https://doi.org/10.31925/farmacia.2025.2.20 **ORIGINAL ARTICLE**

SYSTEM PHARMACOLOGY AND MOLECULAR MODELLING TO IDENTIFY POTENTIAL DRUG CANDIDATES FROM THE *SOPHORA* PLANT GENUS FOR CHRONIC BRONCHITIS TREATMENT

MUNAZZAH MALIK [1], MIAN MUHAMMAD MUBASHER [2], ISHA ZAFAR [1], MUHAMMAD BILAL [1#], QURBAN ALI [3#]*, AJAZ AHMAD [4], SHIMING HAN [5]

[1]*Centre for Applied Molecular Biology, University of the Punjab, Lahore, Pakistan*
[2]*Department of Information Technology, University of the Punjab, Lahore, Pakistan*
[3]*Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan*
[4]*Department of Clinical Pharmacy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia*
[5]*School of Biological Sciences and Technology, Liupanshui Normal University, Liupanshui 55300, China*

**corresponding author: drqaliuop@gmail.com*
[#]Authors with equal contribution.

Manuscript received: December 2024

Abstract

Chronic bronchitis (CB) is a lung condition marked by prolonged, persistent coughing and excessive mucus secretion, which reduces life quality and increases the risk of exacerbations. To identify possible therapeutic targets for chronic bronchitis, the current study employs a network pharmacology approach. The ADMET characteristics of phytochemicals from the *Sophora* genus were assessed using data from multiple sources. Swiss Target Prediction was also used to determine the targets of the selected compounds, and DAVID was used to do enrichment analysis. A phytochemical library from the genus *Sophora* was examined for ADME characteristics. Swiss Target Prediction, DisGeNet, and GeneCards were used to identify the gene targets of selected phytochemicals. The target genes were then screened for overlapping targets. Enrichment analysis was conducted using the DAVID database. At the same time, STRINGS was employed to generate a protein-protein interaction (PPI) network, which was subsequently analysed with Cytoscape, and hub genes were deduced using Cytohubba. AutoDock Vina was then used to dock the final genes and phytochemicals. Because of their high binding affinity, acacetin-CFTR, luteolin-MMP9, and acacetin-MPO complexes have been proposed as potential targets against bronchitis. Molecular dynamics (MD) simulations were performed to evaluate the binding stability and adaptability of the active and therapeutic target compounds. In conclusion, the study focuses on the possible pharmacological effects of bioactive phytochemicals from the *Sophora* genus on chronic bronchitis. It supports its usage as a promising treatment for the condition that may be further investigated through *in vitro* and *in silico* experiments.

Rezumat

Bronșita cronică (BC) este o afecțiune pulmonară caracterizată prin tuse prelungită, persistentă și secreție excesivă de mucus, care reduce calitatea vieții și crește riscul de exacerbări. Pentru a identifica posibile ținte terapeutice pentru bronșita cronică, acest studiu utilizează o abordare farmacologică de rețea. Caracteristicile ADMET ale substanțelor fitochimice din genul *Sophora* au fost evaluate utilizând date din mai multe surse. *Swiss Target Prediction* a fost, de asemenea, utilizat pentru a determina țintele compușilor selectați, iar DAVID a fost utilizat pentru a efectua analiza optimizării. O bibliotecă fitochimică din genul *Sophora* a fost examinată pentru caracteristicile ADME. Swiss Target Prediction, DisGeNet și GeneCards au fost utilizate pentru a identifica genele țintă ale substanțelor fitochimice selectate. Genele țintă au fost apoi examinate în vederea detectării suprapunerii țintelor. Analiza optimizării a fost efectuată utilizând baza de date DAVID. În același timp, STRINGS a fost utilizat pentru a genera o rețea de interacțiune proteină-proteină (PPI), care a fost ulterior analizată cu Cytoscape, iar genele hub au fost deduse utilizând Cytohubba. AutoDock Vina a fost apoi utilizat pentru a conecta genele finale și substanțele fitochimice. Datorită afinității lor ridicate de legare, complexele acacetin-CFTR, luteolin-MMP9 și acacetin-MPO au fost propuse ca ținte potențiale în bronșită. Au fost efectuate simulări de dinamică moleculară (MD) pentru a evalua stabilitatea și adaptabilitatea de legare a compușilor activi și a țintelor terapeutice.

