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INVESTIGATING MULTI-TARGET PHARMACOLOGY OF FENUGREEK AGAINST LUNG CANCER: A NETWORK PHARMACOLOGY AND MOLECULAR DOCKING APPROACH

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ABSTRACT: Background: Lung cancer is a malignant tumor with the highest death and morbidity rates among all cancer types worldwide. Chemotherapeutic medications often cause adverse effects and resistance, resulting in a low survival rate. Herbal compounds that target multiple pathways can offer safer and more effective alternatives. Trigonella foenum-graecum L., commonly known as fenugreek, is a traditional medicinal plant in India known for its antidiabetic and anticancer properties. Although its anticancer effects against lung cancer have been reported in previous clinical studies, the underlying mechanisms and active phytochemicals responsible remain unclear. Therefore, the present study aimed to identify the bioactive phytoconstituents of fenugreek using an in-silico network pharmacology and molecular docking approach to explore its anticancer potential. Methods: A multi-target network pharmacology and molecular docking approach was employed to identify fenugreek's active compounds and mechanisms. Bioactive components were collected from databases, screened, and analyzed. Cytoscape was used to build compound-target-disease networks, and core lung cancer targets were identified. Enrichment analysis was performed using R, and molecular docking confirmed compound-target interactions. Result: Twelve potential bioactives were selected based on ADMET properties. Apigenin, Daidzein, Gomisin N, and Genistein interacted with key proteins (EGFR, MET, ALT1, TP53) and showed strong binding affinities. Conclusion: Fenugreek exhibits anti-lung cancer potential via multi-target modulation of disease pathways, supporting its role in combination therapies and preventive strategies.

Keywords: Bioinformatics, fenugreek, lung cancer, molecular docking, network pharmacology.

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1. INTRODUCTION

According to the Global Cancer Observatory 2022 report, lung cancer ranks as the second most common cause of cancer-related deaths among women and the primary cause among men [1]. Since 1985, the number of cases of lung cancer in developing nations has significantly increased, making it a serious health concern [2]. The most significant risk factor for lung cancer is tobacco use; with additional risk factors including exposure to radiation, second-hand smoke, HIV infection, occupational exposure, and air pollution [3]. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two main types of lung cancer that develop from respiratory epithelium cells [4]. Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are the three main pathologic subtypes of non-small cell lung cancer (NSCLC), accounting for 85% of cases [3]. Currently, the most effective treatment strategy is chemotherapy, although side effects such as adverse impacts on non-targeted organs, recurrence, and drug resistance and their toxic effects result in a survival rate of only 15-18% [3]. Traditional medicine offers complementary approaches to cancer treatment alongside conventional methods [6]. Integrating traditional medicine with modern approaches may lead to the discovery of new, safe, and affordable bioactives for cancer prevention and treatment to address the global cancer burden [5]. Drugs derived from natural products aim to address numerous shortcomings of synthetic chemicals and traditional chemotherapeutic approaches. Research into plant products may, therefore, contribute to the development of effective and novel alternative therapeutics in the search for safer, eco-friendly, low-cost, fast, and lung cancer treatment drugs [3]. Fenugreek (*Trigonella foenum-graecum L.*) is a native plant of South Eastern Europe and West Asia, and is cultivated in India, China, Argentina, Egypt, and Mediterranean and Southeast countries and has been considered a notable multipurpose medicinal and traditional herb for several centuries [7][8]. Fenugreek seeds and leaves have been reported to have anti-cholesterolemic, anti-tumor, anti-inflammatory, laxative, hypoglycaemic properties and contain saponins, flavonoids, and alkaloids that exhibit anti-inflammatory, anti-oxidant activity, demonstrating anti-cancer and pro-apoptotic properties in both *in vitro* and *in vivo* experiments [9][10]. Fenugreek's constituents have been shown to have preventive properties against many types of illnesses, including diabetes, cancer, cardiac problems, neurological diseases, hypolipidemia, gastroprotective effects, arthritis, among others. Its bioactives have also been reported to possess therapeutic potential in cancer management, particularly for lung and breast cancers, through

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various cellular mechanisms including apoptosis induction and antioxidant activities [11]. However, mechanism of the bioactive phytochemical of fenugreek with potential targets of lung cancer needs to be elucidated. Therefore, the present study was undertaken to identify potential multi-targeting, anti-lung cancer agents from phytochemicals of Fenugreek using a combined approach of network pharmacology and molecular docking.

