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MOLECULAR DOCKING AND ADMET-BASED SCREENING OF NATURAL PLANT HORMONES AS POTENTIAL MMP9 INHIBITORS IN CERVICAL CANCER

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Abstract

Cervical cancer is a significant health problem due to late diagnosis and poor treatment, resulting in an increasing death rate each year. This malignancy is caused by a variety of hereditary variables, and matrix metalloproteinase-9 (MMP9) is a crucial protein that contributes to extracellular matrix breakdown, tumor invasion, and metastasis, making it a possible target for therapeutic drugs. This research involved an in silico study to identify essential plant hormones as natural inhibitors against MMP9. This protein's FASTA sequence was obtained from the NCBI database, and GOR I, AlphaFold, and Procheck were used to perform additional structure prediction analysis. ExPASy ProtParam and TMHMM tools were employed to examine physicochemical and transmembrane properties of the protein. The strongest anti-cancer drugs were identified through molecular docking using PyRx and CB Dock2, which were downloaded from the PubChem database. Among these substances, luteolin, curcumin, quercetin, resveratrol, and epigallocatechin gallate, luteolin had the strongest hydrogen bond interactions with the target protein and the highest binding energy of -9.5 kcal/mol. Luteolin's pharmacokinetic characteristics were examined using further ADMET analysis, which showed that it is a promising therapeutic candidate. Finally, a safety assessment was conducted using Protox II for toxicity analysis. Consequently, luteolin has demonstrated an effective natural inhibitor of MMP9, warranting further experimental validation for its potential application in the management of cervical cancer.

Keywords: *Binding energies, Cancer, Metabolism, Molecular docking, Structure prediction, Toxicity*

INTRODUCTION

Significant advancements in the treatment of cervical cancer have been accomplished in accordance with the World Health Organization's aim to eradicate the disease. Because of its well-established etiology, cervical cancer, which is mostly caused by human papilloma virus (HPV) infection, is seen as preventable and treatable. Traditional screening techniques like HPV nucleic acid testing and cytology have been enhanced by developments in precision screening technologies including DNA methylation triage, HPV integration detection, liquid biopsies, and artificial intelligence-assisted diagnostics. Therapeutic approaches that target the elimination of HPV and the reversal of precancerous lesions have been developed as essential steps in the prevention of the illness (1, 2).

Therapeutic approaches that target the elimination of HPV and the reversal of precancerous lesions have been developed as essential steps in the prevention of the illness. Finding the best candidates for conservative, minimally invasive techniques without sacrificing oncological results is at the center of the debate surrounding surgery for early-stage cervical cancer. The development of systemic treatments for advanced cervical cancer has shown encouraging outcomes from recent clinical trials. Cervical cancer treatment has advanced significantly thanks to immunotherapies such immune checkpoint inhibitors (ICIs), antibody-drug conjugates (ADCs), and targeted therapy (3).

Cancer chemotherapy has been started in 1940 with the introduction of different types of medicines including nitrogen mustards and antifolate medicines. Major developments in cancer medications have

made it possible to treat a number of cancer types. Several traditional treatments, including stem cell therapy, surgery, chemotherapy, radiotherapy, and natural antioxidants, are still utilized for early detection and treatment of cervical cancer. These therapies may lessen the consequences of cancer, but they can have some negative side effects (4). The primary objective in this instance is to find and create effective solutions that could reduce the incidence of cancer without having any negative effects. Recent years have seen the emergence of computational research as a new and cutting-edge subject that is crucial in identifying the primary cause of cancer at an early stage and in developing low-cost, naturally occurring plant-based treatments with few side effects (5).

The purpose of this work is to investigate the structure and function of the MMP9 enzyme that contributes to cervical cancer and to use *in-silico* research to identify some anti-cancer chemicals from various plant sources. Molecular docking and ADMET analysis assisted in identifying the best and most promising medication candidates for the treatment of cervical cancer.