Keywords: chronic bronchitis (CB), chronic obstructive pulmonary disease (COPD), genus *Sophora*, network pharmacology, molecular docking, molecular dynamics simulations

Introduction

Chronic obstructive pulmonary disease (COPD) is a type of lung disease that causes breathing problems and airflow obstruction [1]. Chronic bronchitis (CB) is a common and variable component of COPD, which leads to a decline in lung function, an increased risk of ventilation obstruction in smokers, an increased susceptibility to lower respiratory tract infections, and an increase in exacerbation frequency and mortality [2]. Goblet cells overproduce and secrete

mucus, which obstructs small airways, modifies airway walls, and decreases surface tension, resulting in the collapse of airways. Patients with chronic bronchitis may wheeze and produce mucus for an extended period before experiencing shortness of breath. Chronic bronchitis is characterised by difficulty breathing, low levels of oxygen, bluish skin, nails, and lips, wheezing, crackling respiration, swollen feet and heart failure [3]. Bronchitis caused by an infection can be acute or chronic. The most prevalent viral and bacterial causes are Influenza A and B, *Staphylococcus*, *Streptococcus*, and *Mycoplasma pneumoniae*. Chronic bronchitis is associated with asthma, bronchiectasis, and cystic fibrosis [4]. Airway epithelial cells in chronic bronchitis produce proinflammatory factors like interleukin-8 and colon-stimulating factor in response to detrimental or infectious stimuli. Meanwhile, the release of angiotensin-converting enzyme and neutral endopeptidase decreases [5]. In chronic bronchitis, the air sac-lining alveolar epithelium initiates and is affected by the inflammatory process. As chronic bronchitis progresses, the bronchial mucosa swells and becomes inflamed, affecting the mucociliary system. The obstruction of airflow by debris in restricted airways aggravates discomfort. The excessive mucus secretion associated with chronic bronchitis is also responsible for the characteristic cough [6].

Chronic bronchitis (CB) affects both smokers and nonsmokers, with or without COPD, and its prevalence in the mature population spans 3.4 to 22%, depending on the term used. Smoking, indoor and outdoor air pollution exposure and occupational exposure are all risk factors for CB. Despite CB's substantial impact on both illness and mortality, there has been limited success in devising functional treatments [7]. The primary objectives of CB treatment are to alleviate symptoms, minimise adverse effects and delay the disease progression. The key goals of therapy are to decrease excessive mucus production, manage inflammation and decrease wheezing [8]. CB has either not investigated common COPD treatments, such as inhaled β2-agonists, anticholinergics, and glucocorticoids, or has produced contradictory results. Although the phosphodiesterase-4 inhibitor roflumilast has been demonstrated to be useful in the treatment of CB, its use is restricted due to its adverse effects [3]. Pulmonary rehabilitation, which includes education, exercise, and changes in daily living, can be recommended. The other components of CB treatment include physical activity, and avoiding contact with toxins is crucial [9]. Many CB patients continue to experience significant symptoms regardless of following guidelines and having treatment [9].

Historically, natural products and their structural analogues have made a significant contribution to pharmacotherapy and have the potential to act synergistically on multiple targets at once, making it extremely difficult to elucidate and comprehend the molecular mechanisms by which they act on their respective molecular targets [10]. In 2008, the concept of network pharmacology, which links medicine and computer science, was first proposed [11]. In the past few years, network pharmacology has been effectively used to illustrate the mechanisms of action of natural products in the context of multiple diseases by projecting target genes and respective pathways [12, 13]. Network pharmacology was utilised to identify the active components of the *Sophora* genus. Employing KEGG and GO pathway enrichment analysis, an interactive network of drug-component-target-pathway disease was generated, followed by molecular docking and simulation studies, and the potential mechanisms of the *Sophora* genus in treating and managing CB symptoms were investigated. Our research may serve as a guide for future basic experimental research.

Materials and Methods

Sophora bioactive compounds screening

All the data regarding the bioactive compounds in the *Sophora* genus were obtained from published research. *Sophora*, the plant's genus name, was used to search PubChem for phytochemicals, while PubMed and Google Scholar were used to analyse the literature. A library containing 449 phytochemicals was generated. The PubChem database was queried using the specific name of each phytochemical to determine its three-dimensional structure. Using the PubChem database, the canonical SMILES for each of the active substances were identified. The canonical SMILES were then subsequently employed to determine the pharmacokinetic properties of every secondary metabolite.