2. MATERIALS AND METHODS

2.1 Screening of active constituents of Fenugreek

The active compounds of Trigonella foenum-graecum L. were obtained from IMPPAT (https://cb.imsc.res.in/imppat/), PCIDB, and Duke's Phytochemical databases. The structural information of the 139 active constituents was retrieved using PubChem in the form of SMILES notation, and phytochemicals for which no chemical information was available were discarded. Using pkCSM database, drug-likeness properties and ADMET analysis were performed.

2.2 Intersection of Fenugreek and Lung Cancer Targets

The selected phytochemicals of Fenugreek were used to predict their potential gene targets using STITCH, DrugBank (https://go.drugbank.com/) and SwissTargetPrediction database. GeneCards and OMIM were used to retrieve lung cancer related genes of Homo sapiens using the keywords ‘lung cancer’. Venny 2.1, an online application for making Venn diagrams, was utilized to visualize the overlap between the target proteins of lung cancer and selected bioactives.

2.3 Construction of the D-C-T-D and PPI Networks of Therapeutic Targets for Fenugreek against Lung Cancer

The common targets and bioactives were imported into Cytoscape (version 3.10.2) to generate the Drug-Compound-Target-Disease network, and its topological characteristics were examined using the Network Analyzer function [12] STRING ver.12.0 was used to analyze protein-protein interactions, with a minimum combined score of 0.400.

2.4 Gene Ontology and KEGG Pathway Enrichment Analysis

GO functional enrichment analysis was conducted using the clusterProfiler package in R (ver. 4.3.3) (R Foundation for Statistical Computing) [13] and its components - Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) were identified using EnrichR. Biological annotations of significance ($p < 0.05$) were selected for further study from the top 10 modules. KEGG pathway enrichment analysis was used to study the primary signaling pathways implicated in fenugreek's anti-lung cancer properties and "ggplot2", an R package, was used to analyze the enriched data. RAWGraphs were used to illustrate the relationships between the targets and pathways.

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2.5 Molecular docking

The molecular docking of bioactive phytochemicals of Fenugreek, the commercially available drugs for lung cancer and identified targets for the disease was performed using the AutoDock Vina software ver. 1.2. The RCSB PDB (https://www.rcsb.org) and PubChem databases were searched for extracting the 3D structures of the targets and bioactive, respectively. Using Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download), the docking results were visualized.

3. RESULTS AND DISCUSSION

3.1 Active constituents of Fenugreek dataset preparation

Putative bioactives derived from Fenugreek were obtained from IMPPAT, PCIDB and Duke's Phytochemical databases. Using pkCSM tool, the 139 bioactives were analysed for drug likeliness on the basis of Lipinski's rule of five and ADMET properties. Only 49 phytochemicals negative for Hepatotoxicity and Ames toxicity, with a permeability coefficient > 0.9 were selected [14] for subsequent analysis (Table I).

Table I. Table of drug-likeliness and ADMET analysis of 12 bioactives of Fenugreek

S.No.	Phytochemicals	SMILES Notation	Caco2 permeability
1.	Genistein	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O	0.9
2.	Apigenin	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O	1.007
3.	Carvone	CC1=CCC(CC1=O)C(=C)C	1.413
4.	Daidzein	C1=CC(=CC=C1C2=COC3=C(C2=O)C=CC(=C3)O)O	0.903
5.	Falcarindiol	CCCCCCCC=CC(C#CC#CC(C=C)O)O	1.608
6.	Flavanone	C1C(OC2=CC=CC=C2C1=O)C3=CC=CC=C3	1.219
7.	Formononetin	COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)O	1.253
8.	Gomisin N	CC1CC2=CC3=C(C(=C2C4=C(C(=C(C=C4CC1C)OC)OC)OC)OC)OCO3	0.98
9.	Isoliquiritigenin	C1=CC(=CC=C1C=CC(=O)C2=C(C=C(C=C2)O)O)O	0.955
10.	Scopoletin	COC1=C(C=C2C(=C1)C=CC(=O)O2)O	1.184
11.	Thymol	CC1=CC(=C(C=C1)C(C)C)O	1.606
12.	Yuccagenin	CC1CCC2(C(C3C(O2)CC4C3(CCC5C4CC=C6C5(CC(C(C6)O)O)C)C)C)OC1	1.264

*These bioactives followed Lipinski's rule of five and were negative for toxicity.