MATERIALS AND METHODS

DATA COLLECTION

The primary sequence of MMP9 enzyme in FASTA format was taken from National Center for Biotechnology Information database. NCBI https://www.ncbi.nlm.nih.gov/ is a primary database that contains information related to any gene, protein, nucleotide or genome of any organism (6).

STRUCTURE PREDICTIONS AND VALIDATION

The MMP9 protein's secondary structure was predicted using the GOR IV server https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_gor4.html that determines protein secondary structure elements such as α-helices and β-sheets based on position-specific scoring matrices (7). The three-dimensional structure of MMP9 was then predicted with AlphaFold https://alphafold.ebi.ac.uk/ , an innovative protein structure prediction system that uses deep learning techniques to build extremely accurate structural models. The predicted structure was subsequently checked using the PROCHECK tool https://saves.mbi.ucla.edu/ , which evaluates stereochemical quality using a Ramachandran plot and confirms the stability of the modeled protein conformation (8).

PROTEIN PHYSICOCHEMICAL CHARACTERIZATION

ExPASy ProtParam https://web.expasy.org/protparam/ is an online tool used to determine the physiochemical parameters of a target enzyme. It describes protein properties such as molecular weight based on the amount of amino acids in the protein's main sequence, isoelectric point, and number of positively and negatively charged residues, GRAVY, and aliphatic index (9).

TRANSMEMBRANE HELIX PREDICTION

The TMHMM server https://services.healthtech.dtu.dk/services/TMHMM-2.0/ is an online and freely accessible tool for analyzing a protein's transmembrane regions. It describes how many protein residues are present in transmembrane, outer, and intracellular areas (10).

RETRIEVAL OF ANTI-CANCER COMPOUNDS

Recent research has found that some natural compounds have substantial anti-cancer and anti-inflammatory properties, which play an important role in cancer treatment. Based on prior studies, five anti-cancer chemicals were downloaded from the PubChem database https://pubchem.ncbi.nlm.nih.gov/ including curcumin, quercetin, resveratrol, epigallocatechin gallate, and luteolin (11).

MOLECULAR DOCKING AND INTERACTION ANALYSIS

The CB-dock2 https://cadd.labshare.cn/cb-dock2/index.php online cavity-based detection tool was used to carry out molecular docking. To do this, the 3D structure of the target protein was uploaded first, followed by the 3D structure of the ligand after purification using Discovery Studio to remove water

molecules and other contaminants. It offers the best model with robust interactions with the target protein, which was then examined to verify amino acid residues and bond interactions (12).

TOXICITY ANALYSIS

Protox III https://tox.charite.de/protox3/ is used to determine the toxicity of specified chemicals. This program predicts the toxicity profile of compounds in several organs such as the liver, heart, brain, and lungs to ensure that the natural chemicals utilized as therapeutic treatment candidates are safe for human consumption and do not cause any side effects (13).

ADMET ANALYSIS

SwissADME https://www.swissadme.ch/ is an online tool for predicting the ADMET profile of a natural inhibitor to determine its adsorption, distribution, metabolism, excretion, and toxicity. The canonical smiles of compounds were obtained from PubChem and pasted into this program (14).

RESULTS

DATA COLLECTION

FASTA sequence of MMP9 protein was retrieved from NCBI database under the accession number of NP_004985.2.

STRUCTURE PREDICTION AND VALIDATION

The secondary structure of the target protein examined using the GOR IV tool is depicted in Fig. 1. According to this analysis, protein comprises 121 alpha helices, 154 beta strands, and 432 random coils in a high ratio. Fig. 2 illustrates the protein tertiary structure predicted by the AlphaFold program. Further validation was performed using Ramachandran plot analysis, which revealed that 94% of amino acids are in the most favored region, with 5.1% in the additional allow region. Only 0.5% were in generously allowed locations, while 0.2% were in disallowed region as shown in Fig. 3.

