

Exploring Therapeutic Potential of Tephrosia calophylla Phytochemicals as Natural Epidermal Growth Factor Receptor (EGFR) Inhibitors in Oral Squamous Cell Carcinoma

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ABSTRACT

Aim: This study aimed to evaluate the potential of Tephrosia calophylla phytochemicals as natural inhibitors of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma (OSCC) using an integrated in silico and in vitro approach.

Materials and Methods: Fourteen compounds, including ten Tephrosia phytochemicals and four comparative agents (gefitinib, methotrexate, fluorouracil, curcumin), were screened through molecular docking against EGFR (PDB ID: 6LUD). Drug-likeness and pharmacokinetics were assessed using ADME filters and Lipinski, Veber, Egan, Ghose, and Muegge rules. The top-binding phytoconstituents were further analyzed for bioavailability and binding interactions. In vitro assays using KB oral carcinoma cells evaluated the antiproliferative and colony-forming inhibition of crude Tephrosia calophylla extract.

Results: Docking revealed that phytochemicals such as tephrosin, deguelin, elliptinone, and luteolin demonstrated strong binding affinities within the EGFR active site, forming stable hydrogen bonds and hydrophobic interactions comparable to gefitinib. ADME predictions indicated favorable oral absorption and bioavailability for most compounds, with several meeting multi-rule compliance for drug-likeness. In vitro experiments showed that Tephrosia calophylla extract significantly reduced KB cell proliferation in a dose-dependent manner ($p < 0.05$) and decreased colony formation at 50 $\mu\text{g/mL}$ over 10 days.

Conclusion: Tephrosia phytochemicals display promising EGFR inhibitory potential supported by favorable pharmacokinetic properties and in vitro cytotoxicity. These findings highlight their value as natural alternatives to conventional EGFR-targeted therapies in OSCC, warranting further validation through molecular dynamics, preclinical models, and clinical translation.

Keywords: Oral squamous cell carcinoma; Tephrosia calophylla; Phytochemicals; Molecular docking, human, health, Cancer

INTRODUCTION

Oral squamous cell carcinoma (OSCC) arises from a series of genetic and epigenetic alterations that disrupt the equilibrium between oncogenic signaling, tumor suppressive checkpoints and cell growth regulatory pathways.^{1,2} The mutations often target tumor suppressor genes such as TP53, inactivation compromises DNA damage responses, and CDKN2A (p16INK4a), deletion leads to loss of control over G1-S checkpoint control.^{3,4} One of the central pathways driving this process is the epidermal growth factor receptor (EGFR) signaling axis.⁵ EGFR, encoded by ERBB1, is a receptor tyrosine kinase that becomes hyperactivated in a majority of OSCCs through overexpression, gene amplification, or ligand overproduction.⁶ Once activated, EGFR phosphorylates docking proteins such as GRB2, SOS1, and GAB1, channeling signals into the RAS-RAF-MEK-ERK cascade, which stabilizes transcription factors like ELK1 and c-FOS to drive proliferation.^{7,8} Parallel activation of the PI3K-AKT-mTOR pathway enhances survival, metabolic adaptation, and protein synthesis, while the JAK-STAT and SRC family kinase branches reinforce angiogenesis and immune evasion.^{9,10} Crosstalk with integrins and growth factors such as VEGFA and FGFR further amplifies these

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oncogenic networks.¹¹ The collapse of tumor suppressive barriers compounds these effects. Loss of RB1 activity, whether through hyperphosphorylation by CDK4/6-Cyclin D1 (CCND1) complexes or mutational inactivation, releases E2F transcription factors and accelerates DNA synthesis.¹²

NOTCH1 mutations reconfigure differentiation programs, FAT1 alterations destabilize cell adhesion and polarity, and

CASP8 loss suppresses apoptotic responses.¹³ Together, these lesions combine with EGFR hyperactivation to produce cells capable of sustained proliferation, epithelial–mesenchymal transition (EMT), and metastatic spread.¹⁴

