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Original Article

ELLAGIC ACID IN POMEGRANATE SEEDS AS A POTENTIAL THERAPEUTIC FOR ORAL CANCER VIA THE PI3K/AKT PATHWAY: AN *IN SILICO*

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ABSTRACT

Objective: This study aims to evaluate the anticancer potential of ellagic acid, derived from pomegranate seeds, against oral cancer. Molecular docking was selected to predict the interaction between ellagic acid and the PI3K/AKT pathway, a critical signaling cascade in oral carcinogenesis.

Methods: An in silico approach was employed using MOE 2022 software to perform molecular docking simulations. Ellagic acid was docked against key proteins in the PI3K/AKT pathway to assess binding affinities and interaction dynamics. Additionally, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties were predicted to evaluate the compound's drug-likeness and safety profile.

Results: The molecular docking analysis revealed that ellagic acid exhibits a binding affinity of-6.1004 kcal/mol to the PI3K protein, with an RMSD refinement value of 1.1516, indicating a stable interaction. ADMET predictions suggest favorable pharmacokinetic properties, including high human intestinal absorption and non-inhibitory effects on cytochrome P450 enzymes, implying low potential for drug-drug interactions. Toxicity assessments indicated no significant risks, supporting the compound's safety profile.

Conclusion: The in silico findings suggest that ellagic acid from pomegranate seeds may serve as a promising anticancer agent against oral cancer by effectively targeting the PI3K/AKT pathway. These results contribute to the existing literature by providing computational evidence of ellagic acid's mechanism of action and support further *in vitro* and *in vivo* studies to validate its therapeutic potential.

Keywords: Anticancer agent, Ellagic acid, Oral cancer, Pomegranate seed, Punecalagin

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INTRODUCTION

Oral cancer remains a significant global health concern, with high mortality rates and an estimated 377,000 new cases and nearly 177,000 deaths reported in 2020 [1–3]. The five-year survival rate is approximately 40%, underscoring the critical need for early diagnosis and effective prevention strategies to improve patient outcomes and increase life expectancy [2, 4, 5]. This impact is particularly pronounced in regions with limited healthcare access, such as rural areas, where early detection and specialised care are often unavailable. A major concern with oral cancer is its poor prognosis, especially when diagnosed at a late stage, which is often the case. Notably, 90–95% of these cases are Oral Squamous Cell Carcinoma (OSCC), further emphasising the need for preventive strategies targeting modifiable risk factors to curb the rising incidence [6]. The global burden of oral cancer continues to grow, with stable age-standardized mortality rates indicating a need for focused public health interventions. By addressing risk factors and implementing targeted prevention strategies, healthcare systems worldwide can work towards reducing the incidence and improving the prognosis for those affected by this life-threatening disease [7]. Invasive treatments for oral cancer, including surgery, chemotherapy, and radiotherapy, are standard but have notable limitations. Surgery can lead to significant functional and aesthetic impairments, affecting speech and swallowing [8]. Chemotherapy often causes side effects such as mucositis, infections, and salivary gland dysfunction, leading to secondary issues like dehydration and nutritional deficiencies [9]. Radiotherapy can damage healthy tissues, resulting in complications like mucositis, infections, and changes in saliva production [10]. These challenges highlight the need for alternative therapies that effectively manage cancer while minimizing adverse effects. Consequently, there is a growing interest in alternative therapies, particularly those involving herbal compounds, which have shown promise in clinical settings. For instance, studies have indicated that natural flavonoids can induce apoptosis in oral cancer cells, suggesting their potential as adjunctive treatments [11].

Indonesia, as a megadiverse nation with a vast agricultural economy, covers over 10.45 million hectares of cropland annually. Among its main agricultural products are biopharmaceutical plants, such as the pomegranate (*Punica granatum L.*), which grows throughout the region [12]. The fruit is well-known for its high phytonutrient content, supporting its medicinal qualities and making it a popular option for health-conscious consumers [13]. However, consumer favourability often leads to the disposal of pomegranate seeds as major organic waste, representing one-fifth of the whole fruit. Pomegranate seeds present a significant amount of punicalagins, which, after metabolic processes, transform into ellagic acid. Ellagic acid has demonstrated anticancer activity through the inhibition of molecular pathways such as PI3K/Akt and MAPK, which are crucial in triggering oral cancer progression. [14] Moreover, ellagic acid exhibits antioxidant and anti-inflammatory benefits through mechanisms such as COX-1 inhibition [6, 15]. Ellagic acid, derived from pomegranate seeds, is imperative to evaluate and promote this herbal agent extensively and commercially to raise societal awareness of oral health and explore its role as a complementary approach in oral cancer prevention and treatment.

