

Anticancer Potential of *Tephrosia maxima*: Inhibition of Cell Viability and Colony Forming Ability in Oral Squamous Cell Carcinoma Cell Lines

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) continues to be a significant global health challenge, especially in developing areas, where lifestyle associated risk factors increase its prevalence. Even with improvements in surgery, radiotherapy, and chemoradiation, toxicities related to treatment and resistance still undermine clinical outcomes, driving the quest for safer, plant-derived supplements. The *Tephrosia* genus, characterised by a rich content of rotenoids, flavonoids, and isoflavones, has demonstrated promising anticancer capabilities in various models, yet *Tephrosia maxima* remains largely unexplored. **Materials and Methods:** The present study investigated the anticancer potential of *T. maxima* through molecular docking analysis followed by *in vitro* evaluation in oral cancer cells. Three primary phytoconstituents, specifically tephrosin, deguelin, and lupeol, were docked to Epidermal Growth Factor Receptor (EGFR), which is a crucial oncogenic factor in OSCC. Based on these *in silico* findings, the biological activity of *T. maxima* extract was further evaluated using KB oral squamous carcinoma cells through cell viability and clonogenic assays. **Results:** Molecular docking revealed that deguelin exhibited the highest affinity (-7.90 kcal/mol), followed by tephrosin (-7.20 kcal/mol) and lupeol (-6.40 kcal/mol), with docking configurations showing stabilizing hydrogen bonds and hydrophobic interactions in the ATP-binding pocket. *In vitro* tests indicated a dose-related decrease in cell viability, with an IC₅₀ of roughly 96 µg/mL after 24 hr. Clonogenic assays also showed significant reduction in colony forming ability post-treatment, suggesting a decrease in long-term proliferative potential. **Conclusion:** These findings collectively emphasize *T. maxima* as a valuable source of bioactive phytochemicals that can induce cytotoxic and anti-proliferative effects on OSCC, likely via interactions targeting EGFR. Although these findings correspond with recognized anticancer properties of similar *Tephrosia* species, this research provides the preliminary evidence of such effects in *T. maxima*. Further *in vivo* and clinical investigations are necessary to confirm these findings and assess its potential for translation into oral cancer treatments.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, Cancer, Disease, Health, Human, Illness, Medicine, Plant-based, *Tephrosia maxima*.

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INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is the most prevalent malignancy affecting the oral cavity. With the exception of certain regions of France, the disease is relatively rare in the industrialised world, whereas it is widespread in the developing world, particularly in the Indian subcontinent and Southeast Asia. Although its incidence is increasing among young individuals, oral cancer predominantly affects men over middle age, individuals who smoke, and those lower social economic

backgrounds (Scully and Porter, 2001; Dee *et al.*, 2025). According to estimates, infections, poor diet, habit, and tobacco chewing and sedentary lifestyle, sedentary lifestyle combined with alcohol consumption or areca nut usage account for approximately 43% of cancer deaths globally. These risk factors contribute to chronic exposure of oral mucosa to carcinogens leading to genetic and epigenetic alterations. Consequently, the multistep process of oral carcinogenesis, along with genetic alterations, results in progressive dysplasia, uncontrolled cell proliferation, and eventual malignant transformation (Dutta *et al.*, 2012; Gupta *et al.*, 2022; Chang *et al.*, 2017). Despite several therapeutic advancements, complete cure or prevention of cancer still remains elusive (Dee *et al.*, 2025; Mohamad *et al.*, 2023).

The development and progression of OSCC involve complex molecular alternations that disrupt normal cellular regulatory pathways. These include dysregulation of cell proliferation,



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apoptosis, angiogenesis and key signaling pathways such as Epidermal Growth Factor Receptor (EGFR), MAPK and PI3K/Akt pathways, which play crucial roles in tumor initiation and progression. Consequently, many therapeutic strategies have been developed to target these molecular mechanisms in order to inhibit tumour growth and improve treatment outcomes. Despite these advancements the overall survival rate of OSCC remains relatively low and the disease is often associated with poor prognosis due to later stage diagnosis and high recurrence rates.