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3.2 Intersection of Fenugreek and Lung Cancer Targets

Lung-cancer related targets were retrieved from the GeneCards and OMIM databases and targets related to the bioactives of Fenugreek were retrieved from the SwissTargetPrediction, DrugBank, and STITCH databases. The Venny tool was used to identify seven overlapping common targets at the intersection of the 56 targets associated to lung cancer and the 293 targets connected to Fenugreek compounds (Table II and Figure 1).

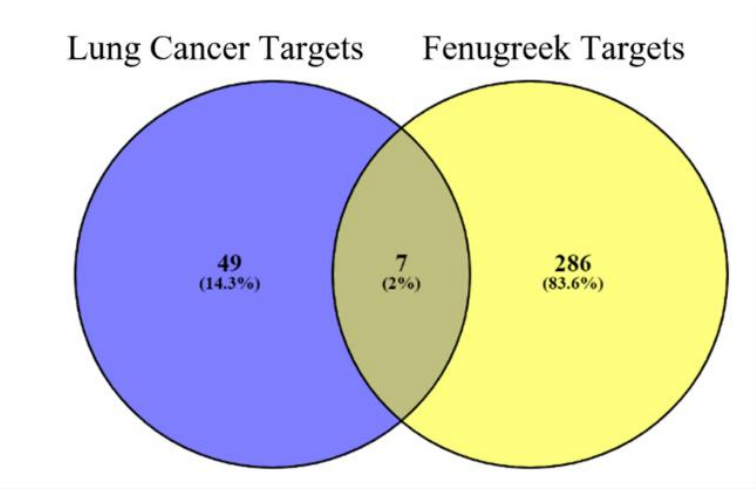

Fig. 1 Venn Diagram showing common target proteins between Fenugreek-targeted genes and Lung Cancer genes.

3.3 Construction and Analysis of the D-C-T-D Network

The Drug–Compound–Target–Disease network of the 7 common protein targets between lung cancer and Fenugreek and the bioactive phytochemicals from Fenugreek was constructed using Cytoscape. The resulting network had 74 edges, each of which represented the interaction between Fenugreek and the following nodes: 49 bioactive nodes, 7 target nodes, 1 Fenugreek node, and 1 Lung Cancer node. Each of the 74 edges (one for fenugreek and one for lung cancer) shows the connection between fenugreek and its bioactives, bioactives and common targets, and the common targets and lung cancer (Figure 2).

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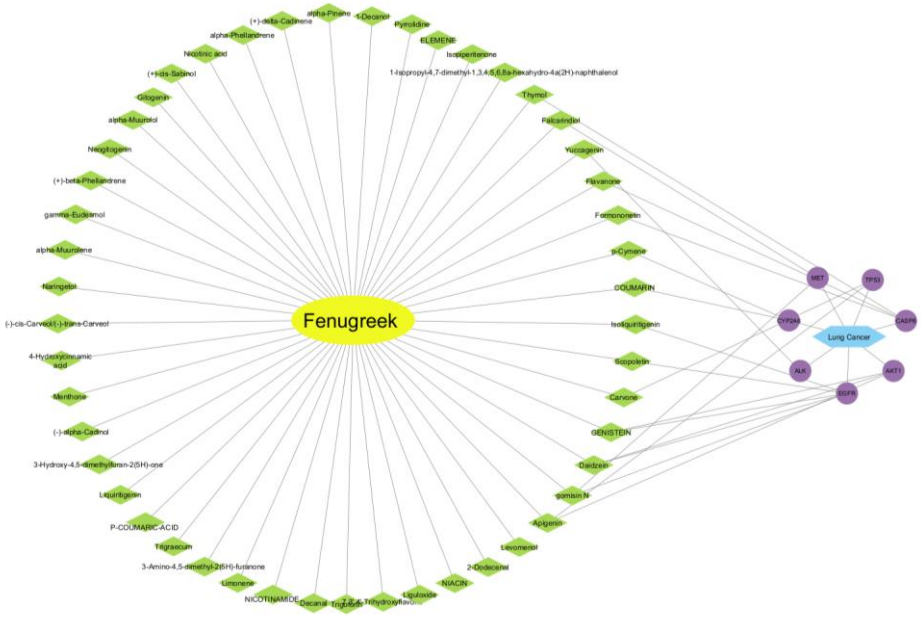