Recent studies have highlighted the significance of the PI3K/Akt and MAPK signalling pathways in the development and progression of oral cancer [12, 14]. The PI3K/Akt pathway is frequently mutated in head and neck squamous cell carcinomas, indicating its pivotal role in tumorigenesis [14, 15]. Targeting this pathway has become a focal point in developing therapeutic strategies for oral cancer.

Pomegranate-derived compounds, particularly ellagic acid, have shown promise in modulating these critical pathways [12, 15]. Research indicates that ellagic acid can inhibit the PI3K/Akt pathway, thereby reducing cancer cell proliferation and inducing apoptosis. Additionally, ellagic acid has been observed to affect the MAPK pathway, further contributing to its anticancer effects [12, 14, 15]. These findings suggest that ellagic acid may serve as a valuable adjunct in oral cancer therapy by targeting key molecular mechanisms involved in disease progression. Furthermore, the

antioxidant and anti-inflammatory properties of ellagic acid enhance its therapeutic potential [6, 15–17]. By scavenging free radicals and inhibiting inflammatory mediators, ellagic acid helps mitigate oxidative stress and inflammation, both of which are implicated in cancer development. This multifaceted approach not only targets cancer cells directly but also modifies the tumor microenvironment, making it less conducive to cancer progression.

In light of these promising findings, further research is warranted to fully elucidate the mechanisms by which ellagic acid exerts its effects on oral cancer cells. Clinical studies are essential to determine the efficacy and safety of ellagic acid as a complementary therapy in oral cancer treatment. By advancing our understanding and application of pomegranate-derived compounds, we can develop more effective strategies to combat oral cancer and improve patient outcomes.

MATERIALS AND METHODS

Materials

Ellagic acid compounds

This study favors the active compound of PS, which was obtained from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/), then prepared as a 2–dimensional figure. The active compound used focuses on ellagic acid (CID 5281855) as the prime component in the PS. The researchers used the 5itd receptor (P27986) as the main regulatory subunit of oral cancer PI3K pathway. This study aims to evaluate the interaction of the compound to the receptors through 3–dimensional (3D) conformation retrieved from RCSB Protein Data Bank web page (https://www.rcsb.org/).

Research tools

This research deploys a computational approach comprising Chem3D. exe and BIOVIA_DS2024 for preparation of test materials and visualisation of docking results under the neglection of water molecules, page Lipinski Rule of Five (http://www.scfbio-iitd.res.in/) for the physicochemical tests, ADMETlab 3.0 (https://admetlab3.scbdd.com) for predicting ADME and toxicity, as well as MOE 2022 to identify active research target sites and obtain molecular docking results.

Methods

Preparation of ligand molecular structure and protein structure

The research commenced with the acquisition of test compounds and target proteins from PubChem and the RCSB Protein Data Bank (PDB), respectively, each compound being appropriately labelled according to its chemical name. Subsequently, docking visualisations of the protein P27986 were performed using Chem3D and BIOVIA Discovery Studio 2024, with water molecules omitted to focus on key interactions. Active site identification and peptide chain sequence optimisation were conducted utilising the Molecular Operating Environment (MOE) 2022 to enhance docking accuracy. MOE 2022 was selected for its comprehensive suite of computational chemistry tools, facilitating accurate modelling and visualisation of molecular interactions. Its robust algorithms and user-friendly interface render it a preferred choice for molecular docking studies. Prior to utilisation, validation studies were performed to ensure the reliability of MOE 2022 in predicting binding affinities and interaction modes [18].