Therapeutically, EGFR represents a validated target. Tyrosine kinase inhibitors such as gefitinib and monoclonal antibodies like cetuximab demonstrate clinical benefit, yet their impact is blunted by resistance.¹⁵ Resistance mechanisms include EGFR T790M and C797S mutations within the kinase domain, compensatory signaling from MET or HER2 (ERBB2), and constitutive activation of PIK3CA, AKT1, or STAT3.¹⁶ This resistance highlights the necessity for alternative agents that can effectively block EGFR signaling.¹⁷

Chemotherapeutic agents remain an integral part of cancer management but are constrained by non-specific cytotoxicity, systemic toxicity, and the emergence of resistance.¹⁸ These drawbacks frequently compromise long-term outcomes and underscore the need for alternative strategies. Plant-derived compounds offer a viable alternative. They exert effects through diverse mechanisms, including modulation of signaling pathways, induction of apoptosis, and suppression of angiogenesis.¹⁹ Their multi-targeted nature and comparatively favorable safety profile strengthen their appeal.²⁰ Several agents in clinical use such as vincristine and vinblastine from *Catharanthus roseus* for hematological malignancies, paclitaxel from *Taxus brevifolia* in breast and ovarian cancers, camptothecin derivatives from *Camptotheca acuminata* for colorectal and ovarian cancers, and podophyllotoxin derivatives

from *Podophyllum peltatum* in testicular and lung cancers, highlight the longstanding contribution of phytochemicals to modern cancer therapy.^{21–26}

Tephrosia calophylla, a member of the Fabaceae family, is traditionally recognized for its rotenoid-rich profile and ethnomedicinal use.²⁷ The genus is commonly called “fish-poison beans,” the Tephrosia family is noted for its diverse bioactive flavonoids with documented pharmacological potential.²⁸ Tephrosia calophylla contains a wide range of bioactive flavonoids and rotenoids with reported antioxidant, cytotoxic, and apoptosis-inducing activity.^{29–31} Molecules such as tephrosin, deguelin, elliptinone, lanceolatin B, rotenone, and flavones like apigenin, chrysoeriol, luteolin, formononetin, isoflavanone have been shown to interfere with kinase activity.³² Kinase regulate apoptotic proteins including BAX and BCL2, and downregulate transcription factors such as NF- κ B and HIF-1 α .^{33,34}

Tephrosia compounds have shown anticancer activity in other tumor systems. Deguelin has been investigated in breast and lung cancer models for its ability to inhibit AKT and HSP90 signaling.³⁵ Tephrosin has demonstrated cytotoxic effects in leukemia cell lines, while rotenone has been reported to induce apoptosis in breast carcinoma cells.^{36,37} These findings suggest that the therapeutic scope of Tephrosia extends beyond a single cancer type and provides a rationale for exploring its activity against EGFR-driven oral cancers.

Molecular docking is a structure-based computational method that predicts the most favorable orientation and

Table 1: shows (a) Molecular Properties & Binding Energy (b) Hydrophobicity (c) Drug -Likeness Rule Evaluation

| Compound | Mol. Weight (g/mol) | Binding Energy (kcal/mol) | Bioavailability | TPSA | H. Donor | H. Acceptor | Hydrophobic | Log P | Lipinski | Ghose | Veber |
|---------------|---------------------|---------------------------|-----------------|--------|----------|-------------|-------------|-------|----------|-------|-------|
| Tephrosin | 330.35 | 7.92 | Low | 93.04 | 1 | 7 | 5 | 2.54 | Yes | Yes | Yes |
| Deguelin | 394.41 | 8.11 | Low | 75.99 | NA | 6 | 5 | 3.61 | Yes | Yes | Yes |
| Elliptinone | 295.33 | 7.89 | Low | 75.1 | NA | 6 | 4 | 2.75 | Yes | Yes | Yes |
| Lanceolatin B | 312.37 | 8.04 | Low | 70.67 | NA | 3 | 2 | 3.23 | Yes | Yes | Yes |
| Rotenone | 394.42 | 8.05 | Low | 66.76 | NA | 6 | 6 | 4.4 | Yes | Yes | Yes |
| Chalcone | 208.25 | 7.58 | High | 43.37 | NA | 1 | 2 | 3.28 | Yes | No | Yes |
| Tectorigenin | 300.27 | 7.85 | Low | 86.99 | 3 | 6 | 3 | 2.7 | Yes | Yes | Yes |
| Isoflavanone | 238.24 | 7.47 | High | 46.53 | NA | 2 | 2 | 3.12 | Yes | Yes | Yes |
| Sitosterol | 414.71 | 7.49 | Low | 20.23 | NA | 1 | 10 | 8.72 | No | No | No |
| Formononetin | 268.26 | 7.64 | High | 58.92 | 3 | 11 | 3 | 2.93 | Yes | Yes | Yes |
| Fluorouracil | 130.08 | 5.78 | High | 61.16 | 2 | 2 | 1 | 0.89 | Yes | No | Yes |
| Curcumin | 368.39 | 7.89 | Low | 93.06 | 3 | 6 | 4 | 3.29 | Yes | Yes | Yes |
| Methotrexate | 454.44 | 8.82 | Low | 210.52 | 3 | 7 | 2 | 0.91 | Yes | Yes | No |
| Gefitinib | 446.9 | 8.44 | Moderate | 90.49 | 3 | 5 | 1 | 2.83 | Yes | Yes | Yes |