Currently, surgical intervention remains the primary treatment modality for OSCC. Chemo radiation or radiation therapy is often employed as an urgent treatment for patients with a high risk of recurrence or as the primary treatment when surgery is not feasible. Although these treatments are integral to cancer management, they are frequently associated with numerous effects that significantly compromise the patient's quality of life. As a result, there is an increasing need for adjunct therapeutic strategies that can effectively manage the disease while minimising treatment-associated toxicity (Kisling and Stiegmann, 2025; Yin et al., 2013; Kumar et al., 2026).

Given the limitations and side-effects of conventional treatment modalities, increasing attention has been directed towards alternative and complementary therapeutic approaches. Over the past two decades, several herbal remedies from Chinese and Ayurvedic medicine have attracted considerable interest due to their ability to enhance therapeutic efficacy while mitigating the toxic effect associated with chemotherapy and radiation therapy in head and neck cancers (Fateen et al., 2025). Phytomedicine has emerged as a promising anticancer strategy because bioactive phytochemicals can promote apoptosis, inhibit angiogenesis and modulate key signalling pathways involved in cancer development (Aryan, 2018; Lee et al., 2015; Arora et al., 2023).

Indeed, numerous natural compounds have successfully progressed to clinical approval as anti-cancer agents. Examples include compounds derived from *Taxis brevifolia*, camptothecin and *Podophyllum peltatum* which have demonstrated significant therapeutic benefits in cancer treatment (Yang et al., 2020; Chazin et al., 2014; Méresse et al., 2004; Kobayashi and Ratain, 2004; Rivera et al., 2004; Postmus et al., 2000). In oral cancer specifically, curcumin has been reported to reduce chemoradiotherapy induced toxicity and promote apoptosis in tumour cells, while other planned derived treatments such as Danshen have demonstrated and inflammatory and anti-proliferative effect through modulation of MAPK signaling pathways (Chaveli-López and Bagán-Sebastián, 2016; Dharman et al., 2021; Hsiao et al., 2018; Hsiao et al., 2018; Chen et al., 2018; Yang et al., 2017; Kumar et al., 2020). Therefore, herbal therapies have been recognised for their potential to reduce treatment related side-effects and enhance host immune responses, highlighting their promise as complementary and alternative therapeutic options (Bae et al., 2017; Nik Nabil et al., 2018; Zhang et al., 2021).

Nevertheless, challenges such as fluctuations in bioactive compound concentrations, lack of standardisation and potential herb-drug interactions remain significant obstacles that warrant further investigation (Shrikant, 2023; Atanasov et al., 2021; Singh et al., 2012).

Within this context, medicinal plants belonging to the genus *Tephrosia* have attracted scientific attention due to their diverse pharmacological activities. The *Tephrosia* genus is characterised by its rich content of isoflavones, rotenoids and flavonoids- bioactive compounds that are relatively uncommon in many medicinal plants and are known for their antimicrobial, antioxidant, hepato- protective and anticancer properties (Obbalareddy et al., 2022). Among the various species within this genus, *Tephrosia maxima* is, at present, a relatively less explored species despite belonging to a group recognised for its anticancer potential in several experimental cancer models.

The limited research available on *T. maxima* combined with the substantial phytochemical diversity, suggests that it represents a promising candidate for further investigation in cancer therapy. The plant may serve as a source of novel bioactive compounds that could offer therapeutic advantages compared to currently studied herbal agents (Gulecha and Sivakumar, 2011).

However, despite the presence of numerous anticancer phytochemicals within the *Tephrosia* genus, the specific anticancer potential of *T. maxima* against oral squamous carcinoma cell lines remains largely unexplored. Additionally, although direct molecular pathway validation in cancer cells was beyond the scope of the study, molecular docking of major phytochemicals against epidermal growth factor receptor, a key oncogenic driver in OSCC, was performed to provide supportive mechanistic insights. Therefore, the present study was conducted to evaluate the cytotoxic and anti proliferative effects of *T. maxima* extract on OSCC cell lines using cell viability and colony formation assays. The objective of the present study was to evaluate the anticancer properties of *Tephrosia maxima* extract on oral cell carcinoma cell lines supported by molecular docking evidence.

