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Chrysopogon zizanioides VOLATILE COMPOUNDS AS POTENTIAL INHALED THERAPEUTICS FOR METASTATIC LUSC BIOMARKER

Muhammad Faisal[1]*, Ilham Hariaji[1], Andy Sulaiman Siregar[2]
[1]Department of Pharmacology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Sumatera Utara, Medan 20217 Indonesia
[2]Master Program in Biomedical Sciences, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
e-mail: muhammadfaisal@umsu.ac.id

Abstract

Lung squamous cell carcinoma (LUSC) remains a major subtype of non-small cell lung cancer with limited therapeutic options, particularly in metastatic stages. This study aimed to identify extracellular matrix (ECM)-associated biomarkers involved in LUSC progression and evaluate volatile compounds from *Chrysopogon zizanioides* as potential inhaled therapeutic candidates through an integrated computational approach. Gene expression and survival analyses were performed using UALCAN and TNMplot databases to identify prognostic ECM-related biomarkers. Volatile compounds of *C. zizanioides* were screened using drug-likeness and toxicity prediction analyses. Molecular docking was subsequently conducted against representative COL6A3 domains, including the N2 domain, Kunitz-associated domain, and microfibrillar structure. Pharmacokinetic (PK) properties relevant to inhalation-based therapy were also assessed. The results demonstrated that COL6A3 was significantly overexpressed in tumor and metastatic LUSC tissues and associated with poor overall survival, suggesting its involvement in tumor microenvironment remodeling and metastatic progression. Among 30 identified volatile compounds, zizanal and khusitone exhibited favorable drug-likeness and low predicted toxicity profiles. Whole-protein and specific-site molecular docking analyses revealed that both compounds showed stronger binding affinities toward the microfibrillar region of COL6A3 than pirfenidone, with khusitone demonstrating the strongest interaction (−7.12 kcal/mol). Interaction analysis identified ARG2811 as a key residue contributing to ligand stabilization. Furthermore, PK prediction suggested favorable pulmonary-oriented characteristics, including efficient membrane permeability, low risk of CYP450 interactions reduced blood-brain barrier penetration, and moderate plasma clearance. Collectively, these findings suggest that zizanal and khusitone may serve as promising lead compounds for inhaled therapeutics targeting COL6A3-associated metastatic LUSC.

Keywords: *Chrysopogon zizanioides; Lung squamous carcinoma; Metastatic; Volatile compounds*

1. INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, with lung squamous cell carcinoma (LUSC) constituting a major subtype of non-small cell

lung cancer (NSCLC) characterized by high morbidity and limited targeted therapeutic options (Alduais, Zhang, Fan, Chen, & Chen, 2023). Despite significant advances in treatment modalities, the prognosis for patients with LUSC remains poor, particularly in advanced and metastatic stages, highlighting the urgent need for innovative diagnostic tools and therapeutic strategies (Peng et al., 2025).

Recent years have witnessed the emergence of several molecular biomarkers proposed for the diagnosis and prognosis of LUSC, although their clinical application remains limited (Ma et al., 2025). Notable among these are tumor suppressor genes such as TP53, which is frequently mutated in LUSC and associated with genomic instability and unfavorable clinical outcomes (Benitez et al., 2024). Additionally, alterations in the PI3K/AKT signaling pathway have been implicated in both therapeutic resistance and tumor progression (Abd El-Salam et al., 2026), while the overexpression of genes such as SOX2 and FGFR1 has been linked to squamous differentiation and tumor growth (Chen et al., 2015). Immune-related biomarkers, including PD-L1, have facilitated the adoption of immunotherapy in clinical practice; however, their predictive value varies significantly among patients (Catani et al., 2025).

Despite these advances, most currently reported biomarkers mainly reflect intracellular signaling pathways or genetic alterations, whereas the contribution of the tumor microenvironment, particularly extracellular matrix (ECM) components, remains insufficiently investigated in LUSC (Zanoaga, Braicu, Nutu, Berindan-Neagoe, & Bender, 2026). Emerging evidence indicates that ECM remodeling not only facilitates tumor initiation and progression but also modulates cancer cell behavior, including proliferation, invasion, and metastatic potential (Lee, Ng, & Bakhtiar, 2025). Thus, the present study focused on ECM-associated components and cell surface receptors to identify gene expression profiles associated with overall survival in patients with LUSC. In addition, potential inhibitors targeting these biomarkers were investigated. Furthermore, the identification of potential therapeutic compounds targeting these biomarkers was also performed to support the development of candidate inhaled therapeutics for LUSC management.

Natural products have historically served as important sources of anticancer

agents, due to their diverse chemical and biological properties. *Chrysopogon zizanioides*, commonly referred to as "akar wangi," contains numerous compounds with documented pharmacological activities, such as antioxidant, analgesic, antimicrobial, anti-inflammatory, anticancer effects, and wound-healing effects (Gunasekar, Majdalawieh, Abu-Yousef, & Al Refaai, 2025). However, the potential of these compounds to target ECM-related biomarkers in LUSC has not yet been thoroughly investigated.

This study aimed to identify key extracellular matrix (ECM) and cell surface receptor biomarkers associated with the progression of lung squamous cell carcinoma (LUSC). It also sought to predict potential inhibitors targeting these biomarkers from *Chrysopogon zizanioides* using an integrated computational approach, including gene expression analysis, molecular docking, and pharmacokinetic (PK) profiling, with the broader objective of identifying candidate compounds for inhalation-based therapy against LUSC.

