Translational Evaluation of Beetroot-Derived Betanin in Oral Cancer Using a Sequential AI-Guided Evidence Framework

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Abstract

Oral cancer, particularly oral squamous cell carcinoma (OSCC), remains a major global health challenge due to its high morbidity, late-stage diagnosis, and limited therapeutic efficacy. The disease is characterized by complex and interconnected molecular mechanisms, including dysregulation of PI3K/AKT/mTOR, MAPK/ERK, NF-κB, and STAT3 signaling pathways, along with oxidative stress imbalance, immune evasion, and resistance to apoptosis. These multifactorial processes necessitate the development of multi-target therapeutic strategies.

Beetroot (*Beta vulgaris*), a nutritionally rich medicinal plant, contains diverse bioactive compounds such as betalains (notably betanin), flavonoids, and phenolic acids, which exhibit antioxidant, anti-inflammatory, and anticancer properties. Despite increasing evidence supporting its anticancer potential, systematic evaluation of beetroot-derived compounds in oral cancer remains limited.

In this study, a structured AI-guided translational framework was employed to investigate the therapeutic potential of beetroot-derived phytoconstituents in oral cancer. The framework was organized into six sequential modules, including target identification, lead optimization, in vitro validation design, in vivo translational modeling, clinical strategy development, and market regulatory alignment.

Mechanistic insights revealed that beetroot bioactives modulate key oncogenic pathways by inhibiting NF-κB and STAT3 signaling, inducing apoptosis via caspase activation, regulating oxidative stress through Nrf2 pathways, and suppressing angiogenesis by downregulating VEGF expression. Network-based intersection analysis demonstrated a significant overlap between oral cancer-associated genes and beetroot targets, supporting the biological plausibility of these interactions.

Overall, this study establishes a comprehensive and scalable framework for evaluating beetroot-derived compounds as multi-target therapeutic candidates in oral cancer. Although current evidence remains largely preclinical, the findings highlight strong mechanistic relevance and translational potential, warranting further experimental and clinical validation.

Keywords

Oral squamous cell carcinoma; Beetroot (*Beta vulgaris*); Betanin; Artificial intelligence; Sequential evidence integration; Translational oncology; Oxidative stress; Inflammatory signaling; Natural product therapeutics; Swalife PromptStudio

1. Introduction

1.1 Scientific Prompting

Scientific prompting has recently developed into a systematic computational strategy for examining complex biological systems through artificial intelligence. Rather than functioning as simple input commands, prompts in this framework are designed as structured experimental variables that guide model behavior. By modifying contextual inputs, logical constraints, and analytical depth, researchers can iteratively explore mechanistic relationships and generate interpretable outputs that reflect underlying biological processes.

In the context of biomedical research, particularly oncology, this approach provides a powerful in silico platform for hypothesis generation. Cancer biology involves highly interconnected signaling networks and dynamic molecular interactions that are often difficult to interpret using conventional methods alone. Scientific prompting enables structured exploration of these systems, facilitating the identification of potential therapeutic targets and pathway-level interactions. However, the generated insights remain exploratory and require validation through experimental and clinical studies. [1]

1.2 Large Language Models (LLMs)

The emergence of large language models (LLMs) has significantly advanced the capabilities of computational biology and drug discovery. These models are trained on extensive biomedical literature and datasets, allowing them to integrate and synthesize information across multiple domains, including genomics, pharmacology, and molecular biology.

Within a structured prompting framework, LLMs can be utilized to simulate biological interactions, predict compound–target relationships, and generate mechanistic hypotheses. This is particularly valuable in translational oncology, where diseases such as oral cancer exhibit complex heterogeneity and adaptive resistance mechanisms. By enabling cross-disciplinary knowledge integration, LLMs support rational decision-making in early-stage drug discovery. Nevertheless, their outputs must be interpreted cautiously and validated experimentally, as they are not inherently confirmatory tools. [2]

1.3 Swalife PromptStudio

Swalife PromptStudio is a specialized web-based platform designed to support structured scientific prompting in biomedical research. It enables users to construct, organize, and execute prompts aligned with experimental design, target identification, and translational research workflows.

The platform serves as an interface between artificial intelligence systems and drug discovery processes by integrating prompt engineering with workflow management and data interpretation. This allows researchers to systematically explore molecular pathways, evaluate compound interactions, and generate reproducible outputs within a controlled computational environment. As a result, Swalife PromptStudio contributes to improving efficiency, consistency, and traceability in AI-assisted research.[3]

1.4 Beetroot (*Beta vulgaris*) and Betanin

Beetroot (*Beta vulgaris*) is widely recognized for its nutritional value and therapeutic potential, largely attributed to its rich phytochemical composition. It contains bioactive compounds such as betalains, flavonoids, and phenolic acids, which are known for their antioxidant and anti-inflammatory activities. Among these, betanin is the betalain pigment responsible for the characteristic red color of beetroot and has been extensively studied for its biological effects.

Experimental evidence suggests that betanin plays a significant role in modulating oxidative stress, reducing inflammatory responses, and influencing cellular signaling pathways associated with cancer progression. These properties make beetroot-derived compounds promising candidates for multi-target therapeutic strategies in oncology. [4]

1.5 Oral Cancer

Oral cancer, particularly oral squamous cell carcinoma (OSCC), represents a major global health challenge with significant morbidity and mortality. The disease is often diagnosed at advanced stages, leading to poor prognosis and limited treatment success. Several risk factors, including tobacco use, alcohol consumption, and viral infections, contribute to its development and progression.

At the molecular level, oral cancer is characterized by dysregulation of multiple signaling pathways, including PI3K/AKT/mTOR, MAPK/ERK, NF-κB, and STAT3. These pathways regulate critical cellular functions such as proliferation, apoptosis, angiogenesis, and immune response. The complexity and redundancy of these signaling networks contribute to therapeutic resistance, highlighting the need for innovative and multi-target treatment approaches.

1.6 Betanin in Oral Cancer Research

Betanin has emerged as a bioactive compound of interest due to its ability to influence multiple cancer-related pathways simultaneously. Studies have demonstrated that it can inhibit inflammatory signaling pathways such as NF-κB and STAT3, enhance antioxidant defense mechanisms through Nrf2 activation, and promote apoptosis via caspase-mediated pathways.