MATERIALS AND METHODS

Molecular docking

Tephrosin, deguelin, and lupeol-three of *T. maxima*'s main phytoconstituents-were chosen for docking investigation. The Protein Data Bank provided the crystal structure of EGFR (PDB ID: 1M17), while PubChem provided the 3D structures of the ligands and their energy was minimized. SwissDock was used for docking, and the grid box surrounding the active location was established. Based on binding affinity scores, the optimal binding conformations were found and displayed using PyMOL to examine hydrophobic and hydrogen bonding interactions.

Cell culture

KB cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, and maintained at 37°C in a humidified incubator with 5% CO₂. The extract of *T. maxima* was initially dissolved in DMSO to create stock solutions, which were subsequently diluted with the culture medium to achieve the necessary concentrations, ensuring that the final DMSO concentration stayed below 0.1% (v/v).

MTT assay

The MTT assay was utilized to evaluate the cytotoxic ability of the *T. maxima* extract. KB cells were plated into 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hr to promote adherence. Cells were subsequently treated with different concentrations of extract (25-200 µg/mL) for 24 hr, using 0.1% DMSO as the control. After treatment, MTT solution (5 mg/mL) was introduced to each well and incubated for 4 hr at 37°C to allow for formazan crystal formation. The supernatant was gently taken out, and the crystals were dissolved in DMSO. Absorbance was determined at 570 nm using a microplate reader. Cytotoxicity (%) was determined using the equation:

$$\text{Cytotoxicity (\%)} = 1 - \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Cell viability (%) was calculated by deducting the cytotoxicity value from 100%. The half-maximal inhibition concentration (IC₅₀) was established

Colony formation assay

Using a clonogenic assay, the cells' capacity to establish colonies following treatment was assessed. After being placed on 35-mm culture plates, KB cells per plate were allowed to adhere overnight. Following that, cells were treated with 50 µg/mL of *T. maxima* extract for 24 hr, while controls were administered 0.1% DMSO. After treatment, a new growth medium was added, and the cells were incubated for ten days. Colonies were fixed with methanol, then stained for 10 min with 0.5% crystal violet, rinsed with PBS, and left to air dry. Colonies with over 50 cells were manually counted. Treatment effects were determined using the surviving percent and plating efficiency.

Statistical Analysis

All experiments were performed in triplicates and expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism. One-way ANOVA followed by *post hoc* test was used. A *p* value ≤0.05 was considered statistically significant. IC₅₀ values were calculated using nonlinear regression.

Ethical statement

This study did not involve human or animal subjects. Experiments were conducted using established cell lines following institutional biosafety guidelines.

RESULTS

Molecular docking analysis

Molecular docking analysis was performed to evaluate the binding affinity and interaction profile of major photo constituents of *T. maxima* with the Epidermal Growth Factor Receptor (EGFR). Among the tested compounds, deguelin showed the highest binding affinity (-7.9 kcal/mol), with tephrosin following (-7.29 kcal/mol) and then lupeol (-7.55 kcal/mol) (Table 1).

Detailed analysis showed that deguelin formed stable hydrogen bonds with key amino acid residues within the ATP-binding site of EGFR, along with significant hydrophobic interactions that contribute to complex stabilisation. Tephrosin also demonstrated favourable interactions, including both hydrogen bonding and hydrophobic interactions, while lupeol primarily exhibited hydrophobic interactions.

The presence of these interactions within the active site suggests a strong potential for these compounds to competitively inhibit EGFR kinase activity. Notably, deguelin showed the most stable binding conformation, indicating its potential as a leading compound among the tested phytoconstituents. These findings highlight the ability of *T. maxima*-derived compounds to target EGFR mediated signaling pathways which play a critical role in the progression of oral squamous cell carcinoma (Figure 1).

Cytotoxicity (MTT assay)

The MTT assay assessed the cytotoxic impact of *T. maxima* extract on KB oral squamous carcinoma cells. Cell viability diminished in a dose-dependent manner across all examined doses (25-200 µg/mL). A marked decrease in growth inhibition was indicated by the IC₅₀ value at 24 hr, estimated to be approximately 96 µg/mL (Figure 2).

Colony formation assay

Using the clonogenic assay, the *T. maxima* extract's capacity to inhibit long-term proliferation was assessed. Colonies were significantly smaller and fewer in number after treatment with 50 µg/mL extract than after DMSO control. The survival fractions dropped dramatically, suggesting a reduced ability to proliferate and self-renew (Figure 3). After treatment, the inhibition of colony formation is evident in representative photos. A clonogenic assay with 50 µg/mL *Tephrosia maxima* for 10 days showed decreased colony formation compared to the control.