ADMET studies

DataWarrior [14] was used to screen compounds based on their drug-like properties. The library of 449 compounds was screened using data warrior. Different parameters such as mutagenic, tumorigenic and drug likeliness were studied. For the screening of oral bioavailability, SwissADME is a web-based application that predicts the ADME (absorption, distribution, metabolism and excretion) properties of compounds to serve as the basis for drug discovery. A good drug candidate possesses the appropriate ADMET properties and is effective against the therapeutic target when administered at therapeutic doses. The oral bioavailability (OB) was set to ≥ 30% [15, 16]. Bioavailability is the amount of a drug or other substance that enters the bloodstream and can be utilised by the body. The compounds that

exhibit good bioavailability are advantageous as they allow the human body to absorb much of the necessary nutrients without consuming significant amounts. Medication similarity quantifies the probability that a chemical will be converted into an orally bioavailable drug [17].

Swiss target prediction

The web tool SwissTargetPrediction determines the potential macromolecular sites of bioactive small compounds. In this study, all results probabilities for "*Homo sapiens*" were collected. SwissTarget Prediction identified multiple targeted genes using the canonical SMILES from PubChem [15, 16]. The keyword "chronic bronchitis" was used to find the targets in GeneCard and DisGeNET [18, 19]. The targets from both datasets were merged, and the duplicate genes were removed from the analysis. Employing the web tool Bioinformatics and Evolutionary Genomics, a Venn diagram of the disorder and drug's mutual targets was created.

Pathway Analysis and Functional Enrichment

DAVID (Database for Annotation, Visualization, and Integrated Discovery) was chosen to conduct enrichment and functional annotation analyses [20]. Biological process (BP), molecular function (MF), and cellular component (CC) levels of function were predicted using DAVID (CC) [20]. With "*Homo sapiens*" as the chosen species, the common targets were put into DAVID for GO annotation and pathway enrichment analysis. The three levels of function predicted by DAVID were cellular component (CC), biological process (BP) and molecular function (MF) [21]. It offers a functional annotation chart with all values smaller than 5×10^{-2} as the inclusion criterion. The KEGG pathway was then run using this data after it was put into the DAVID database. For additional analysis, KEGG pathways with $p < 0.05$ were chosen. The results of the GO and KEGG enrichment studies were displayed in bubble charts made with the ggplot2 R program [22].

PPI network and biomolecular interaction analysis

The database for the study of protein interactions is called STRING (Search Tool for the Conservation of Genes and Interacting Proteins [23]. This was used in the present study to investigate the pocket-patch-patch interaction (PPI) network. "*Homo sapiens*" was the designated species, and "ancient bronchitis" was introduced.

The tool for displaying biological interaction networks and pathways is Cytoscape software (version 3.9.1). This application allows the interaction of different types of molecular networks and the presentation of extensive networks. The Cytoscape program was used to generate a D-C-G-D network to describe the mechanism of action of various phytochemicals [24].

Analysis of hub genes

CytoHubba was used to look for significantly expressed genes and to investigate the degree of connectivity of protein interaction networks. CytoHubba is a plugin of Cytoscape. Data from the KEGG pathway, SWISS Target, and PPI network were used for CytoHubba analysis [25]. A degree of 10 was applied to gene targets to get the top ten gene targets, then a degree of 10 was applied to phytochemical compounds to obtain the top ten active compounds [26]. Finally, the most suitable genes and substances are chosen for further examination.

Molecular docking

Understanding the interactions between ligands and the related proteins is made simpler by molecular docking. The hub gene PDB format files were acquired from the Protein Data Bank (RCSB) PD, and the 3D structures of the phytochemicals were downloaded from the PubChem database in SDF format and optimised. The most appropriate protein crystal structures, which had a lower resolution and included the whole structure of a human protein, were chosen for the docking procedure. AutoDock Tool 1.5.6 was implemented to remove ligands and water molecules and fix the protein structure. A PDBQT file format was constructed for the protein and ligand molecules after the water molecules were removed and polar hydrogens were supplied using the AutoDock Tool. Molecular docking between the protein and the refined components was carried out using AutoDock Vina. The command prompt was used to compute the binding energy and display the docking results. This stage focused on examining the high correlation between chemicals and their targets and their binding energy; the lower the binding energy, the higher the affinity [26]. PDB structures with missing residues were repaired using Modeller [27]. Moreover, energy minimisation of ligands was done using Avogadro software [28].