2. METHODS

2.1 ECM and Cell Surface Receptors Gene Expression

Gene expression data for ECM-related and cell surface receptor-associated genes were retrieved from the UALCAN database (https://ualcan.path.uab.edu) (Chandrashekar et al., 2017), a publicly accessible platform provided by The Cancer Genome Atlas (TCGA). Through this database, we performed data mining to separate the genes that are related to ECM and cell-surface receptors. Then, a total of 67 candidate genes were identified. Subsequently, differential gene expressions among normal lung patients and LUSC patients were analyzed. In addition, the association between gene expression levels and overall survival (OS) in patients with LUSC was assessed. Genes exhibiting the strongest associations, indicated by the lowest *p*-values for both differential expression and OS analysis, were selected for further investigation.

2.2 Identification of Selected Genes in TNM Comparison

Gene expression analysis of the selected biomarkers, COL6A3 and AGRN, was performed using the online interface of the TNMplot platform (https://tnmplot.com/), which enables comparative analysis among normal, tumor, and metastatic tissues. The expression profiles of both genes were evaluated to determine their association with metastatic progression in LUSC.

2.3 Identification, Selection, and Preparation of Volatile Compounds of Chrysopogon zizanioides

The volatile compounds of *Chrysopogon zizanioides* were identified using the KNApSAcK database (https://www.knapsackfamily.com/knapsack_core/top.php) (Afendi et al., 2012). A total of 30 compounds were gathered, and their three-dimensional (3D) chemical structures were retrieved from the PubChem database in Structure Data File (SDF) format. The drug-likeness and toxicity profiles of all compounds were analyzed using ADMETLAB 3.0 (https://admetlab3.scbdd.com/) and ProTox 3.0 (https://tox.charite.de/protox3/) (Banerjee, Kemmler, Dunkel, & Preissner, 2024). Four selected compounds were subsequently converted into Protein Data Bank (PDB) format using CACTUS online structure translator (https://cactus.nci.nih.gov/translate/).

2.4 Protein Structure Preparation and Validation

After COL6A3 was identified as the poorest prognostic biomarker in LUSC. Then, the protein structure of COL6A3 was prepared and validated. Due to the large size and complex multidomain architecture of COL6A3, a domain-based approach was employed. The N2 domain (PDB ID: 6SNK), Kunitz domain (PDB ID: 1KTH), and microfibril structure domain (PDB ID: 9GTU) were selected on their structural availability and functional relevance to ECM interactions and organization, providing representative regions for molecular docking. The three-dimensional protein structures were obtained. All protein structures were retrieved from the RCSB Protein Data Bank (https://rcsb.org) and validated using the publicly accessible online platform SAVES (https://saves.mbi.ucla.edu/) (Bowie, LtCY, & Eisenberg, 1991; Colovos & Yeates, 1993; Laskowski, MacArthur, Moss, & Thornton, 1993). Water molecules and unnecessary ligands were removed prior to docking using BIOVIA Discovery Studio (https://3ds.com) (BIOVIA, 2016). Subsequently, polar hydrogen atoms and Kollman charges were added to the protein structures using Autodock Tools (ADT) version 1.5.7, and the structure was converted to PDBQT format.

2.5 Molecular docking

2.5.1 Whole-Protein Structure Docking Protocol

Molecular docking analysis was conducted using AutoDock Tools (ADT) version 1.5.7 (Morris et al., 2009). Whole-protein docking was employed to allow ligand interaction across the entire protein surface. Docking simulations were performed using the Lamarckian Genetic Algorithm with 50 runs and a population size of 200, while other parameters were maintained

at default settings for energy evaluation and mutation rates (Faisal & Hariaji, 2026). The binding affinity values (kcal/mol) of all tested compounds were subsequently recorded. As comparison, pirfenidone (CID: 40632), a current potent inhibitor of COL6A3, was assigned as a reference ligand. Compounds exhibiting binding affinity values lower than −7.0 kcal/mol and interacting at binding sites similar to those of pirfenidone were considered potential candidates for further investigation (Reifs et al., 2024).

2.5.2 Specific-Site Protein Structure Docking Protocol

Zizanal, khusitone, and pirfenidone were selected for site-specific docking analysis. The docking grid box was centered on the identified active site, which was predicted by PRANKWEB (https://prankweb.cz/) with dimensions of 40 × 40 × 40 Å. Moreover, the docking parameters were identical to those applied in the whole-protein docking protocol, including the settings of the Lamarckian Genetic Algorithm with 50 runs and a population size of 200.

2.6 Molecular Interaction Visualization and Binding Pose Analysis

The binding conformations of the selected compounds were visualized following molecular docking analysis to assess ligand orientation and interaction patterns within microfibrillar structure of COL6A3 binding pocket. Three-dimensional and two-dimensional interaction analyses were conducted using BIOVIA Discovery Studio Visualizer (BIOVIA, 2016). The docking poses of zizanal and khusitone were compared with the reference ligand pirfenidone, to identify similarities in binding sites and interacting amino acid residues. Compounds demonstrating comparable binding orientations and residue interactions with the reference ligand were considered potential candidates for further evaluation.

2.7 Pharmacokinetic (PK) Prediction

The pharmacokinetic (PK) properties of the selected compounds were predicted using the PerMM online platform (https://permm.phar.umich.edu/) and ProTox 3.0 server (Banerjee et al., 2024). The analysis included absorption, distribution, metabolism, and excretion (ADME) parameters. Absorption properties were evaluated through membrane permeability prediction, while distribution properties included transporter inhibition probability analysis. Metabolism profiles were predicted based on cytochrome P450 (CYP) enzyme substrate and inhibitor, including CYP1A2 and CYP3A4. Excretion characteristics were estimated using total clearance (CL) prediction. The PK profiles of the tested compounds were subsequently compared with pirfenidone as the reference ligand.