DISCUSSION

Oral Squamous Cell Carcinoma (OSCC) represents a significant proportion of head and neck malignancies and remains a substantial global health challenge (Nagi *et al.*, 2023). This burden is primarily driven by the aggressive clinical course of the disease, frequent recurrence and inherent or acquired resistance to standard treatment modalities (Fateen *et al.*, 2025; Kumar *et*

al., 2025; Muthukrishnan and Warnakulasuriya, 2018). While surgical intervention, chemotherapy and radiotherapy have seen technical refinements, overall clinical outcomes are frequently hindered by systemic toxicity and therapeutic failure (Kumar et al., 2024). Consequently, there is an increasing shift towards investigating plant derived compounds as adjunct or alternative therapeutic interventions (Srivastava et al., 2025). Natural compounds remain a cornerstone of oncological drug discovery offering a diverse array of bioactive phytochemicals that can concurrently modulate multiple signaling pathways involved in tumorigenesis and cancer progression (Jeyachandran, 2024).

In this study, the *Tephrosia maxima* extract exhibited potent cytotoxic and antiproliferative effects against KB Oral Squamous Cell Carcinoma (OSCC) cells. Quantitative analysis using MTT assay demonstrated a dose dependent reduction in cell viability, yielding a Half Maximal Inhibitory Concentration (IC_{50}) of approximately 96 $\mu\text{g/mL}$ at 24 hr post treatment. These data underscore the significant cytotoxic potential of the extract against the KB cell line.

Furthermore, clonogenic assays revealed a marked concentration dependent inhibition of colony formation following exposure to *T. maxima*. This suppression of Colony Forming Units (CFU's)

indicates a disruption of long term proliferative capacity. Collectively these results suggest that the mechanism of action of the extract involves both the induction of acute cytotoxicity and impairment of malignant cell survival and proliferation.

While literature specifically detailing the pharmacological profile of *T. maxima* remains sparse, the anticancer potential of the Tephrosia genus is well documented across several related species. Extracts from *T. pulcherrima* and *T. pumila* have demonstrated significant inhibitory effects against HT-29 and RAW cell lines underscoring their cytotoxic efficacy (Ganapaty et al., 2008).

Phytochemical characterisation of *T. calophylla* has further identified bioactive benzil and coumestan derivatives with established anti neoplastic properties (Ganapaty et al., 2009). Similarly, investigations into *T. tinctoria* revealed robust biological activity which has been attributed to high concentration of flavonoids and diverse secondary metabolites (Lakshmi et al., 2010). These data support the hypothesis that the Tephrosia genus is a rich source of phytochemicals capable of exerting potent cytotoxicity effects on malignant cell populations.

A key phytochemical frequently identified across the Tephrosia genus is beta sitosterol, a phytosterol extensively characterised for its antioxidant, chemopreventive and anti neoplastic properties. Prior investigations have established that beta sitosterol effectively induces apoptosis, retards tumour progression across diverse malignancies including breast, prostate and colorectal carcinomas (Kishore and Roy, 2011).

Beyond direct cytotoxicity, Tephrosia derived extracts have demonstrated hepatoprotective and preventive efficacy in hepatocarcinoma models, underscoring the broader therapeutic utility in oncology (Rizvi et al., 2018). *Tephrosia purpurea* extracts have shown significant cytotoxic activity against HeLa cervical

Table 1: Binding energy of bioactive compounds of *Tephrosia maxima* with Epidermal Growth Factor Receptor (EGFR).

Compound	Binding Energy (Kcal/mol)	Key Interactions
Tephrosin	-7.29	H-bond, hydrophobic
Deguelin	-7.9	H-bond
Lupeol	-7.55	Hydrophobic