Physicochemical test

To evaluate the drug-likeness of the Pomegranate Seed (PS) test compounds, physicochemical properties were assessed by uploading their two-dimensional conformations to the Lipinski Rule of Five platform. This analysis considered parameters such as molar refractivity, hydrogen bond donors and acceptors, log P values, and molecular mass. A compound was classified as drug-like if it met at least two of the following criteria: molar refractivity between 40 and 130, fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, and a molecular mass under 500 daltons [15]. For pharmacokinetic predictions, ADMET lab 3.0 was employed to analyse parameters encompassing absorption, distribution, metabolism, and excretion. ADME Tlab 3.0 is an updated comprehensive online platform that provides an efficient evaluation of ADMET-related parameters, addressing limitations of previous versions and offering broader coverage and improved performance. Specific metrics included intestinal absorption rates, human fraction unbound percentages, CYP2D6 substrate and inhibitor status, and total clearance rates. Optimal criteria were defined as follows: a compound was considered suitable for systemic circulation if it exhibited an intestinal absorption rate above zero, a human fraction unbound value greater than zero percent, negative CYP2D6 substrate and inhibitor interactions indicating minimal drug-drug interaction potential, and a total clearance rate exceeding zero per minute [20,21]. Toxicity assessments encompassed evaluations for AMES toxicity, human maximum tolerated dose, hepatotoxicity, skin sensitisation, and lowest Observed Adverse Effect Level (LOAEL) chronic toxicity. Compounds were deemed non-toxic if they tested negative for skin sensitisation, hepatotoxicity, and AMES toxicity. Quantitative thresholds for human maximum tolerated dose and LOAEL chronic toxicity were utilised to inform safe dosage calculations for subsequent *in vitro* and *in vivo* studies [22–24].

Molecular docking test

Molecular docking studies were conducted by uploading the target protein, comparator compound, and test compound into MOE 2022. The software was configured to determine binding modes, Root mean Square Deviation (RMSD) values, and binding affinities in kcal/mol. A lower binding affinity indicated a greater propensity for the compound to interact with the target protein, as less energy was required for bond formation. The mode parameter reflected the variability of the bonds formed, while the RMSD values provided insights into the precision and accuracy of the docking predictions [24]. Visualisation of docking results was performed using MOE 2022 and BIOVIA Discovery Studio 2024 to analyse the number, type, and positions of bonds formed between the target protein and test compounds. This process involved uploading the docked conformations into the target protein framework to confirm interactions within the active site. Such visualisations were crucial for understanding the molecular interactions and guiding further optimisation of the compounds [25, 26]. The selection of the P27986 receptor, corresponding to the regulatory subunit alpha of Phosphoinositide-3-kinase (PIK3R1), was based on its biological relevance to oral cancer. Mutations and deregulation in the PI3K–PTEN–mTOR signalling pathway, in which PIK3R1 plays a critical role, are among the most frequently observed alterations in various cancers, including head and neck cancers. This makes PIK3R1 a pertinent target for investigating potential therapeutic agents against oral cancer [27].

RESULTS

Physicochemical test results

Ellagic acid (CID 5281855) exhibits physicochemical properties that align with Lipinski's Rule of Five, a set of criteria predictive of a compound's potential as an orally active drug in humans. Specifically, ellagic acid has a molecular mass of 302.19 daltons, which is well below the 500-dalton threshold, facilitating efficient absorption and distribution within the body. It possesses four hydrogen bond donors and seven hydrogen bond acceptors, both within the acceptable limits of fewer than five donors and ten acceptors, respectively. These characteristics suggest a favorable balance between solubility and permeability, essential for effective bioavailability. The compound's log P value, a measure of lipophilicity, is calculated to be approximately 1.7, comfortably below the maximum recommended value of 5. This indicates that ellagic acid maintains an appropriate balance between hydrophilicity and lipophilicity, promoting adequate membrane permeability without compromising solubility. Additionally, its molar refractivity falls within the range of 40 to 130, further supporting its drug-like potential. Collectively, these properties underscore ellagic acid's compliance with Lipinski's parameters, suggesting its promise as a candidate for oral drug development.