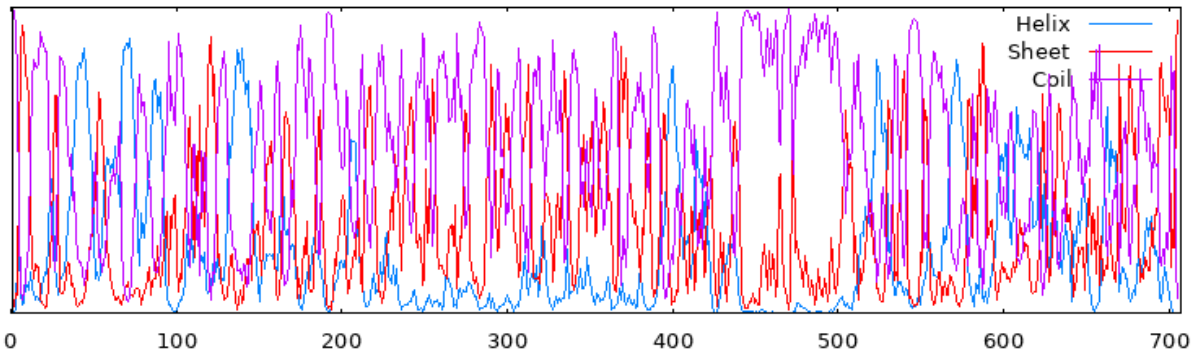


Fig. 1. Secondary structure of MMP9 protein

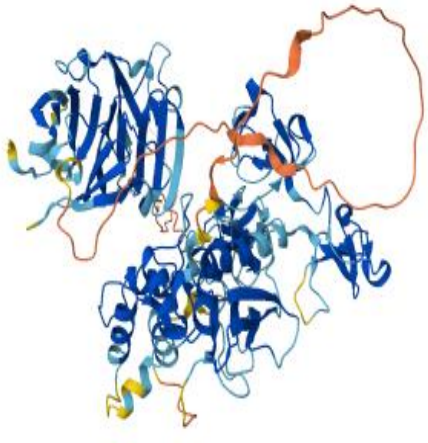


Fig. 2. Tertiary structure of MMP9

PROTEIN PHYSICOCHEMICAL CHARACTERIZATION

Physiochemical parameters of the MPP9 enzyme predicted by the ExPASy ProtParam program have been shown in Table I.

TRANSMEMBRANE HELIX PREDICTION

The TMHMM analysis results shown in Fig. 4 indicate that the MMP9 protein contains no significant transmembrane helices, as reflected by the very low probability peaks near the N-terminal region. Most of the sequence is predicted to be extracellular (outside), while a small portion lies on the cytoplasmic (inside) side. These findings suggest that MMP9 is a secreted or extracellular enzyme, consistent with its role in degrading components of the extracellular matrix.