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3. RESULTS AND DISCUSSION

3.1 Identification of ECM components and Cell Surface Receptors Expression in LUSC

The expression profiling analysis revealed substantial dysregulation of ECM-associated genes and cell surface receptors between normal lung tissues and LUSC samples (**Figure 1**). Several ECM-related genes, including COL6A3, COL1A1, COL1A2, COL5A1, COL6A1, and COL6A2, exhibited markedly elevated expression in tumor tissues compared with normal tissues. Elevating expressions were also observed in several cell surface receptor-associated genes, for instance ITGB1, ITGB5, SDC1, and AGRN. These findings indicate extensive remodeling of the tumor microenvironment in LUSC, particularly involving ECM organization, cell adhesion, and receptor-mediated signaling pathways associated with tumor progression and metastasis.

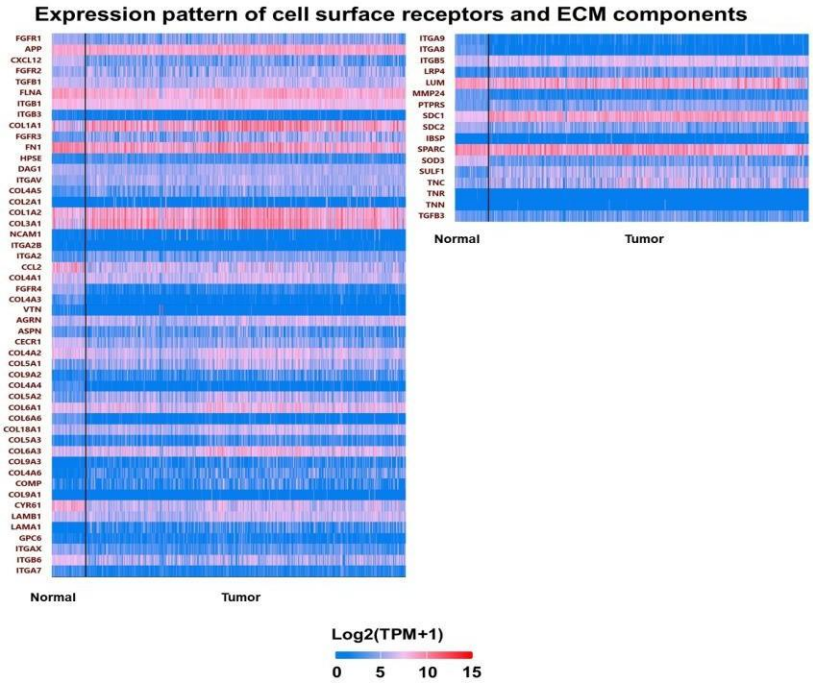

Figure 1. Heatmap of cell surface receptor and ECM gene expression patterns in LUSC.

Further comparative analysis using the UALCAN platform demonstrated that both COL6A3 and AGRN were expressed at higher levels in primary tumor tissues than in normal lung tissues (**Figure 2A**). Nevertheless, COL6A3 showed a substantially greater elevated expression fold than AGRN. This finding suggests that COL6A3 may have a stronger associated with tumor-associated ECM remodeling in LUSC.

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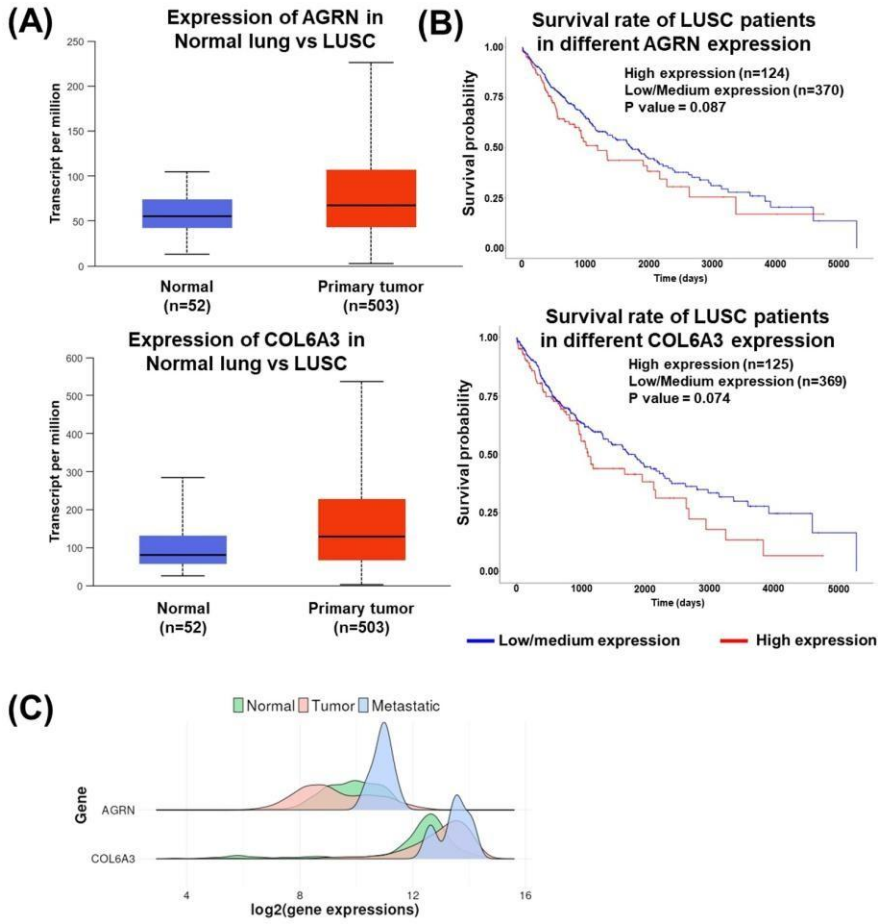

Figure 2. Biomarker analysis of the selected gene COL6A3 and AGRN, **(A)** both gene expression in normal lung vs LUSC, **(B)** OS trend in different both gene expression levels, and **(C)** both gene expression in T (tumor), N (normal), and M (metastatic) tissues.