ADMET prediction results

ADME results

Table 1: Physicochemical test result of PS ellagic acid using Lipinski rule of five (RO5)

Compound	Molecular mass	Hydrogen bond donor	Hydrogen bond acceptor	Log P	Molar refractivity	Drug likeness
Standard	*≤ 500 Da*	*≤ 5*	*≤ 10*	*≤ 5*	*40–130*	+
Ellagic Acid	302.194 Da	4	8	1.3128	77.146	+

Table 2: ADME prediction test results of PS ellagic acid

Compound	Internal absorption (%)	Human fraction unbound (Fu)	CYP2D6 substrate and inhibitor	Total clearance (log ml/min/kg)
Ellagic Acid	88.834	0.083	Negative	0.637

Ellagic acid exhibits a pharmacokinetic profile that underscores both its therapeutic potential and the challenges associated with its clinical application. An intestinal absorption rate of 88.834% indicates efficient uptake through the gastrointestinal tract, facilitating its entry into systemic circulation. However, the compound's low fraction unbound in plasma (0.083) signifies that only 8.3% remains free to interact with biological targets, as the majority is bound to plasma proteins. This high protein binding could limit the bioactive fraction available for therapeutic action. The substantial protein binding of ellagic acid is consistent with existing literature, which reports that approximately 50–60% of the compound is bound to serum proteins following oral administration, with a half-life of about 8.4±1.8 h. This extensive binding may impede the free drug's availability to exert its therapeutic effects [28]. Moreover, ellagic acid is rapidly metabolised and eliminated from the body. Studies have detected maximum plasma concentrations approximately one-hour post-ingestion, with the compound being undetectable after four hours [29]. This swift elimination necessitates frequent dosing to maintain therapeutic levels, which could pose challenges in clinical settings. The limited bioavailability of ellagic acid is further compounded by its poor solubility and permeability, which restrict its absorption and systemic availability. These pharmacokinetic limitations have prompted the exploration of various formulation strategies to enhance its bioavailability. Approaches such as solid dispersions, micro and nanoparticles, inclusion complexes, self-emulsifying systems, and polymorphs have been investigated to improve solubility, stability, and absorption. [30] In summary, while ellagic acid demonstrates promising pharmacokinetic properties, including efficient intestinal absorption, its high plasma protein binding, rapid metabolism, and elimination, coupled with poor solubility and permeability, present significant challenges. Addressing these issues through advanced formulation strategies is essential to fully realise the therapeutic potential of ellagic acid as an orally administered agent.

Notably, ellagic acid is neither a substrate nor an inhibitor of the cytochrome P450 2D6 (CYP2D6) enzyme, which reduces the likelihood of drug-drug interactions involving this pathway. This characteristic is particularly advantageous in clinical settings where patients may be on multiple medications. The compound's total clearance rate is 0.637 (log ml/min/kg), indicating a moderate elimination speed from the body. This rate suggests that ellagic acid is neither rapidly cleared, which could necessitate frequent dosing, nor slowly eliminated, which might raise concerns about accumulation and potential toxicity. In summary, ellagic acid's high intestinal absorption and minimal interaction with CYP2D6 are promising for oral administration. However, its substantial plasma protein binding warrants consideration, as it may impact the effective concentration of the drug available for therapeutic action. These pharmacokinetic insights are crucial for informing dosing strategies and anticipating the compound's behaviour in clinical applications.

Table 3: Toxicity prediction test result of PS ellagic acid

Compound	AMES toxicity	Hepatotoxicity	Human maximum tolerated dose (log mg/kg/day)	Skin sensitisation	Loael chronic toxicity (log mg/kg_bw/d)
Ellagic Acid	Negative	Negative	0.478	Negative	2.893

Toxicity result

The toxicity assessment of ellagic acid (CID 5281855) provides crucial insights into its systemic safety profile, particularly in its potential application as an oral therapeutic agent. The negative result for AMES toxicity suggests that ellagic acid does not exhibit mutagenic properties, indicating a low risk of genetic mutations or DNA damage upon exposure. This finding is essential in assessing the compound's carcinogenic potential, as mutagenicity is often linked to cancer development. Additionally, the absence of hepatotoxicity suggests that ellagic acid does not induce liver damage, a critical consideration for long-term administration. Many pharmacological agents exhibit hepatotoxic effects due to metabolic activation in the liver, leading to oxidative stress and cellular damage; however, the negative hepatotoxicity result implies that ellagic acid does not interfere with hepatic enzymatic functions or cause significant liver toxicity. The Human Maximum Tolerated Dose (HMTD) is recorded at 0.478 log mg/kg/day, reflecting a moderate safety margin for systemic exposure. This parameter is crucial in determining the upper limit of a compound's dosage before adverse effects manifest in human physiology. Although this value suggests an acceptable threshold, further pharmacokinetic studies are required to establish an optimal therapeutic dose that balances efficacy and safety. The negative skin sensitisation result indicates that ellagic acid does not induce allergic or hypersensitivity reactions upon dermal exposure. This is particularly relevant in pharmaceutical formulation, as compounds with skin sensitisation potential can pose risks in topical applications or during systemic absorption. The absence of skin irritation further supports its suitability for oral administration without significant risk of cutaneous adverse effects. Lastly, the Lowest Observed Adverse Effect Level (LOAEL) chronic toxicity value of 2.893 log mg/kg_bw/day provides insights into long-term exposure risks. This parameter reflects the lowest dose at which adverse effects are observed in chronic toxicity studies. A higher LOAEL value generally indicates a favourable safety profile, suggesting that ellagic acid does not exhibit significant toxic effects at standard therapeutic doses. However, additional *in vivo* evaluations are necessary to corroborate these findings and refine dosage recommendations for clinical applications.