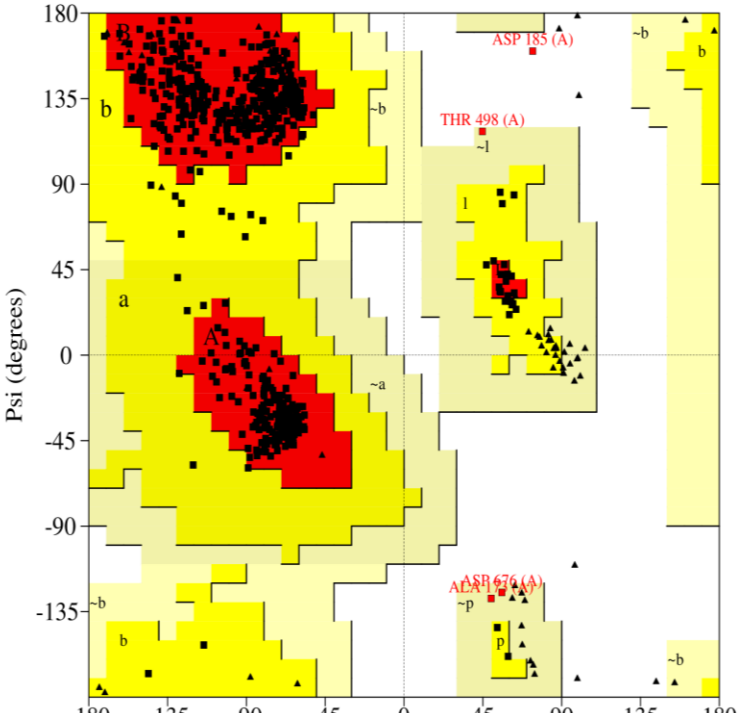


Table I. Physiochemical properties of MPP9 enzyme

Parameters	Values
Molecular Weight	78458.23g/mol
Theoretical pI	5.69
Instability index	41.10
Aliphatic index	65.13
Grand average of hydropathicity (GRAVY)	-0.394
Total number of negatively charged residues (Asp + Glu)	83
Total number of positively charged residues (Arg + Lys)	71

Fig. 3. Ramachandran plot analysis of MPP9 protein

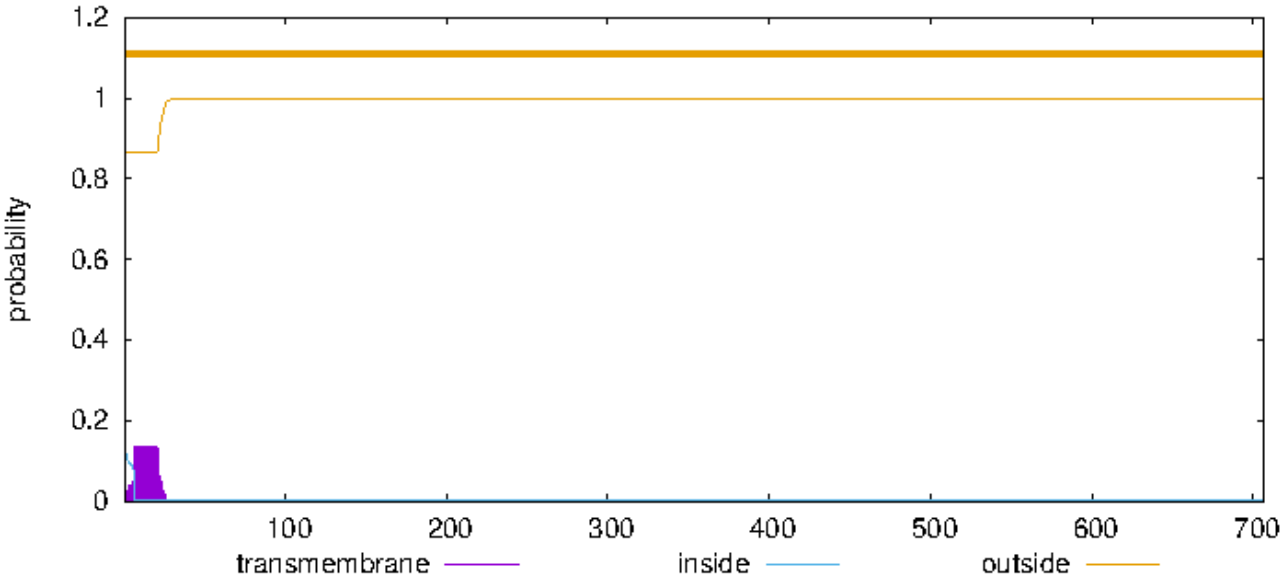


Fig. 4. Transmembrane helix prediction of MPP9 enzyme

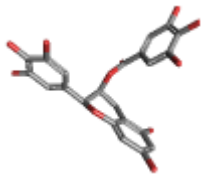
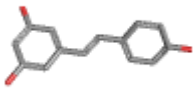
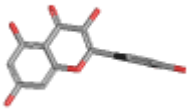
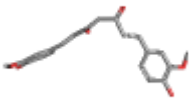
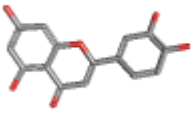
RETRIEVAL OF ANTI-CANCER COMPOUNDS

The phytochemicals with reported anticancer properties were retrieved from the PubChem database, and their molecular weight, compound ID, and 3D structures are depicted in Table II.