Survival analysis indicated that patients with high expression levels of AGRN and COL6A3 tended to demonstrate lower OS than patients with low-to-medium expression levels (**Figure 2B**). Although the observed *p*-value for AGRN ($p = 0.087$) and COL6A3 ($p = 0.074$) did not reach the conventional threshold for statistical significance, both genes demonstrated a clear trend toward poor prognosis. Notably, COL6A3 exhibited a steeper decline in survival probability in the high-expression group, further fostering its potential involvement in LUSC pathogenesis.

The absence of statistical significance may be attributed to biological heterogeneity among LUSC patients, differences in treatment regiments, or sample distribution within the dataset.

Nonetheless, the observed survival trend remains biologically relevant because ECM-associated molecules often exert cumulative effects on tumor progression rather than acting as isolated oncogenic drivers. Prior reports have similarly demonstrated that elevated COL6A3 expression is associated with poor clinical outcomes in multiple cancer types due to its involvement in tumor invasion and metastatic progression (Lingo, Balas, Scherer, & Klein, 2025).

To further evaluate the metastatic relevance of the chosen genes, TNMplot analysis was conducted comparing normal, tumor, and metastatic tissues (**Figure 2C**). The TNMplot analysis showed that COL6A3 expressions in tumor metastatic tissues were higher expression (12-15 log2) than AGRN (<12 log2). The elevated expression of COL6A3 in metastatic tissues supports its involvement in ECM remodeling processes that facilitate tumor cell migration and invasion. Collagen VI has been reported to regulate cellular interactions within the tumor microenvironment, enhance proliferation, angiogenesis, epithelial-mesenchymal transition (EMT), and promote metastatic dissemination through activation of oncogenic signaling pathways (Huang et al., 2018; Iyengar et al., 2005). Particularly COL6A3, the polymorphism of this gene was associated with lung cancer risk and considered as the potential biomarker in Chinese population's lung cancer patients (Duan et al., 2019). Hence, the higher metastatic expression observed for COL6A3 strengthens its potential utility as a prognostic biomarker and therapeutic target in LUSC.

3.2 Chrysopogon zizanioides Compound Identification and First-Screening

Thirty identified compounds inside *Chrysopogon zizanioides* essential oil were found. The comprehensive drug-likeness and toxicity prediction was performed. **Figures 3A** and **B** provide important insight into their therapeutic potential. Most compounds exhibited minimal violations of Lipinski's Rule of Five and Pfizer's drug-likeness criteria, as indicated by the predominance of green and yellow regions in the heatmap. These findings suggest that most of the tested compounds possess favorable physicochemical properties, which are essential for adequate oral bioavailability and successful drug development. Nevertheless, several compounds, including (+)-Isovalencenol and (-)-Khusol, were predicted moderate violations, indicating that although they may retain biological activity, their PK properties could require structural optimization to improve drug-likeness. There are 13 of 30 compounds that meet drug-likeness criteria and are eligible to be assigned for further study.

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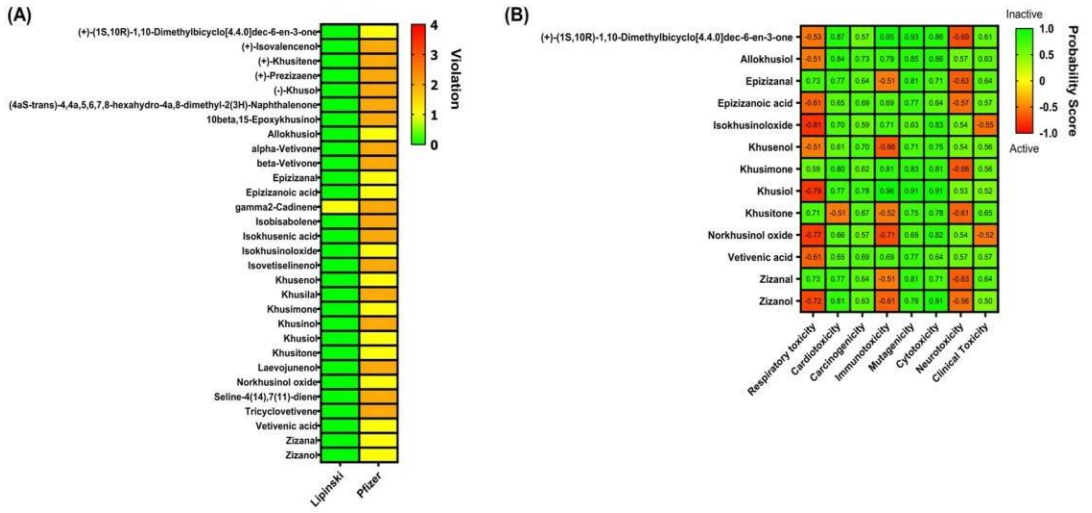

Figure 3. PK and toxicity properties of *Chrysopogon zizanioides* essential oil compounds, **(A)** drug-likeness prediction of 30 compounds, and **(B)** toxicity prediction of 13 compounds after screened by drug-likeness prediction.