binding affinity of ligands within the active site of a target protein, guided by scoring functions and conformational sampling.³⁸ In the present study, this approach was employed to evaluate Tephrosia-derived compounds against EGFR (PDB ID: 6LUD), docking analysis identifies hydrogen bonds, hydrophobic contacts, and conformational fits predictive of inhibitory efficacy. Integration of these computational results with pharmacokinetic profiling and in vitro cytotoxic assays provides a comprehensive platform to evaluate the translational value of Tephrosia compounds as EGFR-targeted agents.

This study aims to investigate whether Tephrosia calophylla phytochemicals can act as effective EGFR inhibitors in OSCC, by integrating in silico docking predictions with in vitro assays.

METHODOLOGY

Protein Retrieval and Preparation

The three-dimensional crystal structure of the tyrosine kinase domain of the Epidermal Growth Factor Receptor (EGFR) was obtained from the RCSB Protein Data Bank (PDB ID: 6LUD). (Figure 1) This structure was chosen as it represents the active conformation of EGFR bound to an inhibitor, providing a reliable reference for defining the ligand-binding pocket. The protein was pre-processed to remove crystallographic water molecules, redundant chains, and heteroatoms not involved in ligand interactions. Hydrogen atoms were added to stabilize the geometry, and Kollman charges were assigned. To correct missing side chains and optimize structural integrity, the protein was refined using BIOVIA Discovery Studio. The active site was identified based on the co-crystallized ligand and corroborated with published binding site data, ensuring accurate docking predictions.

Ligand Retrieval and Optimization

Phytocompounds reported from Tephrosia calophylla were shortlisted through a comprehensive literature survey, with a focus on flavonoids and rotenoids previously documented for cytotoxic or apoptosis-inducing activity. Canonical SMILES and 3D conformations of these compounds were retrieved from the ZINC15 and ChEMBL databases. Ligand structures were downloaded in MOL2/SDF format and subjected to energy minimization using the CHARMM force field in BIOVIA Discovery Studio, ensuring biologically stable conformations. Stereochemistry, tautomeric forms, and protonation states were checked to reflect physiological conditions.

Molecular Docking Simulation

Molecular docking was performed using SwissDock, a web-based docking server powered by the EADock DSS engine. This platform predicts ligand-protein interactions by flexible docking of ligands into the defined binding cavity of the receptor. Each ligand was docked against EGFR (6LUD), generating multiple binding conformations. Docking outcomes were ranked according to binding free energy (ΔG) and fullfitness score, where lower values indicate stronger binding affinity. Among the poses generated, the best conformations were selected for further evaluation, prioritizing those with favorable docking scores and proper alignment within the ATP-binding pocket. (Figure 2)

Docking Analysis

Docked complexes were analyzed using BIOVIA Discovery Studio Visualizer to interpret molecular interactions. Parameters such as hydrogen bonds, hydrophobic contacts, van der Waals interactions, and π - π stacking were systematically evaluated. Residues involved in binding were identified and compared



Fig. 1. Line and Scatter Graph - EGFR - 6LUD

with those of the co-crystallized ligand to validate docking accuracy. Interaction maps were generated to illustrate spatial conformations and the nature of binding contacts. This step provided molecular-level insight into the inhibitory potential of Tephrosia-derived compounds against EGFR.