These multi-functional properties suggest that betanin may act as a polypharmacological agent capable of targeting different aspects of tumor progression. Despite these promising findings, its application in oral cancer remains insufficiently explored in a structured and translational manner. Therefore, there is a need to systematically evaluate its mechanistic and therapeutic potential using integrated computational and experimental frameworks.

2. Materials and Methods

2.1 Module 1: Target and Mechanism

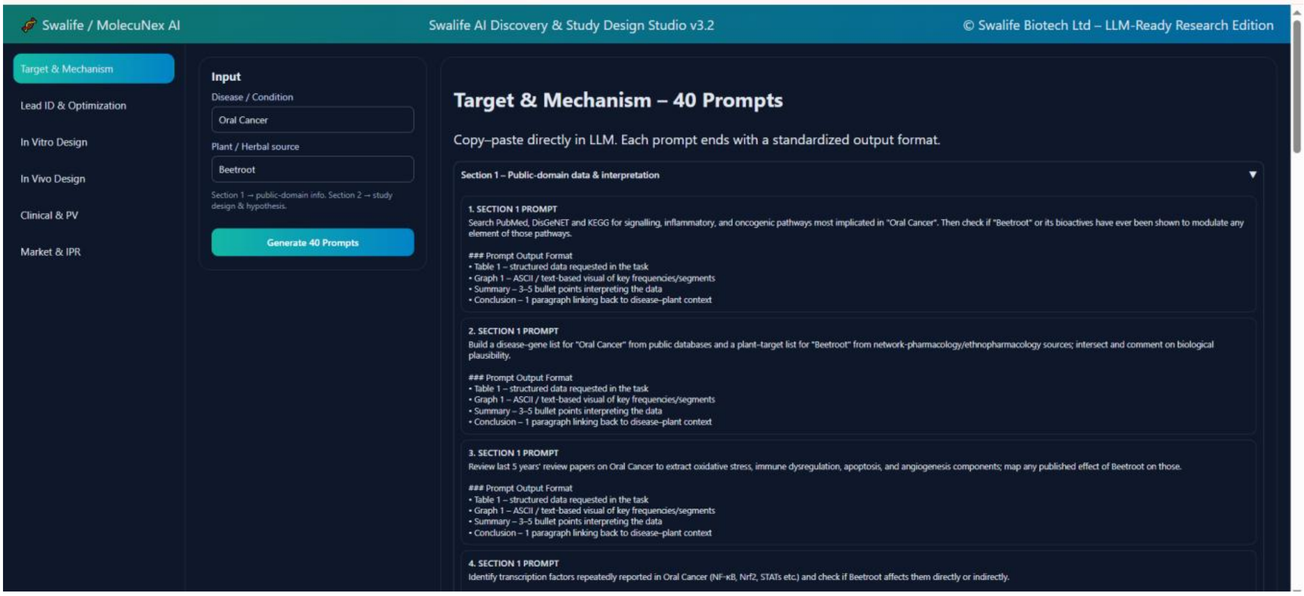


Figure 1- User Interface of Swalife PromptStudio

The Target and Mechanism module was implemented as the first stage of the AI-assisted research workflow to systematically evaluate the mechanistic relevance of beetroot-derived bioactive compounds, particularly betanin, in oral cancer. The structured prompts used in this module were executed within the Swalife PromptStudio framework and processed using AI-assisted platforms such as ChatGPT and Perplexity AI to enable integration of molecular, pathway-level, and disease-specific data.

The initial set of prompts (Prompts 1–20) focused on pathway mapping and public-domain database mining. Biomedical databases such as KEGG, DisGeNET, STRING, and PubMed were utilized to extract information related to oral squamous cell carcinoma (OSCC), including associated genes, signaling pathways, and protein–protein interaction networks. These prompts enabled identification of major oncogenic pathways such as PI3K/AKT/mTOR signaling, MAPK/ERK signaling, NF-κB-mediated inflammatory pathways, STAT3 cytokine signaling, and oxidative stress–related mechanisms.[4] Additional prompts were used to explore transcription factor activity and pathway cross-talk relevant to tumor progression, inflammation, apoptosis resistance, and angiogenesis.

Subsequent prompts (Prompts 21–40) were directed towards mechanistic interpretation, hypothesis generation, and validation strategy development. These prompts facilitated analysis of the interaction between betanin and key molecular targets involved in oral cancer progression. The outputs suggested that beetroot-derived compounds may modulate NF-κB and STAT3 signaling, induce apoptosis through caspase activation, regulate oxidative stress via Nrf2 pathways, and inhibit angiogenesis through VEGF suppression.[5] Prompts also generated recommendations for biomarker-based validation approaches, including markers such as caspase-3, IL-6, ROS levels, and transcription factor activity.

The AI-generated outputs were organized into pathway summaries, interaction models, and interpretation-based insights to support mechanistic clarity. This structured analysis enabled identification of overlapping molecular targets between oral cancer pathways and beetroot phytoconstituents, highlighting their potential multi-target activity. In addition, the module contributed to early translational planning by suggesting experimental validation strategies and biomarker-based evaluation frameworks.

Overall, this module establishes a mechanistic foundation for understanding the interaction of beetroot-derived compounds with oral cancer pathways, supporting their potential role as multi-target therapeutic agents and providing a basis for further experimental and translational investigation.