Table 4: Docking result of PS ellagic acid binding to 5itd receptor (P27986) through the PI3k pathway.

Attempts	Binding affinity	RMSD refinement	Mode
1	-6.1004	1.1516	0
2	-6.0568	2.4477	0
3	-5.8439	1.1561	0
4	-5.8622	2.2106	0
5	-5.5509	2.9100	0
Mean	-5.8468	1.9752	0

The molecular docking results, as tabulated, provide crucial insights into the binding interactions between the ligand and its target protein. The binding affinity values, measured in kcal/mol, represent the thermodynamic stability of the ligand-protein complex, with more negative values indicating stronger binding interactions. Across five repeated docking attempts, the binding affinity demonstrates a gradual decreasing trend, starting from-6.1004 kcal/mol in the first attempt and reducing to-5.5509 kcal/mol in the fifth. This decline suggests that while the ligand consistently maintains a relatively strong affinity for the target, there is variability in binding strength across different docking poses.

To further assess the reliability of these results, statistical analysis was applied to the dataset. The Standard Deviation (SD) of the binding affinity values provides an estimate of the variability between docking attempts. Given the mean binding affinity of-5.8468 kcal/mol, the standard deviation can be calculated to determine the dispersion of values around this mean. A low standard deviation would indicate that the binding affinities are relatively stable across multiple docking runs, while a higher standard deviation suggests greater variability in ligand-protein interactions. Similarly, the Root mean Square Deviation (RMSD) refinement values offer additional insights into the stability of the ligand's binding conformation. The lower the RMSD value, the more stable the ligand's conformation at the binding site. The first and third docking attempts exhibit relatively low RMSD values (1.1516 Å and 1.1561 Å, respectively), suggesting that the ligand maintains a stable conformation in these poses. However, the second, fourth, and fifth docking attempts show increasing RMSD values, with the highest reaching 2.9100 Å. The mean RMSD value of 1.9752 Å, along with its standard deviation, indicates the extent of fluctuation in ligand positioning. A high standard deviation in RMSD values would suggest significant conformational variation across different docking poses, potentially reflecting dynamic binding behaviour. The mode parameter remains consistently at zero across all docking attempts, indicating that the ligand binds in a single binding mode without significant variations. This consistency suggests that despite minor fluctuations in binding affinity and RMSD, the ligand exhibits a preferred binding conformation, reinforcing the reliability of the docking predictions.

By incorporating statistical analyses, such as standard deviation, these docking results gain enhanced interpretability. The standard deviation of binding affinity and RMSD values can provide deeper insights into the robustness of the docking study. A low standard deviation would indicate reproducibility and reliability, while a higher standard deviation may highlight potential binding flexibility or instability. Overall, while the ligand demonstrates a consistent and favourable binding affinity with the target protein, the observed RMSD fluctuations suggest that the stability of the ligand-protein interaction is not uniform across all docking attempts. Further molecular dynamics simulations and additional statistical evaluations would be beneficial to confirm the most stable and biologically relevant binding conformation.