Fig. 2 Drug-Compound-Target-Disease interaction network. Green diamonds are bioactives, the yellow sphere is the compound, purple circles are the common target proteins of the bioactives, and the blue hexagon is the disease. The lines represent interactions between bioactives and targets.

On construction of the PPI network of the 7 common targets of lung cancer and Fenugreek comprised of 7 nodes and 12 edges in STRING, it was observed that CYP2A6 had no interactions with the other 6 proteins - TP53, AKT1, ALK, EGFR, CASP8 and MET that showed direct interactions amongst each other (Figure 3) and also with 12 bioactives of Fenugreek (Figure 4).

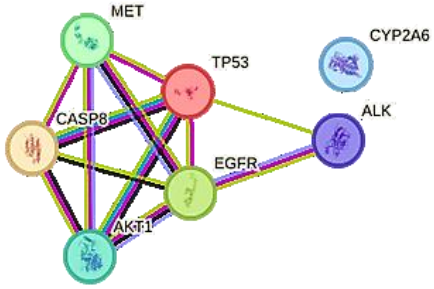

Fig. 3 STRING Network of the common target proteins

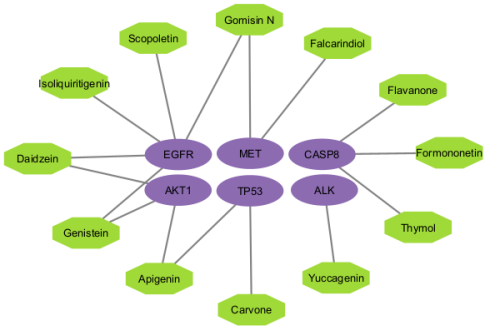

Fig. 4 Six core targets with their respective twelve bioactives of Fenugreek

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3.4 Gene Ontology and KEGG pathway enrichment analysis

Pathway enrichment analyses using Enrichr revealed 510 GO terms ($p < 0.01$) in total, including 416 for Biological Processes (BP), 29 for Cellular Component (CC) and 62 for Molecular Function (MF) and 10 entries with the highest p-value in BP, CC and MF are represented graphically (Figure 5). The gene ontology terms were enriched mainly in positive regulation of kinase and phosphorylation activity, early endosome membrane and mitochondrial outer membrane, endopeptidase activity involved in apoptotic process, and phosphatase binding, respectively. The target-pathway relationship of these targets involved in the top 5 KEGG pathways was represented in the form of a Sankey diagram (Figure 6).