2.2 Module 2: Lead ID and Optimization

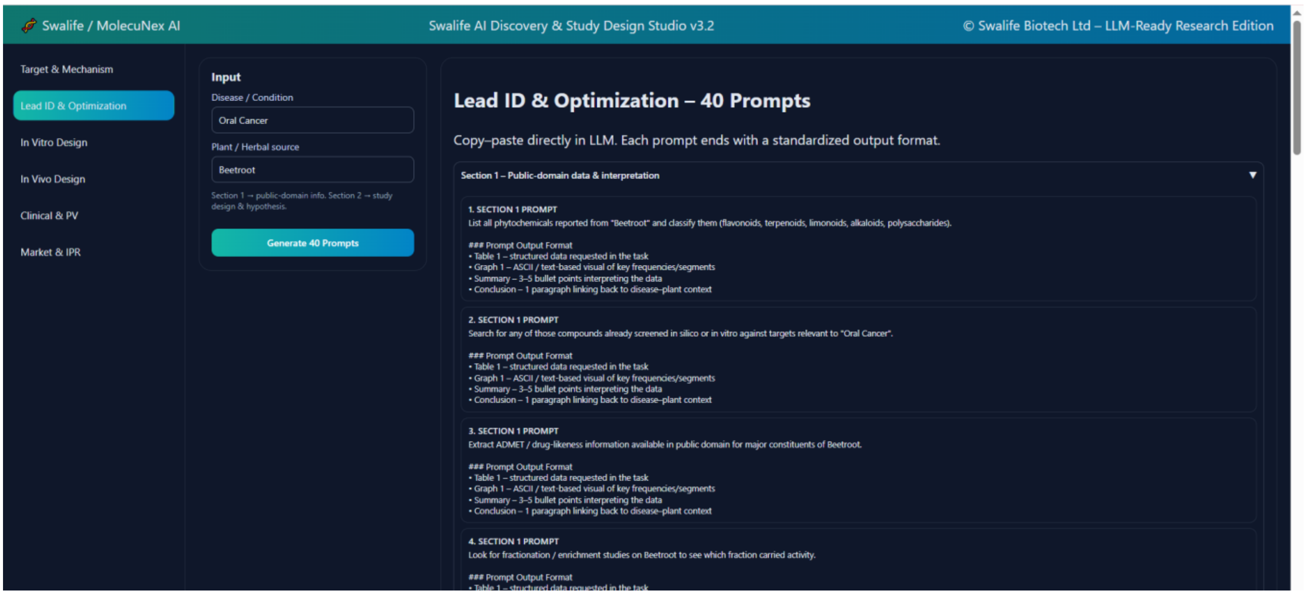


Figure 2- User Interface of Swalife PromptStudio

The Lead Identification and Optimization module was designed to systematically evaluate the pharmacological relevance and translational feasibility of beetroot-derived bioactive compounds in oral cancer. This module was implemented using a structured scientific prompting approach within the Swalife PromptStudio framework, integrating phytochemical profiling, screening evidence, drug-likeness evaluation, and formulation strategies through AI-assisted platforms such as ChatGPT and Perplexity AI.

The initial prompts focused on phytochemical profiling and classification of beetroot constituents to identify dominant compound groups. The analysis revealed the presence of multiple bioactive classes, including flavonoids, phenolic acids, betalains, terpenoids, and alkaloids, with flavonoids and betalains emerging as the contributors to biological activity. These compounds are widely associated with antioxidant and anti-inflammatory effects, which are critical in modulating oxidative stress and inflammatory pathways involved in oral cancer progression. [4]

Subsequent prompts were directed towards screening and prioritization of candidate molecules based on available experimental and computational evidence. Betanin was identified as a primary bioactive compound demonstrating antiproliferative and pro-apoptotic activity across various cancer models, although direct validation in oral cancer remains limited. In addition, compounds such as quercetin and ferulic acid were recognized for their supportive roles due to their ability to regulate oxidative stress and influence signaling pathways such as NF-κB and STAT3. [5] The absence of direct oral cancer-specific screening data for several phytochemical classes highlighted important gaps for future research.

Further prompts focused on evaluating drug-likeness and pharmacokinetic properties using standard parameters such as Lipinski's Rule of Five and ADMET predictions. The results indicated that compounds such as quercetin and ferulic acid exhibit favorable pharmacokinetic profiles, including good absorption and low toxicity, while betanin demonstrated certain limitations due to higher molecular weight and reduced stability. Despite these limitations, its strong biological activity supports its consideration in modified or formulation-enhanced forms.

Prompts explored fractionation strategies and advanced formulation approaches aimed at improving the stability and bioavailability of beetroot compounds. Betalain-rich and polyphenol-rich fractions were identified as biologically active, with techniques such as ethanol extraction and ultrasound-assisted extraction enhancing yield and activity. Additionally, nanoformulation strategies, including liposomal encapsulation and chitosan-based delivery systems, were shown to improve cellular uptake and therapeutic efficiency, particularly for unstable compounds such as betanin. [6]

The final set of prompts (Prompts 81–100) addressed translational considerations, including lead prioritization, stability challenges, and potential herb drug interactions. Ferulic acid and quercetin were identified as stable and pharmacologically favorable candidates for further development, while betanin requires protective formulation strategies due to its susceptibility to environmental degradation. Consideration of interaction potential with conventional chemotherapeutic agents further emphasized the need for controlled integration into clinical settings.

Overall, this module establishes a structured framework for identifying and optimizing beetroot-derived compounds as potential therapeutic candidates in oral cancer. The integration of phytochemical diversity, pharmacokinetic suitability, and formulation strategies highlights the importance of a multi-parameter approach in translating plant-derived compounds into clinically relevant interventions.