ADME and Drug-Likeness Profiling

To assess pharmacokinetic feasibility, docked compounds were screened using SwissADME. Drug-likeness was evaluated

based on Lipinski's Rule of Five and related filters. ADME parameters including gastrointestinal absorption, blood-brain barrier (BBB) permeability, cytochrome P450 enzyme inhibition, and bioavailability scores were recorded. This analysis ensured that shortlisted ligands not only demonstrated strong binding affinity but also possessed favorable drug-like properties. (Figure 3)

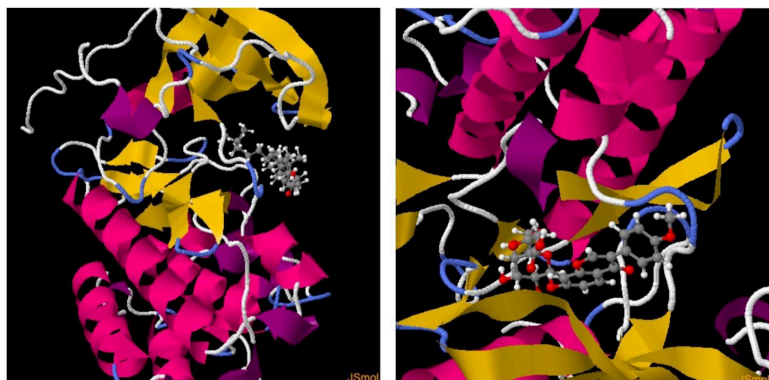


Fig. 2: Molecular docking interactions of Tephrosia phytocompounds with EGFR (α -helices in magenta, β -sheets in yellow) showing ligands bound within the active pocket.

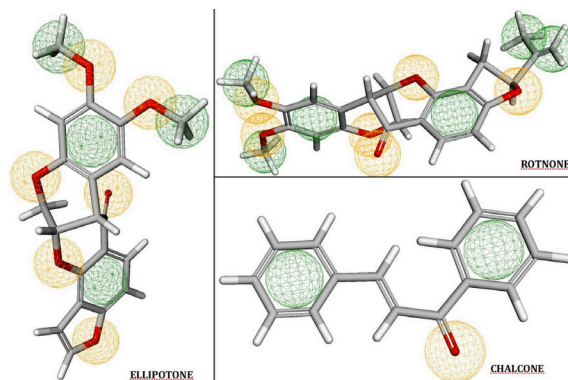


Fig. 3: Binding pocket mapping of Tephrosia phytocompounds with EGFR (green contours: hydrophobic regions; yellow contours: hydrogen bond acceptor sites) confirming multiple pharmacophore-compatible sites