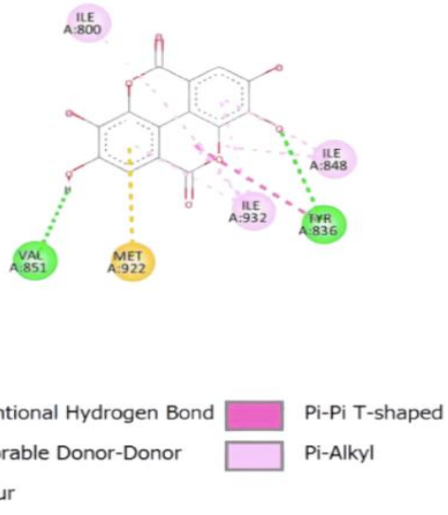

Fig. 1: Visualisation of PS ellagic acid binding to 5itd receptor. Different colors and lines indicate the formation of the type of bond between the test compound and the enzyme peptide. Dark green indicates the formation of a van der Waals bond, pink indicates the formation of pi--alkyl bonds, red indicates the formation of an unfavorable bump bond, yellow indicates the formation of pi–sulphur bonds, magenta indicates the formation of a pi–pi bond

Visualisation test results

The molecular interaction diagram illustrates the binding profile of the ligand within the active site of the target protein, highlighting the key interactions that stabilise the ligand-protein complex. The presence of conventional hydrogen bonds (depicted in green) with residues such as VAL851 and TYR836 suggests strong and specific interactions that contribute to the ligand's affinity. Hydrogen bonds play a crucial role in ligand binding by enhancing specificity and stabilisation within the active site.

Furthermore, pi-sulphur interactions (yellow) are observed with MET922, indicating a stabilising interaction between the aromatic system of the ligand and the sulfur-containing side chain of methionine. This interaction can enhance binding affinity, particularly in environments where sulphur-mediated stabilisation is favoured. The presence of pi-pi T-shaped interactions (pink) and pi-alkyl interactions (light purple) with ILE800, ILE848, and ILE932 suggests that the ligand engages in hydrophobic and stacking interactions with non-polar residues. Pi-pi stacking is particularly relevant in stabilising aromatic ligands within a hydrophobic pocket, which can influence the overall binding energy. Notably, no unfavourable donor-donor interactions (red) are observed, suggesting that the ligand does not experience significant steric or electronic repulsion within the binding site.

Overall, the interaction profile suggests that the ligand exhibits a well-balanced combination of hydrogen bonding, hydrophobic interactions, and aromatic stacking, which collectively contribute to its binding stability. These insights are essential for understanding the ligand's structure-activity relationship and can guide further optimisation in drug design to enhance affinity and selectivity for the target protein.

DISCUSSION

Cancer is a complex and multifaceted disease characterised by the uncontrolled and abnormal proliferation of cells [22]. Its risk factors

encompass genetic predisposition, exposure to carcinogens, lifestyle behaviours, and specific infectious diseases. Cancer initiation often results from the transformation of proto-oncogenes into oncogenes [31]. Oncogenes, which arise from mutated proto-oncogenes, encode overactive proteins that drive oncogenic processes. Oral cancer remains a significant public health concern in Southeast Asia, particularly in Indonesia, where behavioural risk factors such as betel chewing, tobacco use, and alcohol consumption contribute to its prevalence [32–34].

The PI3K pathway plays a pivotal role in oral cancer progression by regulating key cellular processes, including survival, proliferation, invasion, and migration. Notably, PI3K/AKT/mTOR signalling is a central driver of oncogenesis in OSCC, facilitating tumourigenesis and metastatic dissemination. A comprehensive understanding of this pathway provides insights into potential therapeutic targets and diagnostic markers. ATP stimulation has been shown to activate the PI3K/AKT pathway via the P2Y2-Src-EGFR axis, thereby enhancing malignancy by promoting cellular invasion and migration [26, 35–37]. Furthermore, the pathway plays a crucial role in apoptosis regulation, as evidenced by the action of compounds such as 11-epi-sinulariolide acetate, which inhibit PI3K/AKT activation, enabling FOXO proteins to promote apoptosis in oral cancer cells [27, 35]. Although invasive therapies do not directly target this pathway, their effects are closely intertwined with MAPK signalling and the cascading molecular events associated with oral cancer progression and malignancy [35].