MOLECULAR DOCKING AND INTERACTION ANALYSIS

Molecular docking analysis revealed that all five anticancer phytochemicals exhibited strong binding affinities toward the target protein. Among them, luteolin demonstrated the highest binding energy of -9.5 kcal/mol, indicating the most stable interaction. Table III displaying the binding energies of all anti-cancer compounds. Fig. 5 depicting the 2D interaction diagram of MPP9 protein and all natural compounds. The amino acid residues of all substances that form strong hydrogen bonds and other interactions with the target protein (Table III).

Table II. Anti-cancer compounds retrieved from PubChem database

3D structure of Epigallocatechin gallate	**3D structure of** Resveratrol	**3D structure of** Quercetin	**3D structure of** Curcumin	**3D structure of** Luteolin
				
MW=458.4 g/mol CID = 65064	MW=228.24 g/mol CID = 445154	MW=302.23 g/mol CID = 5280343	MW= 368.4 g/mol CID = 969516	MW=286.24 g/mol CID = 5280445

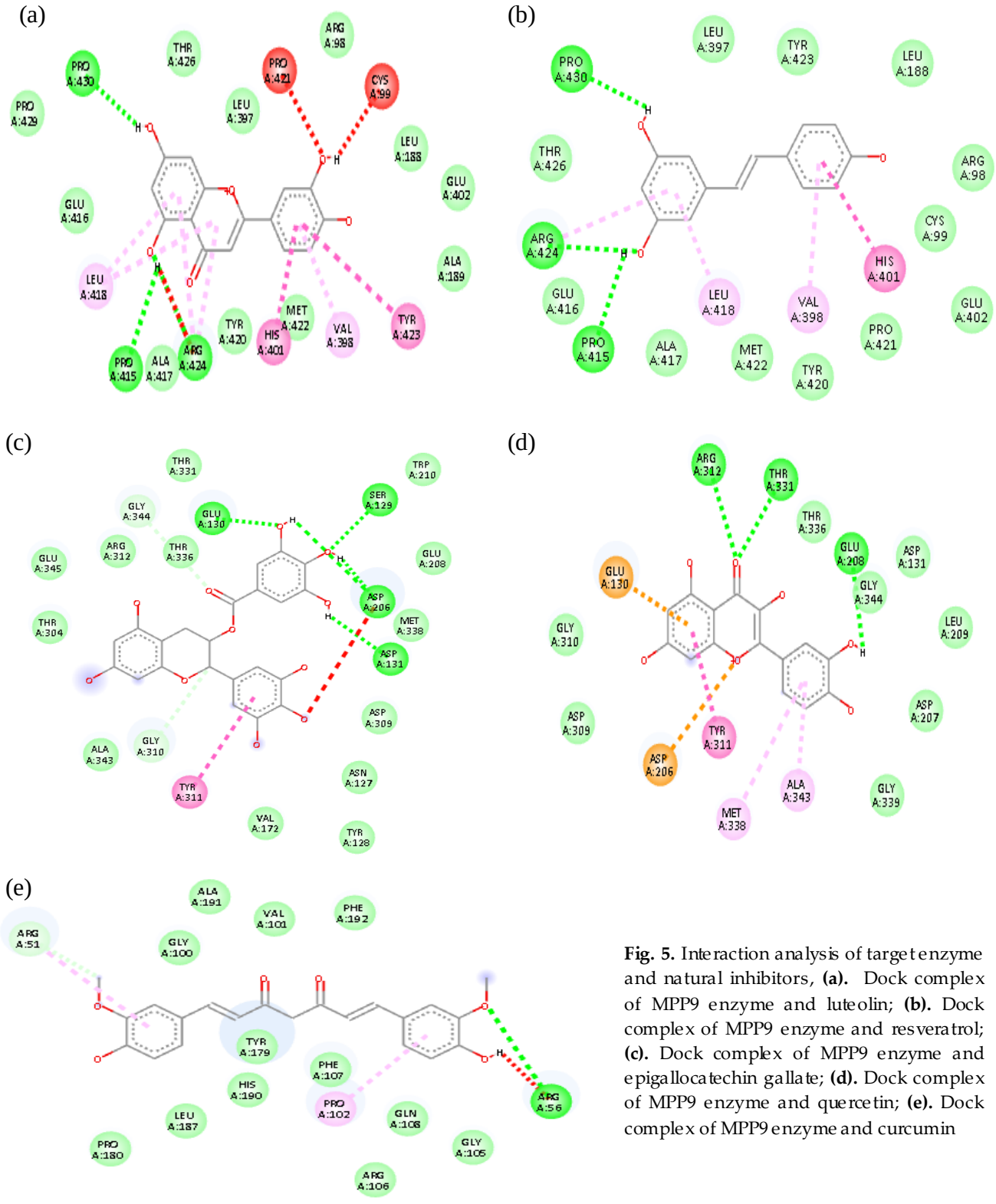


Fig. 5. Interaction analysis of target enzyme and natural inhibitors, **(a).** Dock complex of MPP9 enzyme and luteolin; **(b).** Dock complex of MPP9 enzyme and resveratrol; **(c).** Dock complex of MPP9 enzyme and epigallocatechin gallate; **(d).** Dock complex of MPP9 enzyme and quercetin; **(e).** Dock complex of MPP9 enzyme and curcumin

Table III. List of interacting residues of MPP9 protein with all ligands and their bond interaction