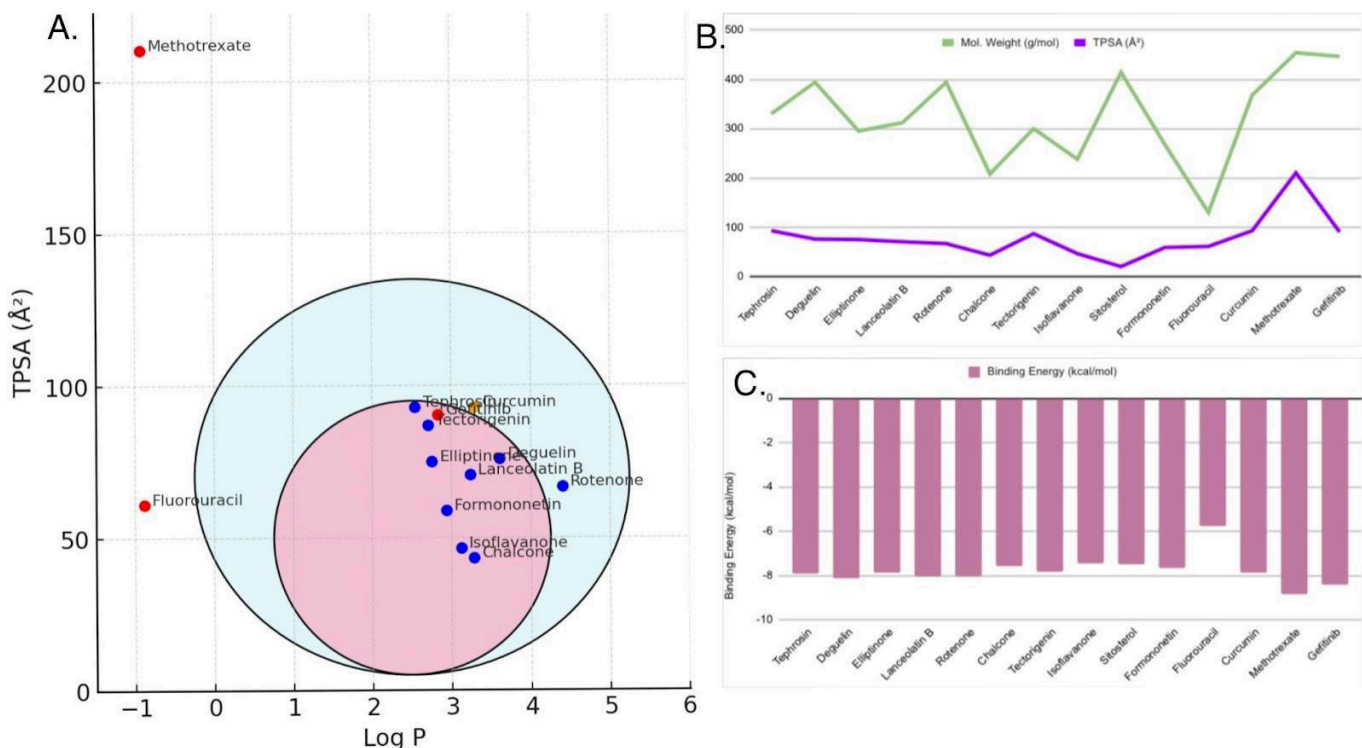


Fig. 4: (A) Half-Boiled Egg Plot for Drug Absorption & Permeability (B) Line Graph- Compound : Log P : TPSA (c) Bar Graph-Compound : Binding energy

Cell Line and Culture Conditions

The KB oral carcinoma cell line was procured from the National Centre for Cell Science (NCCS), Pune, India, a national repository for authenticated cell lines. The cell line is quality controlled by NCCS through standard authentication procedures, including STR profiling and routine mycoplasma testing.

The cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin solution. Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO₂, and cells were routinely sub-cultured upon reaching 70–80% confluence. All experiments were performed using cells in the logarithmic growth phase to ensure reproducibility.

Ethical approval statement

The study protocol was reviewed and approved by the Institutional Ethics Committee (Approval No.: SRB/SDC/OPATH-2302/25/094). All experimental procedures were conducted in accordance with the ethical standards of the institutional research committee.

Cytotoxicity Assessment by MTT Assay

The effect of Tephrosia calophylla extract on KB cell viability was determined using the MTT assay. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere for 24 h. Treatments were carried out with graded concentrations of extract (0–200 µg/mL) for 24 h. Following incubation, MTT solution (5 mg/mL) was added to each well and incubated for 4 h. Formazan crystals formed by metabolically active cells were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Cell viability (%) was calculated relative to untreated controls, and the IC₅₀ value was determined.

Colony Formation Assay

The long-term anti-proliferative potential of Tephrosia calophylla was evaluated through clonogenic assays. KB cells were seeded at low density in six-well plates and treated with

extract at 50 µg/mL for 10 days, with medium renewal every 72 h. Colonies formed were fixed with methanol, stained with 0.5% crystal violet, and counted manually. Both the number and size of colonies in treated groups were compared to untreated controls, providing evidence for the compound's effect on sustained cell survival and proliferative capacity.

Statistical Analysis

Docking scores, interaction residues, and pharmacokinetic profiles were systematically tabulated in Microsoft Excel. In vitro results were expressed as mean ± standard deviation (SD) from three independent experiments. Statistical significance was assessed using one-way ANOVA, with $p < 0.05$ considered significant. Graphical representations of docking energies, cell viability, and colony inhibition were generated for comparative interpretation.