Simulation studies

Molecular dynamics simulation

Following docking, molecular dynamics (MD) simulations of protein-ligand complexes were performed to evaluate the binding kinetics and free energy for the best hit compounds [29]. In the current study, GROMACS version 2022 software was used. The needed ligand topology and MD simulation parameters were constructed using the CGenFF service, while the protein topology was built using the Charmm 36 force field [30]. The MD production run was executed for 200 ns with a time step of 2 fs, and the coordinates of the structure were saved every 10 ps. This was implemented subsequent to the energy reduction and system equilibration. Following a 200 ns MD simulation, the trajectories were utilised to evaluate several aspects of dynamics, such as the root mean square deviation (RMSD) of ligands from the protein backbone. Over

a 200-ns time frame, the quantity of H-bonds between the ligand and proteins was estimated. The ligand-protein interaction energies for Coul-SR and LJ-SR were also determined. In addition to MD simulations, the MM-PBSA approach was also used to investigate biomolecular interactions using gmx_MMPBSA. This approach calculates interaction free energies and is commonly used in computational drug design as a scoring function [31].

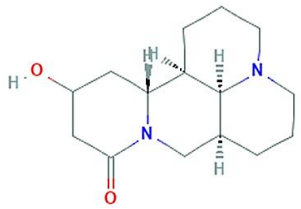
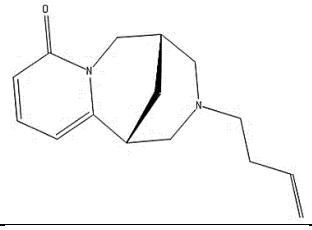
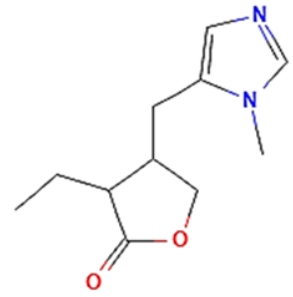
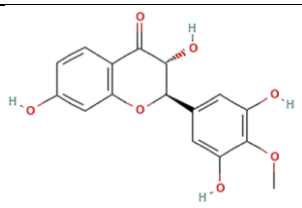
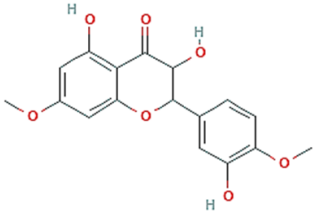
Results and Discussion

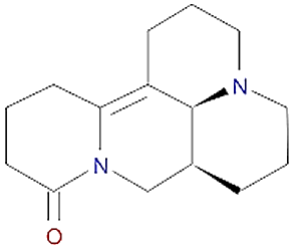
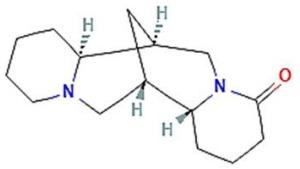
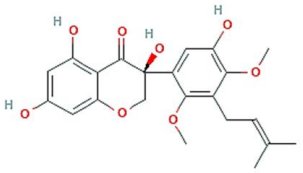
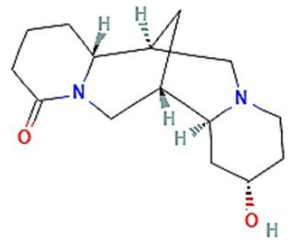
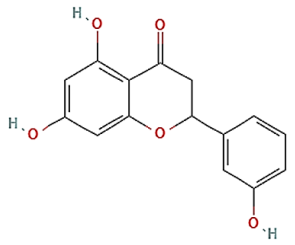
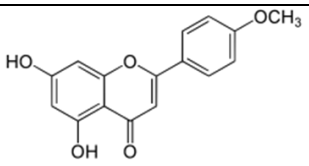
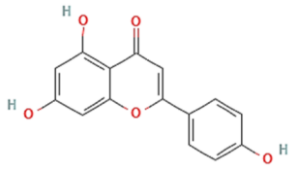
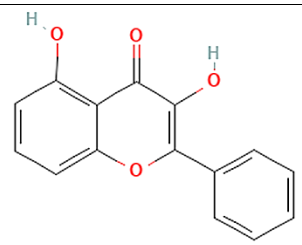
Screening and collection of therapeutic targets of Sophora