Compounds	Amino acid residues	Bond interaction	Docking score (kcal/mol) with target protein
Luteolin	ARG98 CYS99 LEU188 GLU402 ALA189 TYR423 VAL398 MET422 HIS401 TYR402 ARG424 ALA417 PRO415 LEU418 GLU416 PRO429 PRO430 THR426 LEU397 THR426	Van Dar Waals Conventional Hydrogen Bonds Pi- Pi Stacked Pi – Pi T Shape Pi – Alkyl Unfavorable Donor – Donor Unfavorable Acceptor - Acceptor	-9.5
Resveratrol	ARG98 CYS99 LEU188 GLU402 ALA189 TYR423 VAL398 MET422 HIS401 TYR402 ARG424 ALA417 PRO415 LEU418 GLU416 PRO429 PRO430 THR426 LEU397 THR426	Van Dar Waals Conventional Hydrogen Bonds Pi- Pi Stacked Pi – Alkyl	-9.2
Epigallocatechin gallate	TRP210 SER329 GLU208 ASP206 MET338 ASP131 ASP309 ASN127 TYR128 VAL172 TYR311 GLY310 ALA343 THR304 GLU345 ARG312 THR336 GLY344 GLU130 THR331	Van Dar Waals Conventional Hydrogen Bonds Carbon Hydrogen Bonds Pi – Pi T Shape Unfavorable Acceptor - Acceptor	-9.1
Quercetin	ARG312 THR331 THR336 GLU208 GLY344 ASP131 LEU209 ASP207 ASP339 ALA343 MET338 TYR311 ASP206 ASP309 GLY310 GLU130	Van Dar Waals Attractive Charge Conventional Hydrogen Bonds Pi - Anion Pi – Sigma Pi - Pi T Shape Pi – Alkyl	-8.4
Curcumin	ALA191 VAL101 PHE192 GLY100 ARG51 PRO180 LEU187 HIS190 TYR179 PHE107 PRO102 ARG106 GLN106 GLY106	Van Dar Waals Conventional Hydrogen Bonds Carbon Hydrogen Bonds Pi – Alkyl Unfavorable Donor – Donor	-8.3

TOXICITY AND ADMET ANALYSIS

Toxicity studies determined that luteolin is nontoxic to all organs and has an inactive toxicity profile, as shown in Table IV.