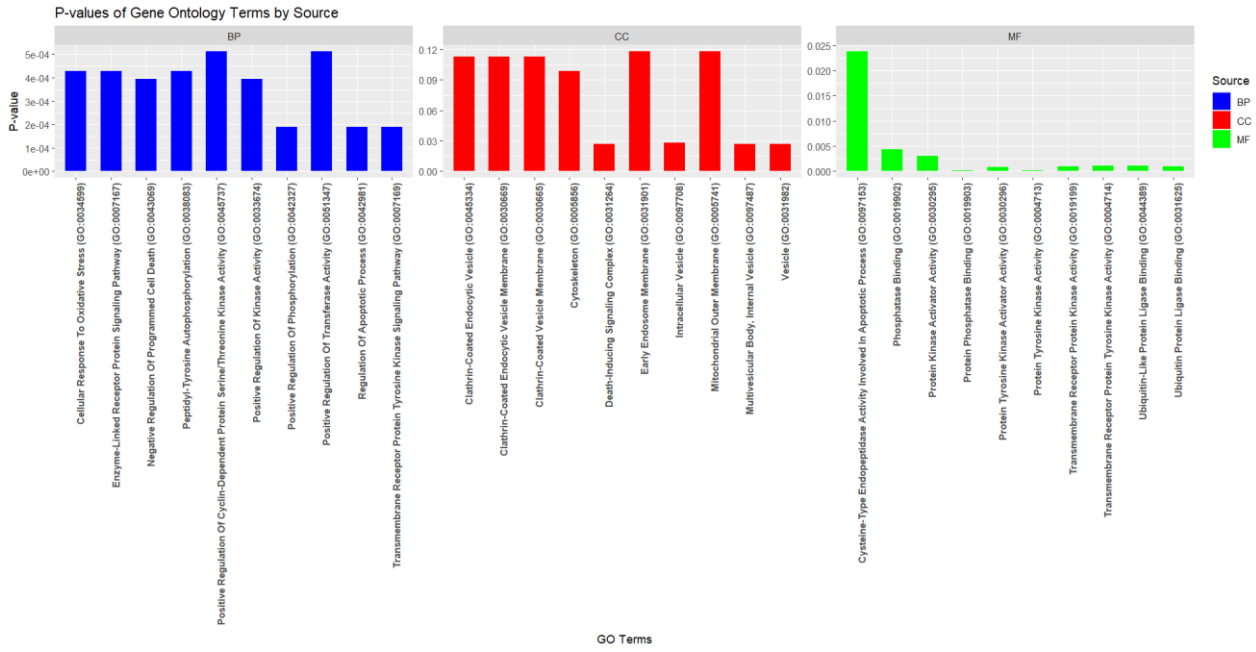

Fig. 5 Visualization of GO terms. The ordinate indicates the gene number of enriched GO terms.

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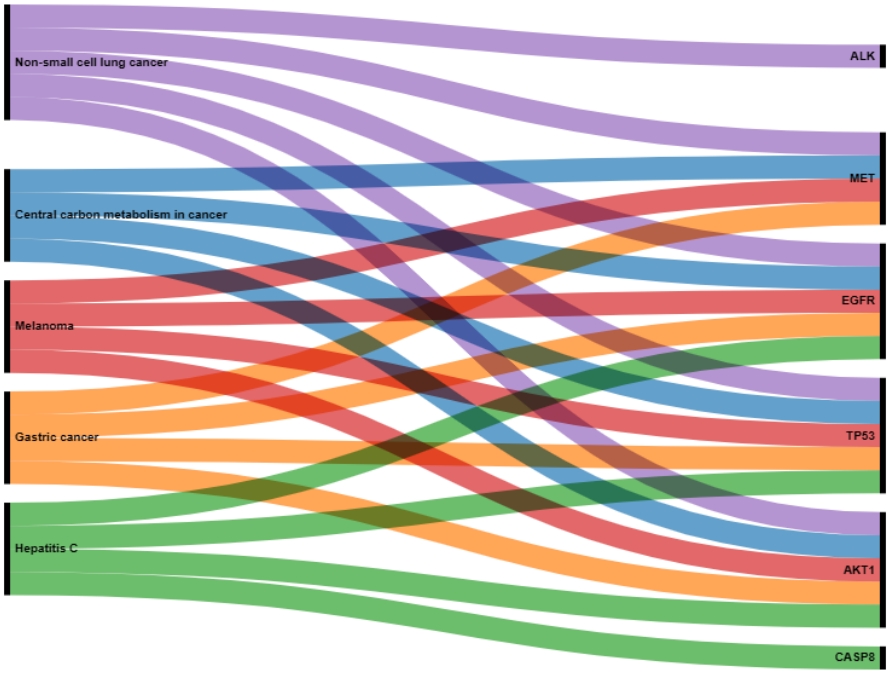

Fig. 6 Sankey Diagram representing the genes involved in the top 5 pathways.

3.5 Molecular Docking

The 3D structures of the six core targets of lung cancer—AKT1, ALK, CASP8, EGFR, MET and TP53, their corresponding bioactives obtained from the Drug-Compound-Target-Disease network (Figure 3, Figure 4) and their interacting residues were retrieved from PDB, PubChem, and PDBSum databases, respectively, followed by their docking using AutoDock Vina software. Commercially available tyrosine kinase inhibitors [15], namely Brigatinib [16], Crizotinib [17][18] and Dasatinib [19], currently prescribed for chemotherapy of lung cancer, were taken as reference drugs for docking studies of EGFR, MET, AKT1, ALK, TP53, and CASP8.