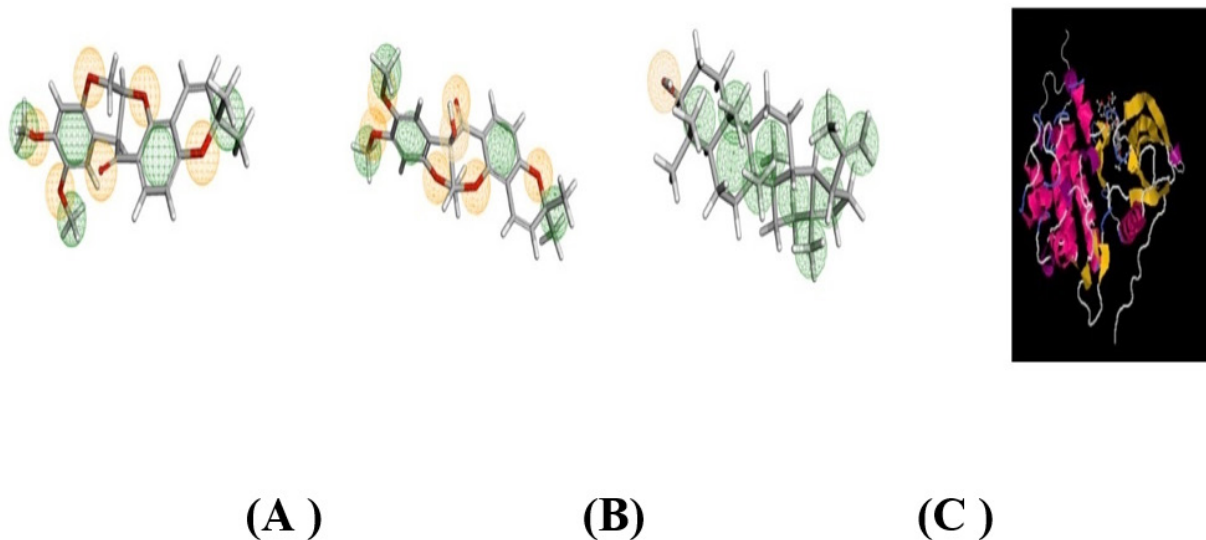


Figure 1: Molecular docking analysis of bioactive compounds from *Tephrosia maxima* with epidermal growth factor receptor (EGFR): (A) Tephrosin, (B) Deguelin, (C) Lupeol, and (D) protein-ligand interaction within the ATP-binding pocket showing hydrogen bonding and hydrophobic interactions.

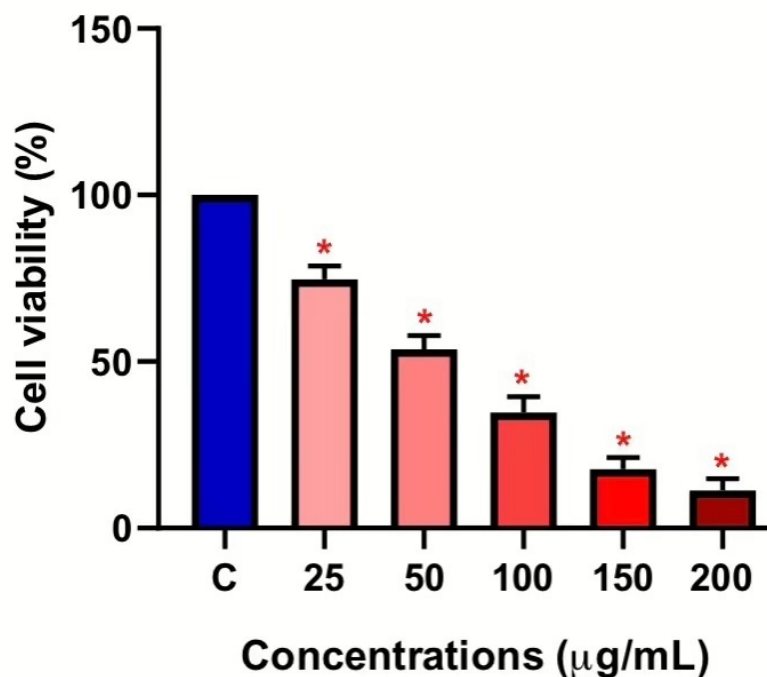


Figure 2: Dose-dependent cytotoxic effect of *Tephrosia maxima* extract on KB oral squamous cell carcinoma cells assessed using MTT assay after 24 hr of treatment. Data are expressed as percentage cell viability.

cancer cells, with the ethyl acetate fraction emerging as the most potent anti neoplastic phase (Shanmugapriya *et al.*, 2011).