ADMET prediction involved various key factors such as gastrointestinal absorption, solubility in water, skin permeability, and bioavailability score as depicted in Table IV. Furthermore, luteolin passes the Lipinski's rule with zero violations, with a molecular weight of 286g/mol, 6 hydrogen bond acceptors, 4

hydrogen bond donors, a molar refractivity value of 76.01, and a Log P (o/w) value of 1.73. Additionally, the boiled egg model was used to measure the GI absorption and blood brain barrier permit. As seen in Fig. 6, luteolin is present in the white region, indicating that it can penetrate the gastrointestinal tract.

Table IV. Toxicity and ADMET analysis of luteolin drug candidate

Toxicity end points	Toxicity	ADMET parameters	Values
Neurotoxicity	Inactive	Molecular weight	286.24 g/mol
Respiratory toxicity	Inactive	GI absorption	High
Cardiotoxicity	Inactive	BBB permeant	No
Hepatoxicity	Inactive	TPSA	111.13 Å²
Nephrotoxicity	Inactive	CYP1A2 inhibitor	Yes
		CYP2D6 inhibitor	Yes
		Skin permeation	-6.25 cm/s

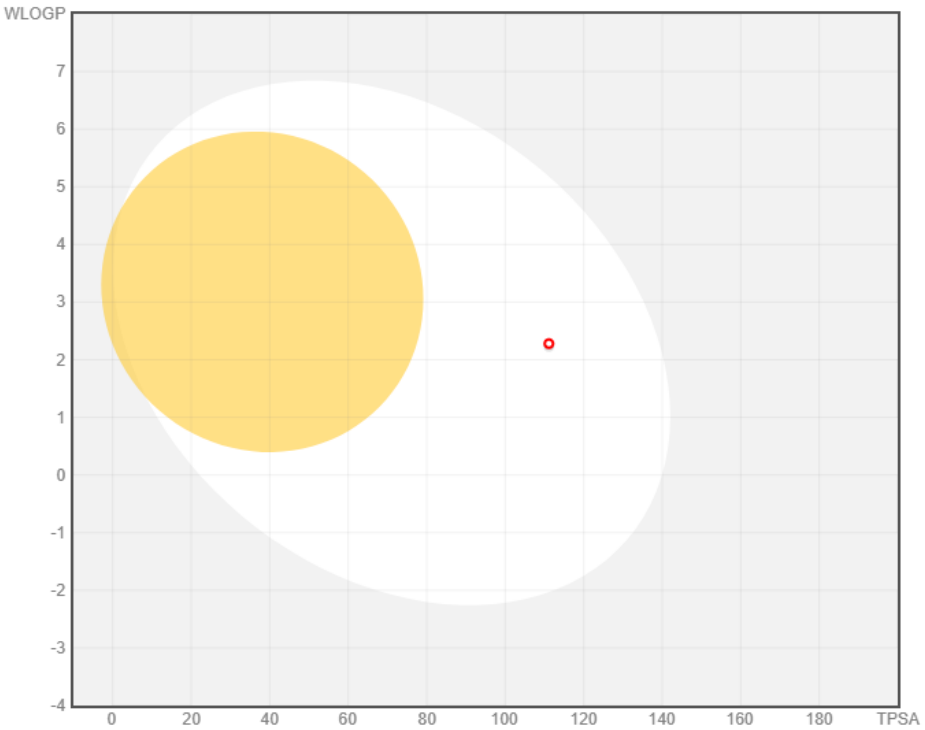


Fig. 6. Boiled model predicting the GI absorption of luteolin

DISCUSSION

Among cancers, cervical cancer is unique since it may be prevented and eradicated, primarily by vaccine and proactive screening. Nonetheless, many women in lower income regions still require cervical cancer screening. There are approximately 1175 genes that are notably down regulated and 698 that are significantly up regulated in cervical cancer samples compared to normal controls. MPP9 was identified as one of the up-regulated genes, which was further validated through computational analysis, leading to the deadly cause of cancer. Despite substantial clinical trials investigating various medicines, there is no effective cure for cervical cancer. For the development of advanced treatment therapies, a more thorough screening of molecular indicators in cervical cancer patients is required to find novel target compounds and characterize pathogenic mechanism (15).

Flavonoids, a class of plant hormones found in various types of plants and foods like fruits, vegetables, herbs and seeds, are well known for their possible beneficial effects on health. Their prominent antioxidant, anti-inflammatory, and anticancer effects have sparked considerable study attention. The numerous biologically active substances present in Melia azedarach are used in molecular docking and QSAR analysis to anticipate beneficial interactions with spinocerebellar ataxia type 3 proteins. Initial findings show strong binding energies, indicating the viability of using natural chemicals for faster medication development against spinocerebellar ataxia type 3. In this work, flavonoids with anti-cancer properties were found to be safe when consumed in diet and were identified as a possible therapeutic candidate against cervical cancer through in silico investigations (16).