The active constituents of Fenugreek showed a significant affinity for all the core targets as indicated by the binding energy values (< -5.0 kcal/mol) [20] (Table 2), compared to the reference drugs (Table III). With reference to the binding energy of the drug Crizotinib, Yuccagenin showed greater affinity with ALK. Docking analysis revealed that four multi-target bioactives of Fenugreek, namely, Apigenin, Daidzein, Genistein and Gomisin N, had good binding affinity with AKT1, EGFR, MET and TP53, the identified core targets of lung cancer.

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Table II. Results of docking and binding affinities for bioactives and drugs with their respective target.

Target (PDB ID)	Reference Drug & Binding Energy (kcal/mol)	Bioactive of Fenugreek	Binding energy (kcal/mol)
EGFR (8A2A)	Brigatinib -7.93	Scopoletin	-6.32
		Isoliquiritigenin	-6.64
		Gomisin N	-5.68
		Daidzein	-6.45
		Genistein	-6.57
MET (3ZZE)	Brigatinib -7.59	Gomisin N	-6.3
AKT1 (4GV1)	Crizotinib -8.05	Genistein	-7.82
		Apigenin	-7.64
		Daidzein	-7.83
CASP8 (1QTN)	Dasatinib (-6.02)	Flavonone	-6.44
		Formononetin	-6.35
ALK (3AOX)	Crizotinib (-3.9)	Yuccagenin	-6.32
TP53 (6GGA)	Crizotinib -6.53	Apigenin	-6.1
		Carvonone	-5.51

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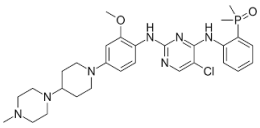
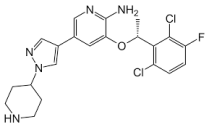
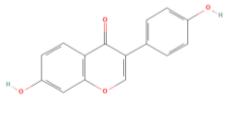
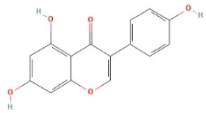
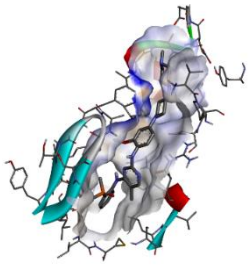
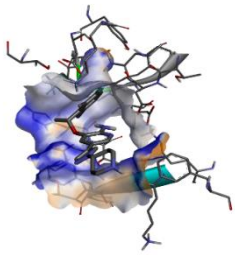
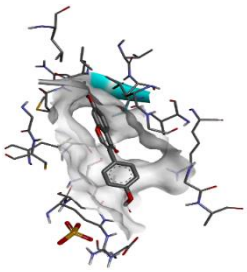
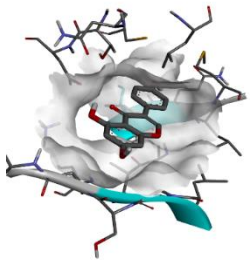
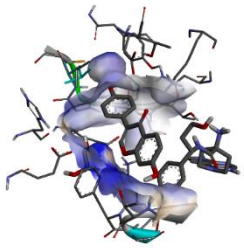
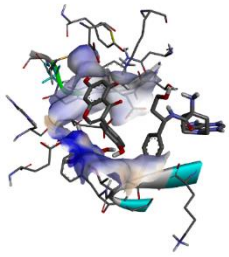
Table III. Visualization of molecular docking of significant multi-targeting bioactives and their corresponding proteins using Discovery Studio Visualizer

Brigatinib	**Crizotinib**	**Daidzein**	**Genistein**
			
			
EGFR-Brigatinib complex	AKT1-Crizotinib complex	EGFR-Daidzein complex	EGFR-Genistein complex
			