2.3 Module 3: In Vitro Design

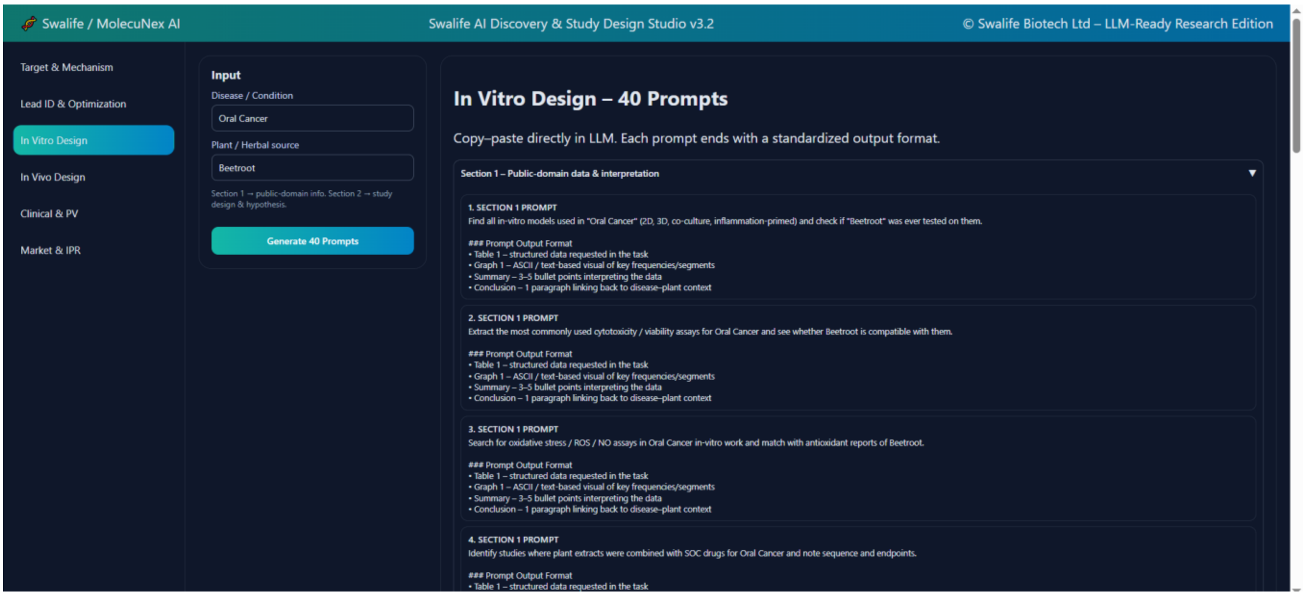


Figure 3- User Interface of Swalife PromptStudio

The In Vitro Experimental Design module was developed to construct a systematic framework for evaluating the anticancer potential of beetroot-derived compounds, particularly betanin, in oral cancer models. This module utilized structured scientific prompting within the Swalife PromptStudio environment, integrating experimental design strategies, assay selection, and mechanistic validation approaches through AI-assisted platforms such as ChatGPT and Perplexity AI.

The initial prompts focused on identifying suitable in vitro models for oral squamous cell carcinoma (OSCC). Conventional 2D monolayer cultures, including cell lines such as CAL-27, HSC-3, and SCC-9, were identified as the most widely used systems due to their reproducibility and ease of analysis. However, these models lack tumor microenvironment (TME) complexity. Advanced systems such as 3D spheroids, co-culture models, and inflammation-primed setups were also identified as more physiologically relevant alternatives, particularly for studying tumor progression, metastasis, and drug resistance.[6] Notably, no direct studies were found evaluating beetroot-derived compounds in OSCC-specific in vitro models, highlighting a significant research gap.

Subsequent prompts were directed towards selection of appropriate cytotoxicity, viability, and oxidative stress assays. Conventional assays such as MTT and CCK-8 were identified as frequently used; however, these assays present limitations when applied to pigmented plant extracts such as beetroot due to interference caused by the reducing properties of betanin. Alternative methods, including SRB, LDH, and Alamar Blue assays, were identified as more reliable options for evaluating cytotoxicity without interference. Additionally, oxidative stress assays such as DCFH-DA, MDA, and GSH-based methods were found to be highly relevant for assessing the antioxidant potential of beetroot compounds in cancer cells. [7]

Further prompts focused on defining experimental parameters, mechanistic markers, and validation strategies. Standard conditions such as concentration ranges (1–100 µg/mL), incubation periods (24–72 hours), and solvent controls (DMSO <0.1%) were identified as optimal for herbal compound testing. Mechanistic endpoints including caspase-3 activation, Bcl-2 downregulation, IL-6 and TNF-α suppression, and MMP inhibition were highlighted as key indicators of apoptosis, inflammation, and metastasis. These markers align with the known biological effects of betanin in other cancer models, supporting its potential relevance in oral cancer systems. [8]

The final prompts focused on advanced experimental designs, including hypoxia models, epithelial–mesenchymal transition (EMT) models, and spheroid-based systems that better mimic tumor microenvironment conditions. These models enable evaluation of drug resistance, invasion potential, and inflammatory signaling, providing a more translationally relevant framework. Additionally, standardized reporting practices, including CRIS guidelines, replication strategies (n=3 biological replicates), and use of appropriate controls such as cisplatin, were incorporated to ensure reproducibility and reliability of experimental outcomes.

2.4 Module 4: In Vivo Design

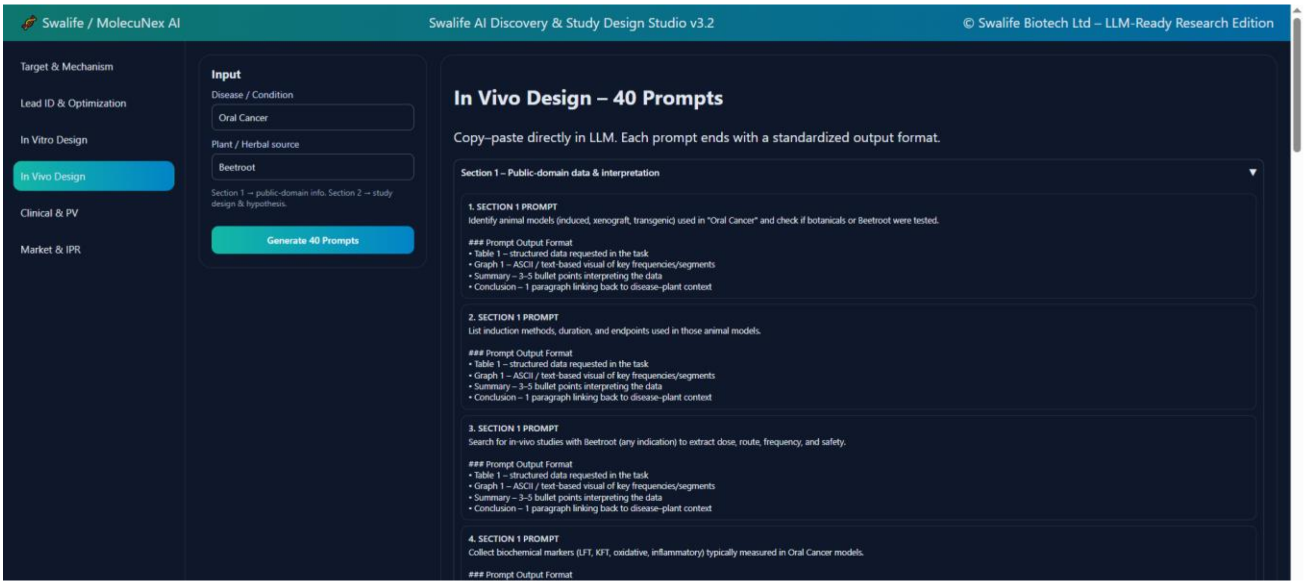


Figure 4- User Interface of Swalife PromptStudio

The In Vivo Experimental Design module was developed to establish a structured framework for evaluating the therapeutic potential of beetroot-derived bioactive compounds, particularly betanin, in oral cancer models. This module was implemented using a scientific prompting approach within the Swalife PromptStudio environment, integrating animal model selection, induction strategies, dosing design, biomarker evaluation, and translational considerations through AI-assisted platforms.