Ellagic acid, a naturally occurring polyphenolic compound found in high concentrations in pomegranate species (PS), has emerged as a promising anticancer agent [17, 38]. Structurally, ellagic acid is a dilactone derivative of Hexahydroxydiphenic Acid (HHDP), a dimeric gallic acid derivative produced through ellagitannin hydrolysis. The compound exerts its anticancer effects through multiple mechanisms, including apoptosis induction, inhibition of proliferation, suppression of angiogenesis, and prevention of migration and metastasis, primarily through modulation of the PI3K pathway [38, 39]. Peng *et al.* (2020) demonstrated that pomegranate extract inhibits MMP-2/-9 activation, as well as cancer cell migration and invasion, by modulating AKT signalling in oral cancer cells [40]. The present study corroborates these findings through molecular docking and visualisation analyses, which demonstrate the stability and consistency of ellagic acid-binding. The docking attempts yielded high binding affinities ranging from-5.5509 to-6.1004 kcal/mol, with an associated error value (standard deviation) of 1.4037, which is well within the acceptable range (≤ 2 kcal/mol), thereby confirming the reliability of the results.

The mechanistic basis for ellagic acid's anticancer activity is further supported by its ability to modulate key oncogenic pathways, particularly through the inhibition of PI3K/AKT signalling. The compound effectively suppresses PI3K and Akt phosphorylation, leading to decreased proliferation and increased apoptosis in cancer cells. Moreover, ellagic acid downregulates critical oncogenic regulators, including PDK-1, mTOR, and HIF-1α, which are essential for tumour growth and angiogenesis [39–41]. Computational docking analyses reveal the compound's robust binding interactions, despite the presence of a single unfavourable bond. The docking studies provide strong evidence for ellagic acid's capacity to significantly disrupt PI3K pathway signalling, as indicated by consistent binding affinities and a tolerated RMSD value of 1.9752.

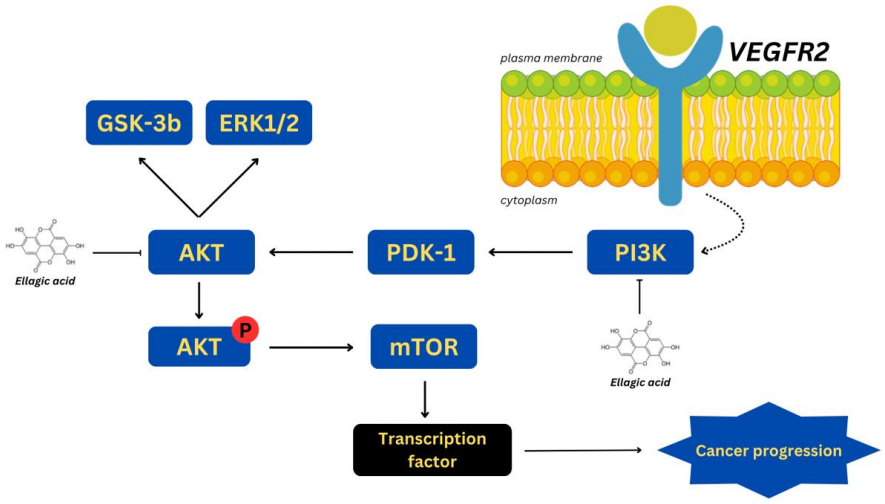

Fig. 2: Modulation of PI3K/Akt pathway by ellagic acid

Beyond its molecular efficacy, ellagic acid demonstrates favourable pharmacokinetic and toxicological properties. According to Lipinski's Rule of Five (RO5), the compound satisfies key criteria for drug-likeness, including molecular mass, hydrogen bond donor/acceptor capacity, log P, and molar refractivity, suggesting high bioavailability and favourable pharmacodynamics. Additionally, ADMETlab 3.0 analyses further support its therapeutic potential by confirming excellent intestinal absorption, high human fraction unbound distribution, and appropriate metabolism through CYP2D6, ensuring efficient systemic clearance. The safety profile of ellagic acid is also favourable, as demonstrated in table 3, where its toxicity parameters, including AMES mutagenicity, hepatotoxicity, and skin sensitisation, are all within acceptable ranges. Moreover, human maximum tolerated dose and LOAEL chronic toxicity evaluations indicate that ellagic acid remains safe even at higher dosages, provided administration remains within the recommended thresholds.