RESULTS

Molecular Docking Analysis

Molecular docking of Tephrosia calophylla phytoconstituents against EGFR (PDB ID: 6LUD) revealed differential binding affinities, with several compounds demonstrating energies comparable to the reference inhibitor Gefitinib (−8.44 kcal/mol) (Table 1). Deguelin (−8.11 kcal/mol), Rotenone (−8.05 kcal/mol), and Lanceolatin B (−8.04 kcal/mol) emerged as the most potent binders within the rotenoid class, showing docking scores that closely approached the benchmark drug. Other flavonoids such as Tephrosin (−7.92 kcal/mol), Elliptinone (−7.89 kcal/mol), and Tectorigenin (−7.85 kcal/mol) also displayed strong binding, suggesting a favorable structural compatibility with the EGFR active site. Although Isoflavanone (−7.47 kcal/mol) and Formononetin (−7.64 kcal/mol) demonstrated relatively lower binding energies, they still exceeded the activity of several plant-derived ligands commonly reported in docking-based cancer studies. (Table 1)

The interaction analysis revealed that high-scoring compounds consistently occupied the ATP-binding cleft of EGFR, establishing key hydrogen bonds and hydrophobic

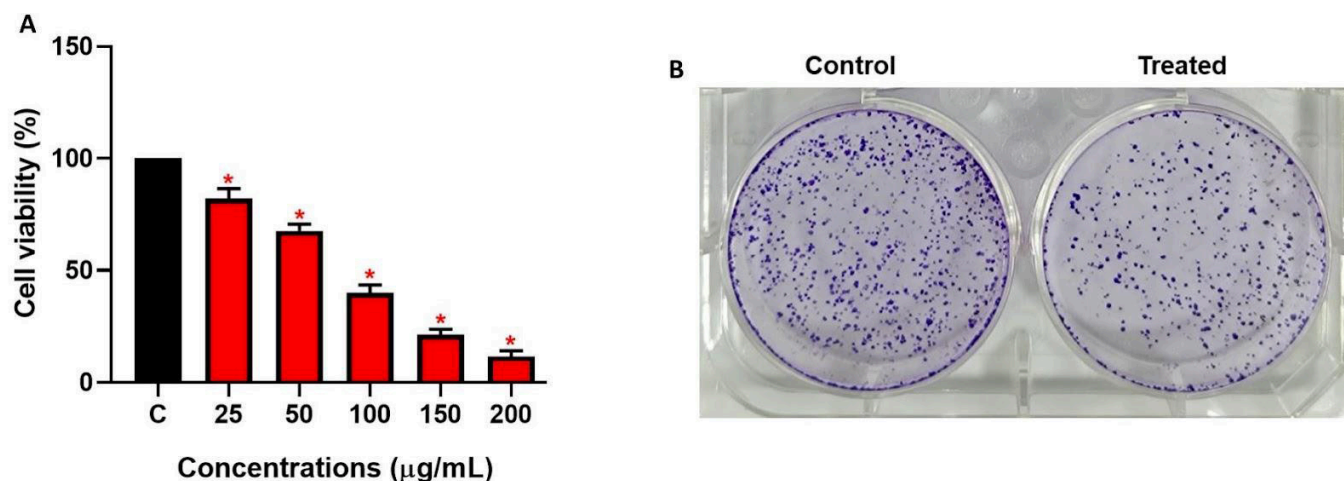


Fig. 5: (A) Effect of Tephrosia calophylla (0–200 µg/mL, 24 h) on KB cell proliferation (* $p < 0.05$); (B) Clonogenic assay with 50 µg/mL Tephrosia calophylla for 10 days showing reduced colony formation compared to control.

contacts with residues implicated in kinase inhibition. Rotenoids in particular exhibited multiple hydrophobic contacts, while chalcone (-7.58 kcal/mol) aligned through aromatic stacking interactions. These findings collectively indicate that phytochemicals from *Tephrosia calophylla* have the structural potential to interfere with EGFR activity at the molecular level. (Figure 4)

Drug-Likeness and ADME Profiling

To evaluate the translational feasibility of the docked ligands, drug-likeness and pharmacokinetic parameters were examined using SwissADME. Most compounds adhered to Lipinski's rule of five, indicating potential oral druggability. Sitosterol, however, showed deviation with an elevated Log P (8.72), reflecting poor solubility and low likelihood of bioavailability. Among the flavonoids, Formononetin exhibited high predicted bioavailability, whereas Tephrosin, Deguelin, and Rotenone were categorized as low, consistent with the general pharmacokinetic limitations of rotenoids. Chalcone, despite a modest docking score, displayed high bioavailability, highlighting its potential as a druggable lead scaffold.