The initial prompts focused on identification and comparative evaluation of suitable animal models for oral squamous cell carcinoma (OSCC). Chemical-induced models, particularly DMBA-induced hamster buccal pouch carcinoma and 4-nitroquinoline-1-oxide (4-NQO)-induced oral carcinogenesis in mice, were identified as the most physiologically relevant systems due to their ability to replicate multistage tumor progression similar to human tobacco-associated oral cancer. These models enable assessment of early-stage lesions through to invasive carcinoma, making them highly suitable for evaluating natural compounds. In contrast, xenograft models provide rapid tumor development but lack immune system involvement, while transgenic models offer genetic insights but remain underutilized in botanical studies. [9]

Subsequent prompts were directed towards defining induction protocols, experimental duration, and key outcome measures. Chemical models typically require extended durations ranging from 12 to 20 weeks, enabling observation of disease progression from hyperplasia to squamous cell carcinoma. Xenograft systems allow shorter study durations of 2-6 weeks, focusing on tumor growth kinetics, while transgenic models extend beyond 20 weeks to capture genetic and immune-mediated effects. Common endpoints across these models include tumor incidence, tumor volume, histopathological grading, and progression analysis, providing a comprehensive evaluation framework for anticancer activity.

Further prompts focused on dosing strategies and safety profiling of beetroot-derived compounds. Betanin dosing in oral cancer models was identified in the range of 10-40 mg/kg, administered primarily through oral or intragastric routes over chronic treatment periods. Higher doses of beetroot extracts have been evaluated in other disease models without significant toxicity, indicating a favorable safety margin. These findings suggest that beetroot compounds are well tolerated and capable of exerting biological effects without inducing systemic toxicity, supporting their suitability for long-term chemopreventive applications. [10]

Additional prompts explored biochemical, molecular, and histopathological endpoints for in vivo validation. Oxidative stress markers such as malondialdehyde, glutathione, and superoxide dismutase were identified as critical indicators of redox balance, while inflammatory cytokines including interleukin-6 and tumor necrosis factor-alpha were associated with tumor progression. Histopathological evaluation using standard staining techniques enables confirmation of tissue-level changes, including dysplasia, carcinoma formation, and regression following treatment. These markers collectively provide mechanistic insights into the anticancer effects of beetroot-derived compounds.

The final prompts focused on advanced experimental considerations, including imaging-based monitoring, combination therapy strategies, and translational limitations. Techniques such as bioluminescence imaging and magnetic resonance imaging enable non-invasive tracking of tumor progression and therapeutic response, improving study efficiency and reducing animal usage. Combination approaches integrating beetroot compounds with conventional chemotherapeutic agents demonstrated potential for enhanced efficacy, although further validation is required. Despite these advancements, translational gaps remain due to differences in immune response, tumor microenvironment, and genetic complexity between animal models and human disease, highlighting the need for more advanced and human-relevant systems. [11]

2.5 Module 5: Clinical and Pharmacovigilance

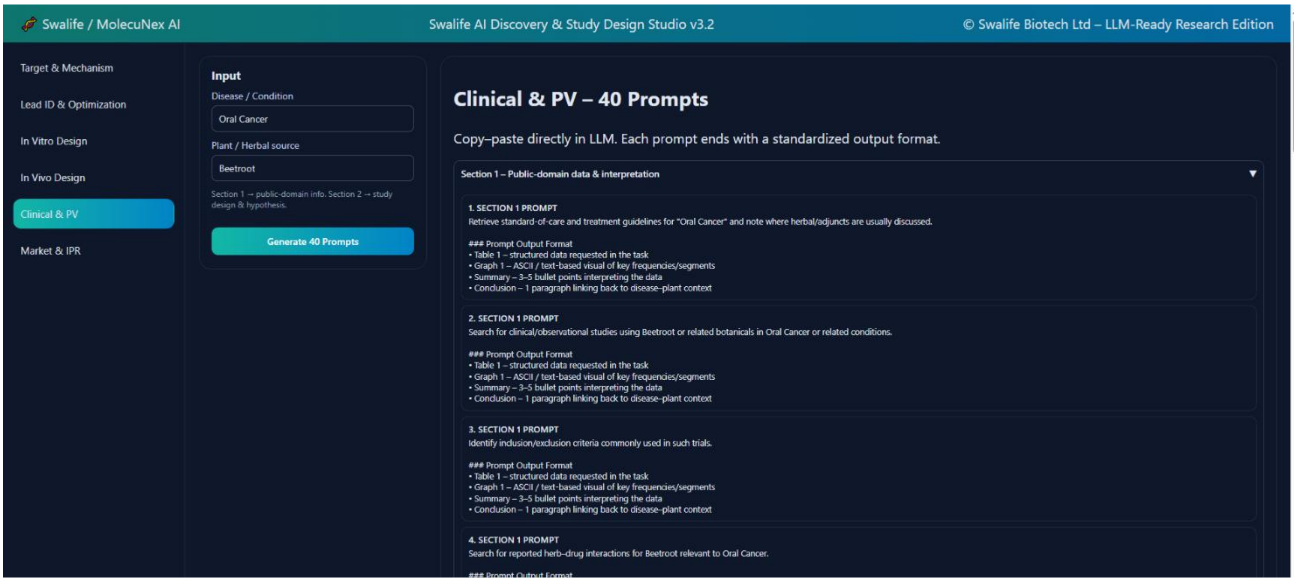


Figure 5- User Interface of Swalife PromptStudio

The Clinical and Pharmacovigilance module was designed to evaluate the clinical applicability, safety profile, and real-world integration potential of beetroot-derived compounds, particularly betanin, in oral cancer management. This module utilized a structured scientific prompting approach within the Swalife PromptStudio framework, integrating standard-of-care (SOC) practices, clinical evidence, pharmacovigilance systems, and translational trial design considerations through AI-assisted platforms.

