Internet]. 2022 Jan 1;29:100894. Available from: https://doi.org/10.1016/j.imu.2022.100894.