In addition, toxicity prediction analysis exhibited that most compounds were associated with low probabilities of adverse effects across multiple toxicity endpoints, including respiratory toxicity, cardiotoxicity, immunotoxicity, mutagenicity, neurotoxicity, and clinical toxicity. In this study, we expect to generate fundamental screening for inhaled medicines, which may treat LUSC through nasal airflow. Thus, we considered eliminating the compound which were predicted to tend 1) 0.5 active probability in respiratory toxicity and 2) more than 0.65 active probability in all toxicity endpoints. This study revealed that epizizanal, khusimone, khusitone, and zizanal met these standards and were further analyzed in molecular docking analysis.

3.3 Protein structure and whole-protein structure docking results

To investigate the potential interaction of volatile compounds with COL6A3, molecular docking analysis was performed against three representative COL6A3-associated domains, namely the N2 domain (PDB ID: 6SNK), Kunitz-associated domain (PDB ID: 1KTH), and microfibrillar domain (PDB ID: 9GTU) (**Figure 4A**). As initial step, we designed this virtual screening to evaluate the binding preference of the compounds across whole-protein docking for predicting favorable binding regions, particularly within proteins that lack clearly established catalytic pockets, for this context is ECM-associated proteins.

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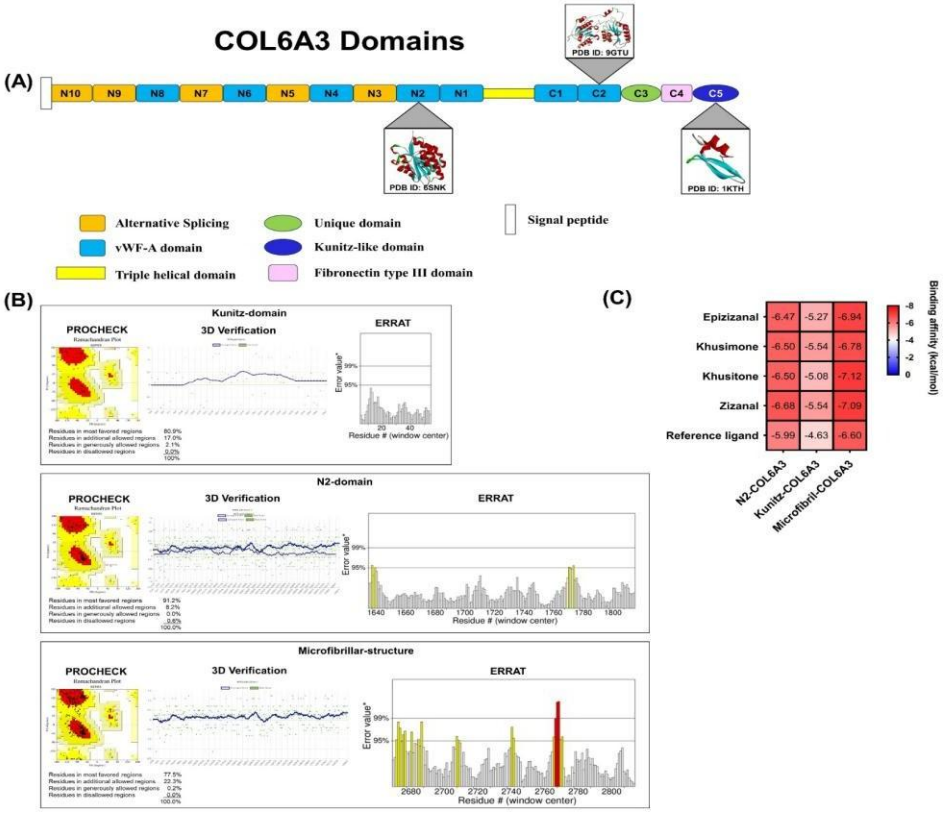

Figure 4. Protein of COL6A3 consists of many domains, **(A)** the representatives of COL6A3 domains are N2 domain (6SNK), Kunitz domain (1KTH), and microfibrillar structure (9GTU), **(B)** protein structure validation, and **(C)** molecular docking results on whole-structure protein of COL6A3 domains.

COL6A3 is a crucial ECM component involved in collagen microfibril organization, stromal remodeling, tumor development, and metastatic signaling. Increasing evidence suggests that COL6A3 overexpression contributes to cancer aggressiveness through EMT, ECM stiffening, angiogenesis, and activation of oncogenic pathways (Huang et al., 2018; Lingo et al., 2025). In addition, the cleavage of COL6A3 generates endotrophin, a bioactive fragment associated with inflammation, fibrosis, and tumor metastasis (Lingo et al., 2025).

Prior to performing docking, we validated the protein structure of COL6A3 domain models using PROCHECK, Verify3D, and ERRAT analyses (**Figure 4B**), which are available through the SAVES online platform. The Ramachandran plot analysis demonstrated that most residues were distributed within favored and additionally allowed regions, indicating acceptable stereochemical quality of the predicted protein structures, indicating acceptable stereochemical quality of the predicted structures. The 3D verification profiles showed that

most amino acid residues possessed compatible structural environments, while ERRAT analysis indicated relatively stable non-bonded atomic interactions throughout the modeled domains. Although several regions exhibited elevated error values, particularly within larger flexible regions, the overall validation results indicate that COL6A3 models were generally feasible for molecular docking simulations.

The docking analysis demonstrated that all tested compounds exhibited negative binding affinities toward the selected COL6A3 domains, indicating favorable interactions (**Figure 4C**). Among the three COL6A3 protein structures, the microfibrillar COL6A3 structure showed the strongest ligand interactions overall, suggesting that the domain may possess more accessible or hydrophobic binding environment favorable for sesquiterpenoid accommodation. In this domain, khusitone had the strongest binding affinity (−7.12 kcal/mol), followed by zizanal (−7.09 kcal/mol), epizizanal (−6.94 kcal/mol), and khusimone (−6.78 kcal/mol). Notably, these values were stronger than the reference ligand, pirfenidone, which exhibited a binding affinity of −6.60 kcal/mol. These findings indicate that the proposed compounds may possess favorable binding stability toward the microfibrillar structure of COL6A3. Since zizanal and khusitone fulfilled the predefined screening criteria, we further evaluated these compounds through site-specific molecular docking analysis targeting the microfibrillar region of COL6A3.