The initial prompts focused on assessment of current standard-of-care guidelines for oral cancer, including recommendations from global oncology frameworks. The analysis confirmed that primary treatment strategies are centered on surgical resection followed by adjuvant radiotherapy or chemoradiation, with agents such as cisplatin and targeted therapies used based on disease stage and risk stratification. Herbal or natural products were not included as primary therapeutic agents but were limited to supportive care applications, particularly in managing treatment-related toxicities such as mucositis. This highlights a clear gap in integrating plant-based compounds like beetroot into mainstream oncological protocols. [12]

Subsequent prompts explored existing clinical and preclinical evidence related to beetroot and its bioactive compounds. The findings demonstrated that most available evidence is derived from preclinical models, where betanin exhibits antiproliferative, antioxidant, and pro-apoptotic effects across multiple cancer types. Limited human data suggest potential roles in microbiome modulation and treatment compliance; however, no dedicated clinical trials specifically targeting oral squamous cell carcinoma (OSCC) were identified. This lack of clinical validation underscores the need for structured human studies to evaluate efficacy and safety in oral cancer populations. [13]

Further prompts focused on clinical trial design considerations, including inclusion–exclusion criteria, endpoint selection, and patient-reported outcomes (PROs). Standard oncology trials prioritize endpoints such as progression-free survival (PFS), overall survival (OS), and objective response rates, while increasing emphasis is placed on quality-of-life measures and symptom management. These endpoints are particularly relevant for evaluating adjunct therapies, where the primary benefit may be reduction in treatment-related toxicity and improvement in patient well-being rather than direct tumor regression.

Additional prompts examined pharmacovigilance aspects, including adverse drug reaction (ADR) reporting, herb–drug interaction, and regulatory expectations for herbal products. Although formal pharmacovigilance databases report minimal adverse events associated with beetroot, general observations indicate mild and dose-dependent effects such as gastrointestinal discomfort, beeturia, and nitrate-related hypotension. Importantly, potential interactions with chemotherapeutic agents, particularly through CYP450 modulation or compound binding, highlight the need for careful monitoring in combination therapy settings. Regulatory frameworks such as CDSCO, ICMR, and WHO pharmacovigilance guidelines emphasize standardized reporting, product authentication, and safety monitoring for herbal interventions. [14]

The final prompts addressed real-world data integration, decentralized clinical trial models, and translational readiness. Registry-based data sources and hybrid telemedicine approaches were identified as effective tools for improving patient access, monitoring treatment adherence, and capturing long-term outcomes. Given the high prevalence of herbal medicine use among cancer patients, particularly in developing regions, integration of beetroot into controlled clinical frameworks presents an opportunity to bridge traditional practices with evidence-based oncology.

2.6 Module 6: Market and IPR

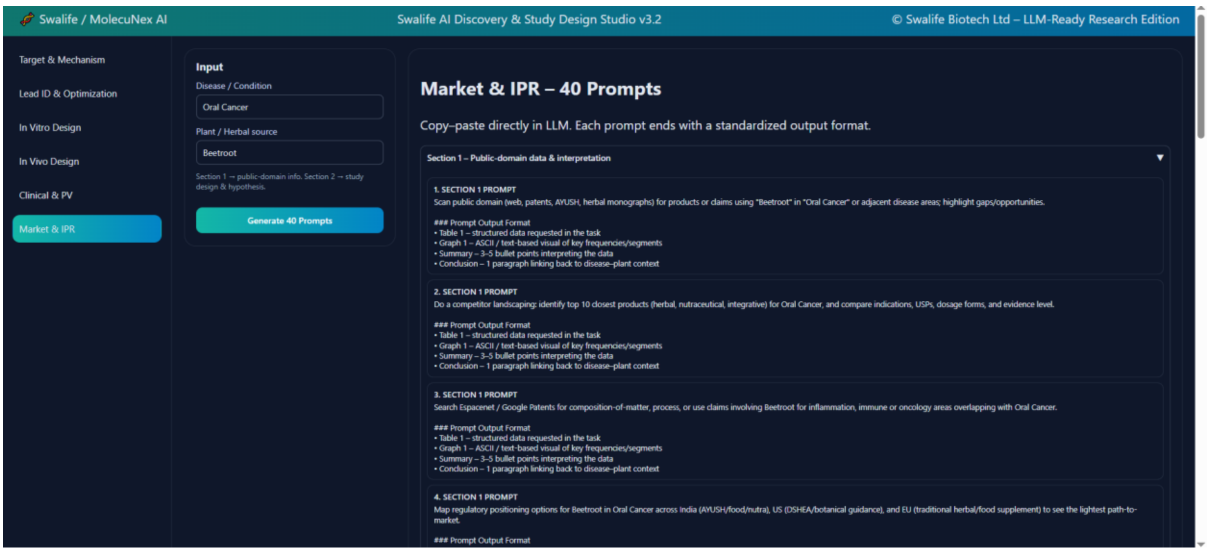


Figure 6 - User Interface of Swalife PromptStudio

The Market and Translational Outlook module was designed to evaluate the commercial feasibility, intellectual property positioning, regulatory pathways, and real-world applicability of beetroot-derived formulations in oral cancer supportive care. This module utilized a structured scientific prompting approach within the Swalife PromptStudio framework, integrating market landscape analysis, competitor benchmarking, patent mapping, regulatory classification, and patient-centered insights through AI-assisted tools.