These cumulative observations align with the current findings and suggest that the cytotoxic profile of *T. maxima* may be attributed to a similar profile of bioactive secondary metabolites such as beta sitosterol or related steroidal compounds.

Additional members of the *Tephrosia* genus further illustrate its robust pharmacological profile. For example, *T. villosa* has demonstrated significant toxicity in brine shrimp lethality assays (*Artemia salina*), a classical indicator of potent bioactive metabolites with potent anti neoplastic utility (Nondo *et al.*, 2011). Furthermore, specific flavonoids isolated from *T. toxicaria* have been characterised for their cancer chemopreventive properties, highlighting the genus's role in interfering with early-stage oncogenesis (Jang *et al.*, 2003; Muralidharan *et al.*, 2023).

Investigations into *T. vogelii* have similarly revealed high biological potency characterised by low LC_{50} values in preliminary screening models (Marango *et al.*, 2017). These findings reinforce the classification of the *Tephrosia* genus as a critical natural reservoir of diverse biologically active secondary metabolites with promising anticancer potential.

Molecular docking analysis conducted in this study provides critical mechanistic insights into the observed cytotoxic activity of *Tephrosia maxima*. *In silico* evaluation of primary phytoconstituents-deguelin, tephrosin and lupeol, revealed

favourable binding orientations with the catalytic domain of the Epidermal Growth Factor Receptor (EGFR). As a well characterised oncogenic driver, EGFR is frequently over expressed in Oral Squamous Cell Carcinoma (OSCC) where its dysregulation orchestrates uncontrolled proliferation, enhanced survival, neoangiogenesis and metastatic progression. Among the ligands screened, deguelin exhibited the most robust binding affinity followed by tephrosin and lupeol. Analysis of the docking indicated that these interactions are stabilized by a network of hydrogen bonds and hydrophobic bonds within the highly conserved ATP- binding pocket of EGFR kinase domain.

These binding characteristics suggest a competitive inhibition mechanism that potentially eliminates receptor activation. These data imply that the antineoplastic efficacy of *T. maxima* may be mediated through the targeted disruption of EGFR dependent signaling cascades, thereby arresting tumour growth and survival.

The integration of cytotoxicity assays, colony formation analysis and molecular docking simulations provides preliminary evidence supporting the antineoplastic potential of *T. maxima*. The significant reduction in cell viability observed via MTT assay highlights potent acute cytotoxic effects, while the concomitant suppression of colony formation underscores the extracts' capacity to inhibit long term tumorigenic potential.

These experimental observations are further corroborated by *in silico* data which elucidate a plausible molecular mechanism centred on the targeted inhibition of EGFR.

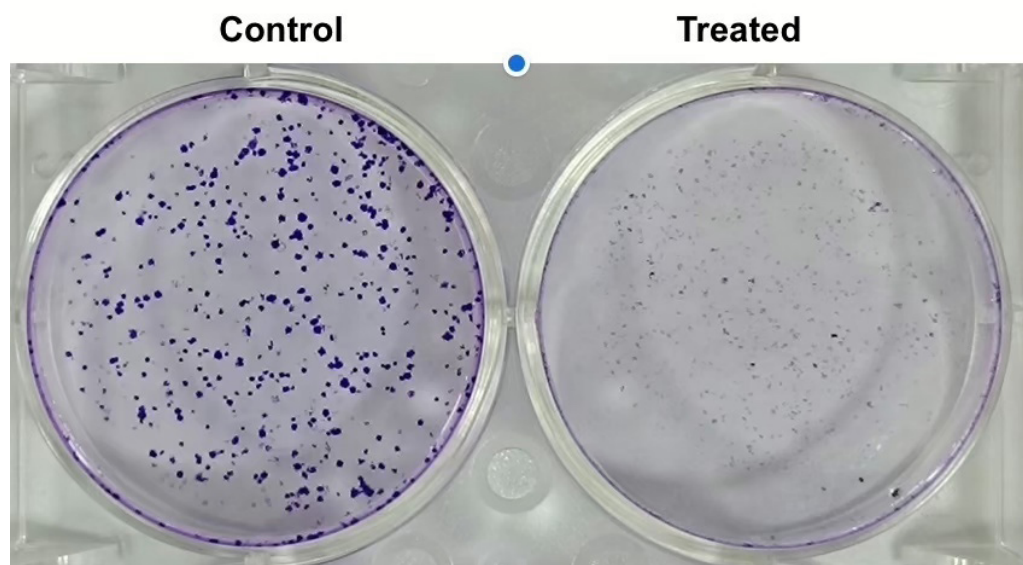


Figure 3: Effect of *Tephrosia maxima* extract on colony formation in KB cells. Treatment with extract significantly reduced the number and size of colonies compared to control, indicating inhibition of long-term proliferative capacity.