3.4 Specific-site Molecular Docking Analysis

To further validate the preliminary whole-protein docking findings, specific-site molecular docking analysis was carried out on the selected binding pocket within the microfibrillar-associated region of COL6A3. The results showed that both volatile compounds exhibited moderately stronger binding affinities than pirfenidone toward COL6A3 microfibrillar structure binding site. Khusitone displayed the strongest interaction with a binding affinity of approximately −7.11 kcal/mol, followed closely by zizanal (approximately −7.09 kcal/mol), whereas pirfenidone displayed a weaker interaction of approximately −5.74 kcal/mol (**Figure 5A**). These findings indicate that the selected sesquiterpenoid compounds have greater predicted binding stability within the targeted COL6A3 pocket compared to pirfenidone.

Molecular docking analysis at specific-site of the microfibrillar-associated COL6A3 region showed that khusitone, zizanal, and pirfenidone all occupied a similar binding site within (**Figure 5B**). This finding indicates that these compounds may interact with shared residues important for ligand stabilization.

Detailed interaction analysis showed that the ligands formed several non-covalent

interactions with key amino acid residues (**Figure 5C**). Zizanal formed conventional hydrogen bonds with ARG2607 and ARG2811, indicating relatively stable polar interactions that may contribute to its favorable docking score. Khusitone also interacted with ARG2811 and GLY2537 through hydrogen bonding and hydrophobic contacts, suggesting that these residues may play important roles in ligand recognition and stabilization within the binding pocket.

Interestingly, ARG2811 appeared as a common interacting residue among the tested ligands, implying that this residue may represent an important hotspot within the COL6A3 microfibrillar domain. Arginine residues are frequently involved in electrostatic stabilization and hydrogen-bond formation due to their positive charge of guanidinium groups (Neves, Yeager, & Abagyan, 2012). Thus, ligands interaction with ARG2811 may enhance ligand retention and binding stability inside the pocket. Similarly, aromatic residues, such as PHE2607 and PHE2606 contributed to π-alkyl interactions, which likely support hydrophobic stabilization of the ligands.

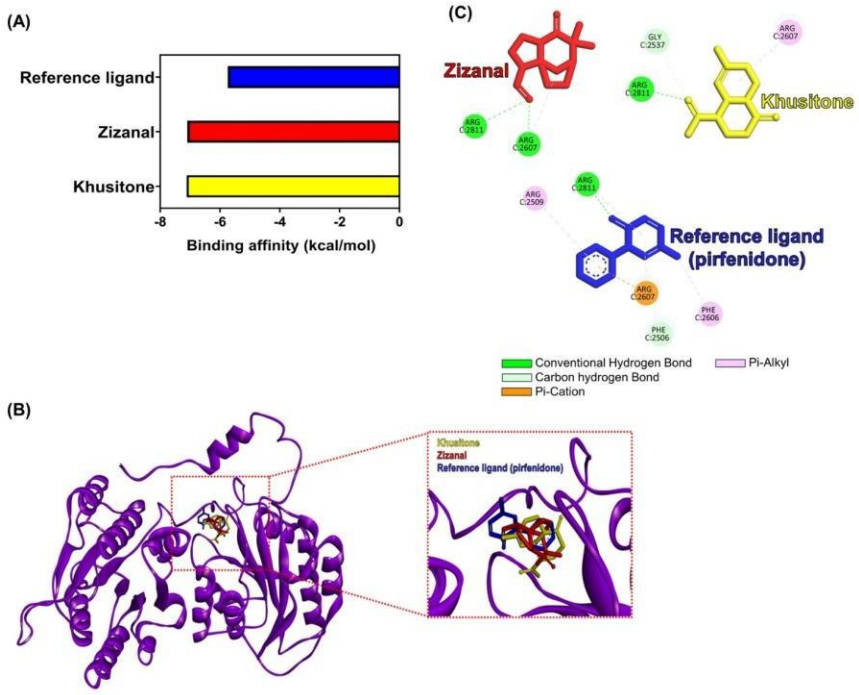

Figure 5. Molecular docking analysis at specific site of COL6A3, **(A)** Binding affinities of zizanal, khusitone, and reference ligand (pirfenidone), **(B)** Binding pose of two compounds and pirfenidone, and **(C)** 2D interaction diagrams comparison among two compounds and pirfenidone.

Meanwhile, the reference ligand pirfenidone demonstrated weaker binding affinity and fewer stabilizing interactions compared with khusitone and zizanal. Although pirfenidone

interacted with ARG2607 and aromatic residues through π-cation and π-alkyl interactions. This finding suggests that this interaction is smaller structure and lower hydrophobic surface area may attenuate its binding complementarity within selected COL6A3 pocket. On the other hand, the sesquiterpenoid scaffold of khusitone and zizanal may provide enhanced hydrophobic compatibility with ECM-associated binding cavity.

3.5 Promising Compounds PK Prediction

After we performed the specific-site molecular docking analysis on the microfibrillar-associated region of COL6A3, additional PK analysis were conducted to evaluate whether the compounds possessed favorable PK properties compared with pirfenidone. Since the investigated compounds are volatile sesquiterpenoids with potential pulmonary applications, the present study pinpointed on absorption behavior, metabolic interaction, distribution properties, and excretion profiles relevant to inhalation-oriented drug development.