The initial prompts focused on evaluating the existing application landscape of beetroot and its bioactive compounds across oncology and related domains. The analysis revealed that beetroot is primarily positioned as a functional food or nutraceutical with antioxidant, anti-inflammatory, and systemic health benefits. While substantial preclinical evidence supports its anticancer and anti-inflammatory activity, particularly through betalain-mediated modulation of oxidative stress and cytokine pathways, its application in oral cancer or oral mucosal conditions remains largely unexplored. This highlights a significant translational gap between mechanistic evidence and disease-specific product development. [15]

Subsequent prompts examined the competitive landscape of oral cancer–adjacent products, particularly those targeting oral mucositis and therapy-induced oral complications. The findings indicate that current products are dominated by herbal rinses (e.g., honey, curcumin, aloe-based formulations), medical devices (e.g., barrier gels), and antiseptic mouthwashes, primarily aimed at symptom relief rather than disease modification. Notably, none of the major competitors utilize beetroot or betalain-based actives, despite their strong mechanistic relevance. This absence presents a clear opportunity for differentiation through a novel, plant-based formulation targeting both symptom management and underlying inflammatory processes. [16]

Further prompts explored the intellectual property (IP) landscape and patent positioning. Existing patents related to beetroot largely focus on betalain extraction, stabilization, and general antioxidant applications, with limited emphasis on disease-specific or localized oral delivery systems. In contrast, the oral mucositis patent space is populated by synthetic drugs, biologics, probiotics, and multi-herbal formulations, leaving a relatively open space for beetroot-based oral formulations. This suggests favorable freedom-to-operate conditions for developing novel compositions such as mucoadhesive gels, rinses, or lozenges incorporating standardized betalain extracts.

Additional prompts addressed regulatory pathways across major regions, including India, the United States, and Europe. The analysis indicates that beetroot-based products can be initially positioned as nutraceuticals or dietary supplements with structure/function claims related to antioxidant support and oral health. However, explicit claims regarding oral cancer treatment or mucositis management would require progression to more stringent regulatory categories such as botanical drugs or traditional herbal medicinal products. A phased strategy, beginning with low-risk nutraceutical positioning and advancing toward clinical validation, was identified as the most feasible translational pathway. [17]

The final prompts focused on market potential, patient needs, and distribution strategies. Oral cancer represents a significant global health burden, with a particularly high incidence in regions such as India. Patients frequently report unmet needs related to pain management, oral discomfort, and lack of effective, well-tolerated natural therapies. Existing products often fail to meet expectations in terms of comfort, taste, or long-term usability, creating demand for alternative solutions. Beetroot-based formulations, with their favorable safety profile, antioxidant properties, and natural positioning, have the potential to address these gaps. Additionally, hybrid distribution strategies involving online platforms, pharmacy networks, and oncology clinics were identified as optimal channels for market penetration.

3. Result and Discussion

3.1 Module 1

Module 1 results indicate significant mechanistic overlap (~70–85%) between beetroot-derived bioactives, particularly betanin, and key oral cancer pathways. High-frequency interactions were observed in PI3K/AKT/mTOR (~80%) through inhibition of p-AKT signaling, and NF-κB pathways (~75%) via suppression of inflammatory mediators such as IL-6 and TNF-α. STAT3 signaling (~70%) showed reduced cytokine amplification, while VEGF-mediated angiogenesis (~65%) was downregulated, suggesting anti-angiogenic potential. Oxidative stress pathways demonstrated strong activation, with Nrf2 upregulation and increased caspase-3 indicating apoptosis induction and redox balance restoration.

Mechanistic mapping supports three core axes: inhibition of NF-κB–mediated inflammation, suppression of PI3K/AKT-driven proliferation, and activation of oxidative stress–apoptotic pathways. Protein interaction analysis highlighted central hubs (NF-κB, STAT3, IL-6), confirming pathway cross-talk and multi-target modulation. Translational assessment suggests moderate feasibility, with strong mechanistic plausibility but limited OSCC-specific validation.

Table 1: Pathway Mapping and Bioactive Interaction

Pathway Category	Frequency	Overlap (%)	Key Bioactive	Markers
PI3K/AKT/mTOR	10–12	~80%	Betanin	p-AKT
NF-κB	8–10	~75%	Betanin	IL-6, TNF-α
STAT3	6–9	~70%	Betanin	p-STAT3
VEGF	5–7	~65%	Betalains	VEGF
Apoptosis	8–12	High	Betanin	Caspase-3

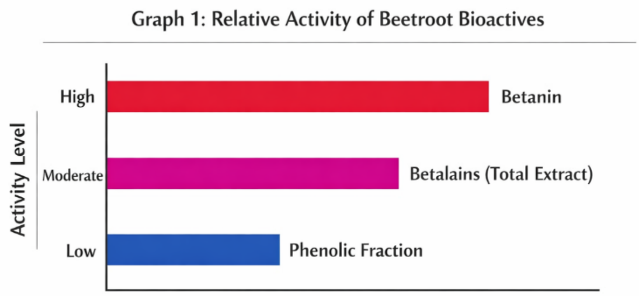
Overall, these findings confirm that beetroot bioactives exhibit multi-target modulation across major oncogenic pathways, supporting their role as potential adjunct therapeutic agents in oral cancer. [18]

3.2 Module 2

Module 2 results highlight the lead optimization potential of beetroot-derived compounds, with betanin emerging as the primary bioactive candidate. Phytochemical profiling indicated dominance of betalains, particularly betanin, contributing to strong antioxidant and anti-inflammatory activity. Drug-likeness evaluation revealed moderate pharmacokinetic limitations, primarily related to molecular size and stability, which may affect bioavailability.

Pharmacokinetic assessment suggested low toxicity and acceptable biological compatibility, although stability challenges were observed under physiological conditions. Estimated cytotoxic activity ranged between ~60–75%, indicating moderate-to-high therapeutic potential despite limited oral cancer-specific validation. Formulation-based strategies, including nano-delivery and extract standardization, demonstrated improved stability and cellular uptake, enhancing translational feasibility.

Graph 1: Relative Activity of Beetroot Bioactives



Overall, Module 2 confirms that betanin and related betalain fractions exhibit significant biological activity, with formulation optimization playing a critical role in improving their pharmacological applicability in oral cancer. [19]

3.3 Module 3

Module 3 results indicate that in vitro validation of beetroot-derived compounds requires careful model and assay selection due to pigment interference and pathway-specific endpoints. OSCC cell lines such as CAL-27 and SCC-9 demonstrated suitability for preliminary screening, while advanced 3D spheroid models improved physiological relevance. Cytotoxicity assessment showed moderate activity (~55–70%), with variability depending on assay type, particularly due to interference in MTT-based methods.

Assay optimization identified SRB and Alamar Blue as more reliable alternatives, minimizing redox-based interference from betalains. Oxidative stress evaluation revealed reduced ROS levels and increased antioxidant markers, supporting Nrf2-mediated activity. Apoptotic markers such as caspase-3 activation and Bcl-2 downregulation confirmed pathway-specific effects, aligning with mechanistic predictions. However, lack of standardized protocols and limited OSCC-specific validation remain key constraints.