Additional descriptors such as topological polar surface area (TPSA) and hydrogen donor/acceptor profiles revealed that ligands with lower TPSA values (e.g., Sitosterol, Chalcone) had improved membrane permeability predictions, while compounds with higher TPSA (e.g., Tectorigenin, Curcumin) may face absorption challenges. The reference drug Gefitinib displayed moderate bioavailability and satisfied all major drug-likeness filters, providing a reliable comparison point for assessing the phytochemicals. Taken together, the ADME analysis supports the feasibility of further development of selected *Tephrosia*-derived molecules, particularly those balancing strong docking interactions with acceptable pharmacokinetic predictions.

In Vitro Cytotoxicity Assay

Biological evaluation through the MTT assay confirmed that *Tephrosia calophylla* extract exerted a dose-dependent inhibitory effect on oral carcinoma KB cells. Exposure to increasing concentrations of extract (0–200 $\mu\text{g/mL}$) for 24 h led to a progressive decline in cell viability, with statistically significant reductions observed at concentrations ≥ 100 $\mu\text{g/mL}$ ($p < 0.05$). At the maximum concentration tested, cell viability dropped markedly compared to the untreated control, reflecting strong cytotoxic activity. The calculated IC_{50} value fell within a pharmacologically relevant range, suggesting that the bioactive constituents of the extract possess therapeutic potential against oral squamous cell carcinoma cells.

The microscopic examination of treated cultures revealed characteristic morphological changes consistent with cytotoxic insult, including cell shrinkage, detachment, and rounding. These observations correlate with the apoptosis-inducing properties previously reported for flavonoids and rotenoids in other cancer models. (Figure 5)

Clonogenic Assay

To assess the long-term proliferative capacity of treated cells, a clonogenic assay was conducted. Treatment with *Tephrosia calophylla* extract (50 $\mu\text{g/mL}$) over a 10-day period

significantly reduced both the number and size of colonies formed compared to the untreated control group. While control wells displayed large, densely packed colonies, extract-treated wells showed sparse and small clusters, indicating a marked inhibition of reproductive viability.

Quantitative analysis revealed a pronounced decline in clonogenic survival, with reductions exceeding 50% relative to controls ($p < 0.05$). This outcome suggests that beyond short-term cytotoxicity, *Tephrosia* phytochemicals are capable of suppressing the ability of cancer cells to undergo sustained proliferation, an essential property for anticancer drug development. (Figure 5)

The combined docking and biological results establish a coherent narrative. Compounds such as Deguelin, Rotenone, and Lanceolatin B demonstrated strong binding affinities to EGFR *in silico*, aligning with the significant cytotoxicity observed *in vitro*. The reduced clonogenic potential of KB cells further supports the hypothesis that *Tephrosia*-derived molecules interfere with growth signaling pathways, consistent with EGFR inhibition. Importantly, while some compounds exhibited bioavailability limitations, others such as Formononetin and Chalcone balanced both favorable pharmacokinetics and acceptable docking scores, highlighting their translational promise.