The absorption analysis (**Figure 6A**) evaluated the membrane permeation behavior of the compounds across the lipid bilayer model by mimicking pH and temperature of lung, 7.4 and 310 K. Both zizanal and khusitone exhibited energetically favorable transmembrane diffusion patterns, with negative energy values observed throughout the membrane penetration pathway. The lowest energy states for zizanal and khusitone were observed around the hydrophobic core region of the membrane, indicating stable membrane partitioning and favorable permeability behavior. Khusitone exhibited slightly stronger membrane interaction energy at several membrane depths compared with zizanal, suggesting enhanced lipophilic compatibility with the phospholipid bilayer. Interestingly, both compounds displayed membrane permeation energies comparable to pirfenidone, indicating that the sesquiterpenoid scaffold may foster efficient passive diffusion across pulmonary epithelial membranes.

Subsequently, we predicted metabolism analysis (**Figure 6B**) demonstrated that zizanal and khusitone exhibited low probability scores as inhibitors or substrates of major cytochrome P450 enzymes, particularly CYP1A2 and CYP3A4 (Deodhar et al., 2020). In contrast, pirfenidone showed markedly higher probabilities as both inhibitor and substrate of those enzymes. It suggests that the volatile compounds may possess lower risks of CYP-mediated drug-drug interactions and reduced metabolic liabilities compared with pirfenidone. Due to inhaled therapeutics are preferably designed to obtain localized pulmonary activity with minimal systemic metabolic burden, low CYP450 interaction potential may represent an advantageous PK characteristic for future inhaled medications.

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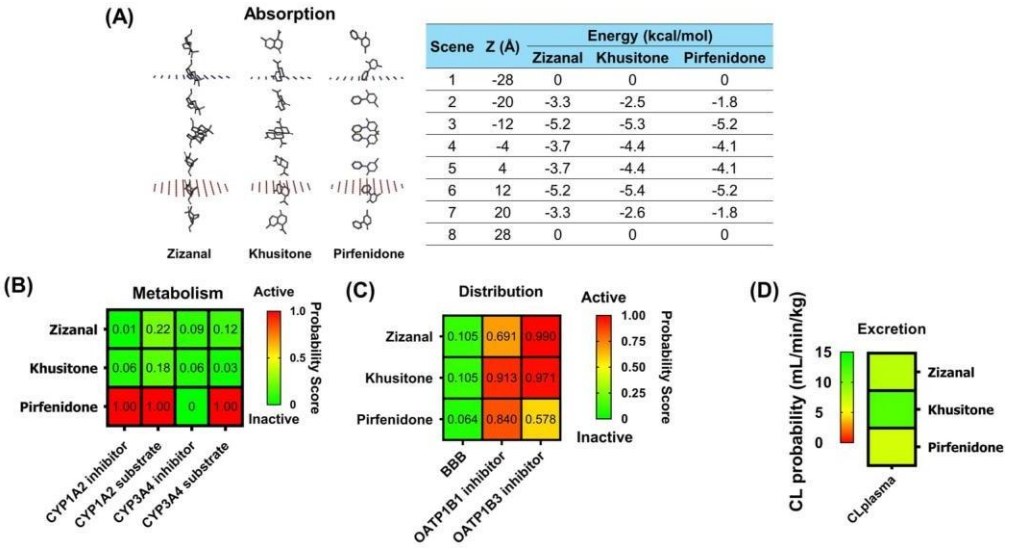

Scene	Z (Å)	Energy (kcal/mol)		
		Zizanal	Khusitone	Pirfenidone
1	-28	0	0	0
2	-20	-3.3	-2.5	-1.8
3	-12	-5.2	-5.3	-5.2
4	-4	-3.7	-4.4	-4.1
5	4	-3.7	-4.4	-4.1
6	12	-5.2	-5.4	-5.2
7	20	-3.3	-2.6	-1.8
8	28	0	0	0

Figure 6. PK prediction analysis of two compounds and reference ligand (pirfenidone), **(A)** absorption, **(B)** metabolism, **(C)** distribution, and **(D)** excretion.

The distribution analysis (**Figure 6C**) exhibited favorable PK properties for the volatile compounds. Both zizanal and khusitone were predicted to be low blood-brain barrier (BBB), which suggests limited central nervous system exposure, although neurological safety requires experimental validation. In addition, both compounds indicated high probabilities of interaction with organic anion transporting polypeptides (OATP1B1 and OATP1B3), suggesting possible clinically relevant drug-drug interactions (Alam et al., 2018).

From an inhalation perspective, there was a report that salbutamol may cause some issues in nervous system. This side effect is triggered by the salbutamol high BBB penetration that made this medication cross BBB easily and accumulated in nervous system (Marques & Vale, 2022). Thus, low BBB active probability is particularly beneficial because inhaled drugs are generally intended to exert localized therapeutic effects in pulmonary tissues rather than systemic neurological activity.

Excretion prediction (**Figure 6D**) revealed that zizanal and khusitone exhibited moderate plasma clearance values comparable to pirfenidone. This observation suggests that the compounds may achieve a balanced elimination profile without excessively rapid systemic clearance. Moderate clearance characteristics are desirable for inhaled medicines (Anderson et al., 2022), which is often necessary to maintain local therapeutic concentration within the lung microenvironment (Guo et al., 2021).