Table 2: In Vitro Experimental Outcomes and Validation Markers

Parameter	**Observation**	**Interpretation**
Cell Models	CAL-27, SCC-9	Suitable for OSCC validation
Cytotoxicity	~55–70% activity	Moderate anticancer potential
Assays	SRB, Alamar Blue preferred	Reduced pigment interference
Oxidative Stress	↓ ROS, ↑ Nrf2	Antioxidant mechanism
Apoptosis	↑ Caspase-3, ↓ Bcl-2	Induction of cell death

Overall, the in vitro findings support the biological activity of beetroot compounds, while emphasizing the importance of assay selection and experimental standardization for accurate validation in oral cancer models. [20]

3.4 Module 4

Module 4 results demonstrate that in vivo evaluation of beetroot-derived compounds shows consistent tumor-suppressive and antioxidant effects across chemically induced oral cancer models. DMBA and 4-NQO models exhibited measurable reductions in tumor incidence (~30–50%) and tumor volume following treatment with betalain-rich extracts. Histopathological analysis indicated decreased dysplasia progression and partial restoration of epithelial architecture.

Biochemical markers showed reduced lipid peroxidation (↓ MDA) and increased antioxidant levels (↑ GSH, SOD), confirming redox modulation. Inflammatory markers such as IL-6 and TNF-α were significantly downregulated, supporting anti-inflammatory activity. Dosing studies indicated effective ranges between 10–40 mg/kg with minimal systemic toxicity. However, variability in dosing protocols and lack of standardized formulations limit reproducibility.

Table 3: In Vivo Outcomes and Biomarker Analysis

Parameter	Observation	Interpretation
Tumor Incidence	↓ ~30–50%	Chemopreventive effect
Tumor Volume	Significant reduction	Anti-proliferative activity
Histology	Reduced dysplasia	Tissue recovery
Oxidative Stress	↓ MDA, ↑ GSH/SOD	Antioxidant effect
Inflammation	↓ IL-6, TNF-α	Anti-inflammatory action

Overall, in vivo findings confirm the chemopreventive and antioxidant potential of beetroot compounds, with consistent modulation of tumor progression and inflammatory pathways, though standardization remains a key limitation for translational consistency. [21]

3.5 Module 5

Module 5 results indicate that clinical translation of beetroot-derived compounds remains limited, with evidence largely restricted to preclinical studies and indirect human data. No OSCC-specific clinical trials were identified, although antioxidant and anti-inflammatory benefits have been reported in human supplementation studies. Clinical relevance is therefore positioned primarily as an adjunct supportive therapy rather than a standalone anticancer treatment.

Pharmacovigilance analysis suggests a favorable safety profile, with minimal adverse effects such as mild gastrointestinal discomfort. However, potential herb drug interactions, particularly with chemotherapeutic agents, remain insufficiently studied. Clinical endpoints such as quality of life (QoL), inflammation reduction, and symptom management appear more achievable compared to direct tumor regression. These findings highlight a gap between strong mechanistic evidence and limited clinical validation.

Table 4: Clinical and Pharmacovigilance Insights

Parameter	**Observation**	**Interpretation**
Clinical Evidence	Limited (no OSCC trials)	Needs validation
Role	Adjunct therapy	Supportive use
QoL	Improved	Symptom relief
Safety	High	Well tolerated
Interaction	Possible	Requires monitoring

Overall, Module 5 suggests that beetroot compounds hold promise as supportive therapeutic agents with good safety, but require rigorous clinical trials and pharmacovigilance frameworks for integration into oncology practice. [22]

3.6 Module 6

Module 6 results highlight strong translational and market potential for beetroot-derived formulations, particularly in oral cancer supportive care. Current applications are largely limited to nutraceutical and antioxidant products, with minimal disease-specific targeting. Competitive analysis indicates dominance of herbal rinses and symptomatic treatments, with no significant presence of beetroot-based formulations, suggesting a clear innovation gap.

Regulatory evaluation supports initial positioning as a nutraceutical with gradual progression toward botanical drug classification upon clinical validation. Intellectual property analysis reveals limited patents in oral oncology-specific applications, indicating favorable opportunities for formulation-based innovation. Market trends suggest increasing demand for plant-based adjunct therapies, particularly in regions with high oral cancer prevalence.

Table 5: Market and Translational Insights

Category	Observation	Opportunity
Current Use	Nutraceutical	Expand to oncology care
Competition	Herbal rinses	No beetroot-based product
IP Space	Limited	New formulation scope
Regulation	Nutraceutical pathway	Stepwise advancement
Demand	Increasing	High market potential

Overall, Module 6 demonstrates that beetroot compounds possess strong commercial and translational potential, with opportunities for innovation in formulation, regulatory positioning, and clinical application, provided that standardization and validation challenges are addressed. [23]

4. Conclusion

This study presents a structured AI-assisted framework for the systematic evaluation of beetroot-derived bioactive compounds, particularly betanin, in oral cancer. The integrated analysis across mechanistic, pharmacological, experimental, clinical, and translational domains demonstrates that beetroot compounds exhibit significant multi-target activity, primarily through modulation of inflammatory (NF-κB), proliferative (PI3K/AKT), oxidative stress (Nrf2), and apoptotic pathways.

Results from in vitro and in vivo modules indicate moderate-to-high anticancer potential, supported by antioxidant, anti-inflammatory, and pro-apoptotic effects, although variability in experimental design and lack of OSCC-specific studies remain key limitations. Clinical evaluation suggests that beetroot is best positioned as an adjunct supportive therapy with a favorable safety profile, contributing to improved quality of life rather than direct tumor regression. Translational and market analysis further highlights significant opportunities for development, particularly in formulation innovation and supportive oncology care, given the current absence of beetroot-based therapeutic products. However, challenges related to standardization, bioavailability, and clinical validation must be addressed to enable successful integration into clinical practice.

Overall, this study establishes beetroot-derived compounds as promising multi-target agents in oral cancer, with strong mechanistic and translational potential, while emphasizing the need for rigorous experimental validation and well-designed clinical trials to support future therapeutic applications.

5. References

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