While ellagic acid demonstrates significant anticancer potential, it is essential to compare its binding affinity with those of standard chemotherapeutic agents targeting the PI3K/AKT pathway, such as rapamycin, wortmannin, or PI3K inhibitors like BEZ235 [15,42–46]. Such comparisons would further elucidate ellagic acid's relative potency and therapeutic viability. Additionally, the present findings provide a foundation for future experimental validation through *in vitro* and *in vivo* studies. Cell culture assays involving OSCC lines could evaluate its pro-apoptotic and anti-proliferative effects, while animal models could determine its efficacy in tumour regression and metastasis inhibition [47–50]. Pharmacokinetic studies assessing bioavailability, metabolism, and systemic clearance *in vivo* will also be crucial in determining its translational potential.

Despite promising findings, this study is limited by the inherent constraints of *in silico* modelling, which may not fully capture the complexity of biological systems. Computational docking and molecular simulations, while valuable for predicting interactions, cannot entirely account for dynamic cellular processes, metabolic stability, or bioavailability in physiological environments. Therefore, future research should focus on experimental validation through *in*

vitro and *in vivo* studies, evaluating ellagic acid's efficacy in clinical settings.

Furthermore, given that PI3K pathway inhibition is often associated with compensatory activation of alternative survival pathways such as MAPK and JAK/STAT, future investigations should assess potential resistance mechanisms and explore combination therapies. Investigating ellagic acid's synergistic effects with existing chemotherapeutic agents could optimise its clinical application, potentially enhancing efficacy while reducing toxicity.

CONCLUSION

The in silico evaluation underscores the promising potential of pomegranate seed–derived ellagic acid as an anticancer agent against oral cancer. Its strong affinity for the PI3K/AKT pathway effectively disrupts key oncogenic processes, including tumour survival, cellular proliferation, and inflammation. Moreover, its favourable pharmacokinetic profile-characterized by excellent biocompatibility, low toxicity, and high intestinal absorption-reinforces its viability as a safe and effective therapeutic candidate. These findings provide a solid foundation for further exploration into the clinical applications of ellagic acid. To translate these computational insights into tangible medical advancements, future research should focus on rigorous *in vitro* and *in vivo* validation. Cell culture studies using OSCC models should be conducted to assess its apoptotic and anti-proliferative effects, while animal studies should evaluate its pharmacokinetics, bioavailability, and systemic efficacy in tumour suppression. Additionally, clinical trials will be necessary to establish optimal dosage, safety margins, and potential side effects in human subjects. Beyond monotherapy, combination therapy strategies should be explored to enhance ellagic acid's therapeutic efficacy. Given the compensatory activation of alternative survival pathways such as MAPK and JAK/STAT in response to PI3K inhibition, investigating synergistic effects with standard chemotherapeutic agents or targeted inhibitors could optimize treatment outcomes while minimizing toxicity. Furthermore, advancements in drug delivery syste.ms-such as nanoformulations, liposomal carriers, and targeted delivery mechanisms should be considered to enhance ellagic acid's solubility, stability, and bioavailability. Such innovations could improve its therapeutic potential and facilitate its clinical transition. In conclusion, while this study provides compelling preliminary evidence for ellagic acid's role in oral cancer therapy, its full therapeutic potential can only be realized through comprehensive preclinical and clinical investigations. A multidisciplinary approach integrating computational modeling, pharmacological research, and translational medicine will be essential in advancing ellagic acid towards clinical application as a viable anticancer agent.

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Nil

AUTHORS CONTRIBUTIONS

BL formulated the research idea, designed the study framework, and supervised the entire research process. He provided intellectual guidance and ensured the study's alignment with relevant scientific methodologies and objectives. PAML was responsible for conducting the in silico simulations, including molecular docking, pharmacokinetic modeling, and ADMET predictions. She also handled data processing, statistical analysis, and visualization of results. KDW contributed to data validation and cross-verification of the computational findings. She played a key role in interpreting the docking outcomes, binding affinities, and pharmacokinetic properties, ensuring scientific accuracy. AACD prepared the manuscript, including the introduction, methodology, results, and discussion sections. He structured the content, integrated relevant literature, and ensured logical coherence in presenting findings. FYM critically reviewed and revised the manuscript for technical accuracy, clarity, and coherence. They also contributed to refining the discussion and conclusion. Additionally, they facilitated funding acquisition and ensured compliance with ethical and institutional research guidelines. All authors have read and approved the final manuscript.

CONFLICTS OF INTERESTS

Authors declare no conflict of interest(s)

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