Molecular docking analysis showed that luteolin demonstrated a robust binding energy of -9 kcal/mol among all other natural compounds via interacting with target pathogenic protein MPP9. While previous medications have proven not to be effective, notably targeting MPP9, this study found a viable candidate for cervical cancer. According to ADMET study, luteolin has drug-like characteristics such as appropriate molecular weight, moderate lipophilicity, high gastrointestinal absorption, along with complete adhering to Lipinski's rule of five. Overall, these data suggest luteolin as a possible lead molecule for the future. Further in vitro and in vivo experiment validations are required.

CONCLUSION

The findings of this study indicate that MPP9 contributes to the development of cervical cancer. Cervical cancer is becoming more common, and there is presently no effective treatment for this kind of disease. Luteolin anti-cancer agents were one possible treatment option against MPP9. The molecular docking and interaction data showed that it has a substantial strong binding affinity when docked to the active site of MPP9 protein. The results show that the pharmaceutical candidate is non-toxic, pharmacophore, and does not violate Lipinski's criterion.

Limitations of the study:

This study has several limitations. Being a cross-sectional study, it cannot establish causal relationships between dietary patterns and metabolic outcomes. Additionally, the dietary behavior measures used in the analysis were self-reported, which may be influenced by recall bias or social desirability bias. The dataset lacked detailed information on physical activity and familial metabolic history, which are important determinants of MetS. Furthermore, the study was conducted in a specific area with only 280 participants, limiting the generalizability of findings.

Recommendations:

Future research should employ longitudinal or intervention study designs to examine whether adolescents with different dietary patterns respond differently to interventions. Including additional variables such as levels of physical activity and parental metabolic syndrome could provide a more comprehensive understanding of MetS risk. Furthermore, considering psychological and environmental factors may offer important insights into how and why adolescents' food habits change over time. School-based health promotion, public awareness campaigns, and policy measures such as warning labels on processed foods can collectively help reduce the burden of early-onset cardiometabolic illnesses.

Conflict of interest:

Authors declared no conflict of interest.

Authors' contribution:

SZ Conceptualization, project supervision, manuscript drafting, and final approval; AA Data curation, formal analysis, and visualization; HT Methodology development and software validation; LA Investigation and resources; NeH Writing and original draft preparation; KA Review & editing; AA Software and docking simulations; SJ ADMET analysis and interpretation; Aa Literature search and data collection; KF Visualization and figure preparation; AM Methodology and formal analysis; MY Data validation and manuscript proofreading; FJ Project administration and correspondence.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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