DISCUSSION

The present study provides integrated computational and biological evidence supporting the anticancer potential of *Tephrosia calophylla* phytochemicals against EGFR-driven oral squamous cell carcinoma (OSCC). Docking studies demonstrated that several rotenoids and flavonoids from *Tephrosia* possess binding energies comparable to the clinically established inhibitor Gefitinib. Among them, Deguelin, Rotenone, and Lanceolatin B consistently occupied the ATP-binding cleft of EGFR with stable interactions, indicating their capacity to interfere with receptor kinase activity. These findings align with previous studies highlighting the ability of rotenoids to modulate tyrosine kinase pathways in breast and lung cancer models.^{35,36}

Drug-likeness and ADME profiling refined the translational perspective of these ligands. While compounds such as Sitosterol showed physicochemical limitations due to high lipophilicity, others like Formononetin and Chalcone balanced favorable pharmacokinetics with acceptable docking scores. This distinction underscores the importance of integrating computational predictions with pharmacological filters before nominating candidate molecules for drug development. The identification of Chalcone as a bioavailable scaffold is particularly noteworthy, as chalcone derivatives have been advanced in preclinical pipelines for various malignancies, including hepatocellular carcinoma and leukemia.³⁹

The *in vitro* assays reinforced the computational observations. Treatment with *Tephrosia calophylla* extract significantly reduced KB cell viability in a dose-dependent manner, with an IC_{50} value that falls within the range observed for plant-derived experimental therapeutics. Importantly, the clonogenic assay demonstrated that beyond acute cytotoxicity, the extract suppressed the long-term proliferative potential



of KB cells. This effect indicates a sustained impairment of tumorigenic capacity, a hallmark that strengthens the relevance of these phytochemicals as anticancer agents. The morphological changes observed in treated cells cell shrinkage, rounding, and detachment are consistent with apoptosis, further supporting mechanistic parallels with EGFR inhibition.

When considered alongside existing literature, the findings emphasize that Tephrosia-derived compounds may complement or provide alternatives to current EGFR inhibitors.¹⁸ Small-molecule inhibitors such as Gefitinib and Erlotinib, while clinically valuable, are limited by resistance arising from secondary mutations in EGFR and compensatory activation of parallel signaling pathways.^{15,34} Natural molecules, by their polypharmacological activity, may overcome these barriers by targeting multiple nodes within the signaling network.³³ The multitargeted activity of rotenoids and flavonoids therefore presents a therapeutic advantage, although their low bioavailability remains a recognized limitation.

The present work also contributes to the growing evidence that molecular docking, when paired with in vitro validation, offers a rational framework for natural product drug discovery. While docking alone cannot fully capture the dynamics of protein ligand interactions in a cellular context, its predictive power is strengthened when biological assays confirm cytotoxic and anti-proliferative effects. Studies with integrated approaches combining docking and in vitro validation have already been applied in breast cancer cell line models, demonstrating improved reliability in identifying biologically relevant anticancer activity⁴⁰⁻⁴¹. The consistency between the docking predictions and the cell-based outcomes in this study provides confidence in the translational relevance of the selected phytochemicals.

In the present study, in vitro assays were carried out using the crude Tephrosia calophylla extract, while molecular docking was performed on individual phytochemicals reported from the plant. This approach was chosen to establish an initial biological effect of the extract while simultaneously exploring the role of its individual phytochemical constituents.

Future studies should evaluate the isolation and purification of high-affinity phytochemicals, including deguelin, tephrosin, and lanceolatin B, followed by their independent biological evaluation to directly link molecular inhibition with cellular responses. Pharmacokinetic studies and comparisons with standard drugs will support translational relevance, while early clinical trials can help establish their safety and therapeutic potential.

CONCLUSION

This study demonstrates that phytochemicals from Tephrosia calophylla possess significant potential as natural inhibitors of EGFR in oral squamous cell carcinoma. Molecular docking revealed strong binding affinities for rotenoids such as Deguelin, Rotenone, and Lanceolatin B, with interactions comparable to the standard inhibitor Gefitinib. ADME profiling highlighted compounds like Chalcone and Formononetin as favorable candidates with better drug-likeness properties. In vitro assays confirmed that Tephrosia calophylla extract exerts dose-

dependent cytotoxicity and suppresses long-term clonogenic survival of KB cells, indicating both immediate and sustained anticancer effects. The EGFR inhibitors are increasingly used in combination with chemotherapy, radiotherapy, and other targeted agents to address resistance, the multitargeted nature of plant-derived phytochemicals may offer advantages within combination regimens and personalized treatment strategies guided by tumor molecular profiling. These findings support further isolation and mechanistic validation of Tephrosia phytochemicals as potential therapeutic leads for EGFR-driven OSCC.

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