		AKT1-Daidzein complex	AKT1-Genistein complex

Studies on fenugreek, a medicinal herb, have demonstrated its pharmacological effects, including anti-inflammatory, antibacterial, antioxidant, chemotherapeutic, and antidiabetic qualities. The therapeutic properties of fenugreek are primarily ascribed to its chemical constituents, including proteins, fatty acids, essential oils, fibers, and steroid saponins [21]. Fenugreek seed contains chemicals like galactomannan and steroid sapogenin, recognized for antioxidant properties. Among them, amino acids such as 4-hydroxyisoleucine possess anti-diabetic and hypocholesterolemic qualities, offering potential for treating disorders like leukemia, infertility, obesity, and cancer [22]. By triggering apoptosis, fenugreek has shown benefits against malignant cells in numerous trials [23]. Its steroidal saponin, diosgenin, exhibits anticancer effects, specifically against lung, breast,

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and colon malignancies and has been reported to suppress telomerase activity in lung cancer by downregulating hTERT gene expression in A549 cells [24]. Although fenugreek seed extract's effects on cancer cells have been studied, less is known about its powerful phytochemicals, potential target genes, and mode of action in lung cancer. The active constituents of fenugreek and their pharmacological mechanism in lung cancer treatment were systematically examined in this study. Four active compounds—Gomisin N, Daidzein, Apigenin, and Genistein—were identified and tested negative for hepatotoxicity and AMES toxicity in the ADMET analysis conducted during initial screening. These compounds demonstrated anti-lung cancer effects by influencing core target proteins, including EGFR, AKT1, MET, and TP53, altering their roles in various signaling pathways, primarily involved in non-small cell lung cancer (NSCLC). Molecular docking confirmed strong binding affinities between core targets and multi-targeting active molecules, further supporting the results from network pharmacology. GO and pathway enrichment analyses revealed that fenugreek modulates key biological processes relevant to NSCLC, including regulation of cyclin-dependent kinase (CDK) activity, transferase function, and oxidative stress response. CDK4/6 inhibition, known to induce cell cycle arrest, is a therapeutic strategy in lung cancer [25], and fenugreek's modulation of CDK activity may support either growth arrest under oncogenic stress or restoration of normal proliferation. Additionally, fenugreek impacts critical oncogenic pathways such as p53 inactivation, EML4-ALK fusion, and EGFR overexpression [26]. The core targets identified in the present study, EGFR, MET, AKT and TP53, through network pharmacology, are reported to be involved in key pathways of NSCLC. About 50–70% of NSCLCs show abnormal PI3K/Akt pathway activation, and approximately 50% harbor mutations in the tumor suppressor gene p53. Genetic alterations in upstream regulators like NF1, MET, ERBB2, and RIT1 further drive PI3K signaling [27]. PI3K/Akt hyperactivation, often driven by EGFR overexpression, has been reported to enhance invasion, proliferation, and resistance to apoptosis [28]. EGFR mutations lead to persistent activation of PI3K/Akt, MAPK/ERK, and STAT3 signaling, contributing to tumor progression and "oncogene addiction" [29]. EGFR inhibitor resistance and NSCLC progression are also influenced by MET, a receptor tyrosine kinase activated via the PI3K/AKT pathway, where AKT plays a central role in survival, transcription, and proliferation [30][31]. Fenugreek compounds such as daidzein and genistein counter these effects by inducing apoptosis through p53-dependent and independent mechanisms, arresting the cell cycle, and inhibiting metastasis and angiogenesis [32-34]. Together, these findings suggest that fenugreek may exert anti-cancer effects through multi-targeted modulation of PI3K/AKT, P53 oncogenic pathway, and EGFR/MET-driven signaling in NSCLC. The study is the first of its kind to provide a theoretical foundation by computationally predicting the crucial role performed by active components of fenugreek that have been shown to be beneficial in NSCLC. Although these results are encouraging,

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we recommend conducting *in vitro* and *in vivo* studies to comprehensively establish fenugreek's potential as a clinically applicable therapeutic drug for lung cancer.

4. CONCLUSION

In conclusion, the present study utilized network pharmacology and molecular docking to elucidate the multi-target mechanism of fenugreek's bioactives in the treatment of lung cancer by targeting multiple pathways, offering an overview of its biological activity, possible interaction targets, and mechanism of action against lung cancer. The results provide a theoretical basis to suggest that bioactives in fenugreek, namely, Genistein, Gomisin N, Apigenin and Daidzein, may contribute in regulating various signaling pathways by multi-targeting core targets such as EGFR, MET, TP53, and AKT1, which are reported to be associated with lung cancer.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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