Collectively, these findings are consistent with the established pharmacological profiles of related *Tephrosia* species and underscore the therapeutic promise of *T. maxima* as a candidate for further drug development and targeted therapy in oral oncology.

Despite the promising results, several limitations of the current study warrant acknowledgement. The experimental evaluations were conducted exclusively *in vitro* utilising a single OSCC cell line (KB cells). These findings may not fully encapsulate the complex, multi-dimensional biological interactions and systemic pharmacokinetics inherent to *in vivo* environments. While the molecular docking analysis offers robust predictive data regarding ligand receptor affinity, these *in silico* interactions require empirical validation through molecular and biochemical studies.

Future investigations should prioritise the bioassay guided fractionation and structural characterisation of the primary bioactive constituents within *T. maxima*. Additionally, comprehensive studies are required to elucidate the specific molecular pathways beyond EGFR that may be modulated by these compounds followed by evaluation of therapeutic efficacy and safety profiles in preclinical animal models.

CONCLUSION

The present study highlights *Tephrosia maxima* as a potent pharmacological candidate against Oral Squamous Cell Carcinoma (OSCC). Molecular docking simulations which revealed that key phyto constituents specifically deguelin,

tephrosin and lupeol exhibit high binding affinities for the Epidermal Growth Factor Receptor (EGFR). This interaction points towards a probable mechanism of action involving the disruption of EGFR mediated signaling cascades, which are frequently overexpressed in oral malignancies. Supporting these *in silico* findings, *in vitro* evaluation demonstrated a significant dose-dependent reduction in neoplastic cell viability, characterized by an IC_{50} value of approximately 96 $\mu\text{g/mL}$. Beyond immediate cytotoxicity the extract markedly suppressed the colony forming potential of OSCC cells suggesting a robust inhibition of long term proliferative pathways essential for tumour progression.

While these findings underscore the potential of *T. maxima* as a rich source of bioactive photochemicals with anti-proliferative properties, the scope of the present study remains limited to *in vitro* assays and computational modeling. Consequently, the transition from laboratory procedures to clinical application necessitates further rigorous investigation including *in vivo* studies to assess systemic efficacy and safety as well as detailed mechanistic evaluations to fully elucidate the apoptosis mechanisms or cell cycle arrest pathways involved. Such validation is essential to confirm the therapeutic viability of *T. maxima* and to establish its role as a herbal adjunct in the comprehensive management of oral squamous cell carcinoma.

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ABBREVIATIONS

MAPK: Mitogen-Activated Protein Kinase; **PI3K/Akt:** Phosphoinositide 3-Kinase/Protein Kinase B; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **ATP:** Adenosine Triphosphate; **PBS:** Phosphate-Buffered Saline.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study evaluates the anticancer potential of *Tephrosia maxima* against Oral Squamous Cell Carcinoma (OSCC) cell lines using an integrated *in silico* and *in vitro* approach. Initially, molecular docking analysis demonstrated that key phytoconstituents like tephrosin, deguelin and lupeol exhibited favorable binding affinity towards epidermal Growth Factor Receptor (EGFR), suggesting possible inhibition of oncogenic signalling pathways.

Based on these findings, the biological activity of *T. maxima* extract was further assessed in KB oral cancer cells. The MTT assay revealed a dose dependent reduction in cell viability with an IC_{50} of approximately 96 μ g/mL. Additionally, clonogenic assays showed a significant decrease in colony forming ability, indicating impaired long-term proliferative capacity.

Collectively, the results suggest that *T. maxima* possesses notable cytotoxic and antiproliferative effects, potentially mediated through EGFR inhibition. These findings highlight its promise as a source of bioactive compounds for future development of adjunctive therapies in oral cancer, although further *in vivo* and other advanced studies are required.

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