Overall, this study identified COL6A3 as a promising ECM-associated biomarker and therapeutic target in LUCS due to its elevated expression in tumor and metastatic tissues, as well as its association with poor patient survival. Computational screening of compounds from *C. zizanioides* essential oil revealed zizanal and khusitone as the most promising candidates based on favorable drug-likeness, low predicted toxicity, and strong binding affinities toward the microfibrillar region of COL6A3. Both compounds demonstrated stronger binding affinity than pirfenidone, particularly through interaction with key residues such as ARG2811, suggesting their potential to interfere with COL6A3-associated tumor microenvironment remodeling. Furthermore, PK prediction analysis indicated favorable pulmonary-oriented characteristics, including efficient membrane permeability, low CYP450 interaction risk, reduced BBB penetration, and balanced plasma clearance profiles.

4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The present study identified COL6A3 as a promising ECM-associated biomarker in LUSC due to its elevated expression in tumor and metastatic tissues, as well as its association with poor overall survival. Among the volatile compounds identified from *Chrysopogon zizanioides* essential oil, zizanal and khusitone demonstrated favorable drug-likeness and toxicity profiles, along with strong binding interactions toward the microfibrillar-associated region of COL6A3 in both whole-protein and specific-site molecular docking analyses. In addition, pharmacokinetic (PK) predictions suggested advantageous membrane permeability, low CYP-mediated metabolic liability, moderate distribution behavior, and balanced excretion properties supportive of inhalation-oriented drug development. Collectively, these findings suggest that zizanal and khusitone may represent promising lead compounds for the development of inhaled therapeutics targeting COL6A3-associated ECM remodeling and metastatic progression in LUSC. Furthermore, this study proposed a potential mechanism of COL6A3 reduction in lung cancer treatment using inhaled medicine from *Chrysopogon zizanioides* essential oil compounds (**Figure 7**).

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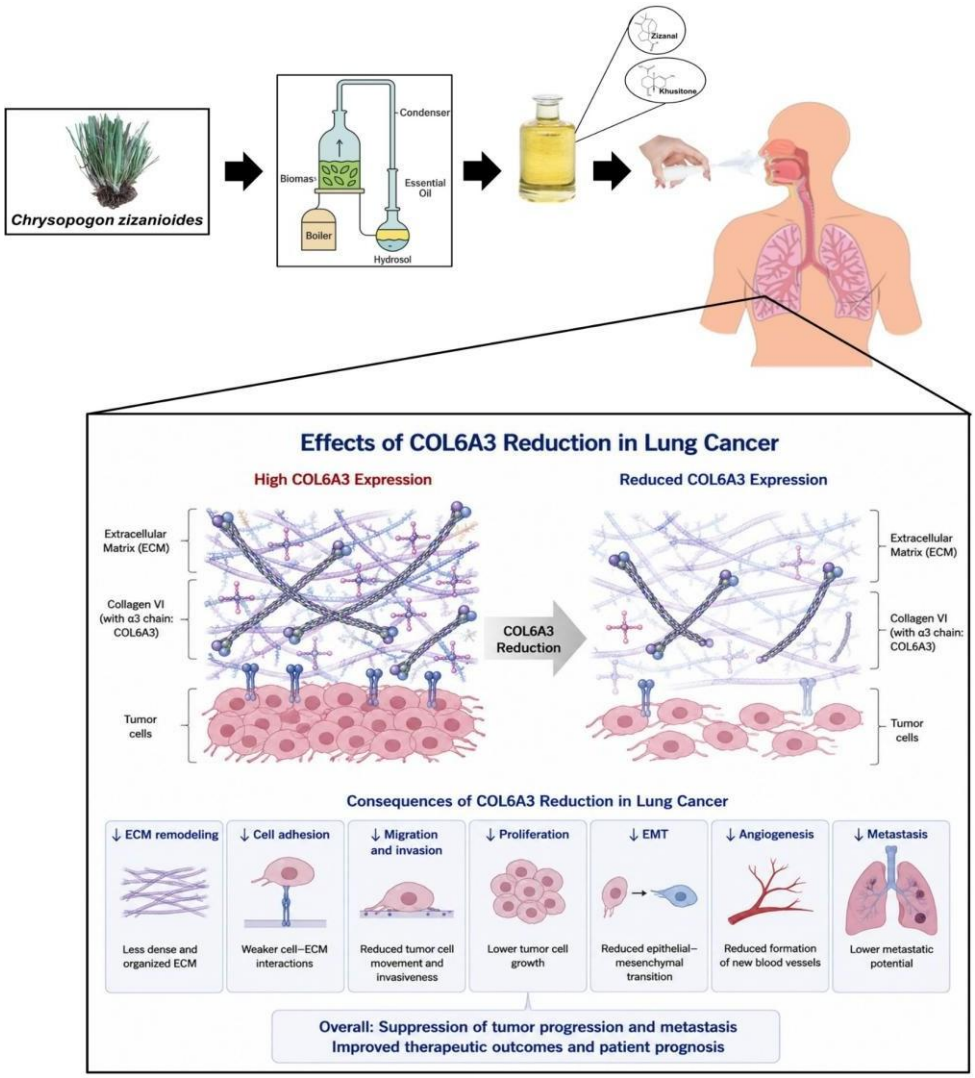

Figure 7. Possible mechanisms of *C. zizanioides* essential oil as inhaled medicine in lung cancer treatment through COL6A3 inhibition.

4.2 Recommendation

Further investigations are recommended to validate the biological relevance and translational feasibility of zizanal and khusitone as inhaled therapeutic candidates against LUSC. Future studies should include molecular dynamics simulation, binding free-energy analysis, aerosolization, and lung deposition studies, pulmonary epithelial permeability assays, and experimental validation using COL6A3-overexpressing lung cancer models. Moreover, mechanistic studies focusing on ECM remodeling,

endotrophin-associated signaling, EMT regulation, and pulmonary retention behavior are necessary to confirm the therapeutic potential of these compounds in inhalation-based anticancer strategies.

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