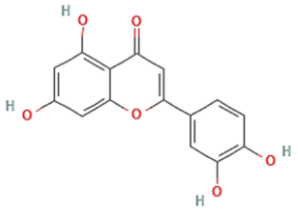
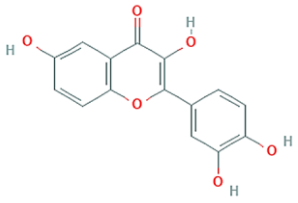
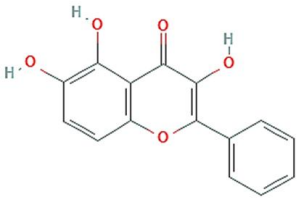
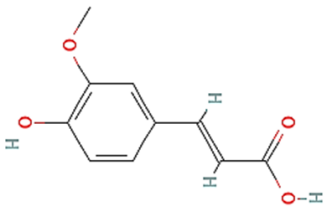
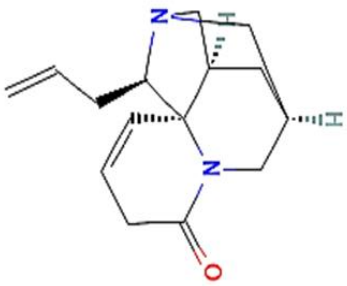
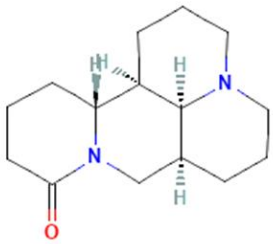
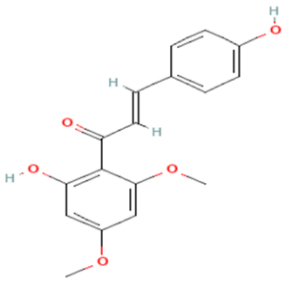
In total, 449 compounds of *Sophora* were collected from the literature and various databases after removing duplicates. Then DataWarrior was performed on these 449 compounds, and 61 active compounds were collected based on different parameters. These parameters are molecular weight, number of H bond acceptors and donors, consensus log p, and number of rotatable bonds. The most important parameters are the molecular weight and log p. Drug likeliness and oral bioavailability parameters (DL $\geq$ 0.18 & OB $\geq$ 30) were used to select 22 active compounds of *Sophora* (Table I) as effective components after performing ADMET analysis on 449 compounds. PubChem was used to determine the chemical structure of these active compounds.

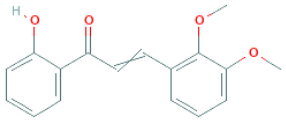
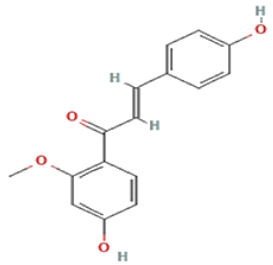
Table I

Oral bioavailability, drug likeness and chemical structures of selected phytochemicals

Compound name	Total mol weight	Bioavailability score	Drug likeness	Chemical structures
Hydroxymatrine	264.368	0.55	0.98781	
Rhombifolin	244.337	0.55	0.85636	
Adenocarpine	296.413	0.55	0.71396	
Sepinol	318.28	0.55	0.56808	
Sophorayunnanol	332.307	0.55	0.56808	

Compound name	Total mol weight	Bioavailability score	Drug likeness	Chemical structures
Dehydromatrine	246.353	0.55	0.56704	
Lupanine	248.369	0.55	0.5543	
Sophoronol D	416.425	0.55	0.54618	
hydroxylupanine	264.368	0.55	0.49571	
trihydroxyflavanone	272.255	0.55	0.44477	
Acacetin	284.266	0.55	0.40331	
Apigenin	270.239	0.55	0.28194	
Dihydroxyflavone	254.24	0.55	0.28194	

Compound name	Total mol weight	Bioavailability score	Drug likeness	Chemical structures
Luteolin	286.238	0.55	0.28194	
Tetrahydroxyflavone	286.238	0.55	0.28194	
Trihydroxyflavone	270.239	0.55	0.28194	
Ferulic acid	194.185	0.85	0.27506	
Tsukushinamine A	244.337	0.55	0.26504	
Matrine	248.369	0.55	0.2541	
Dihydroxy dimethoxychalcone	300.309	0.55	0.21902	

Compound name	Total mol weight	Bioavailability score	Drug likeness	Chemical structures
Hydroxy dimethoxychalcone	284.31	0.55	0.21902	
Methylisoliquiritigenin	270.283	0.55	0.21902	

The SwissTargetPrediction database retrieved 2000 targets from the selected 22 phytochemicals from the *Sophora* genus. The GeneCard and DisGeNet databases generated 1392 and 118 potential gene targets for chronic bronchitis, respectively. The prospective mapping of such active ingredient targets from GeneCard and DisGeNet to chronic bronchitis gene targets produced ten common targets, or Venn common targets (Figure 1), which were deemed possible chronic bronchitis targets.

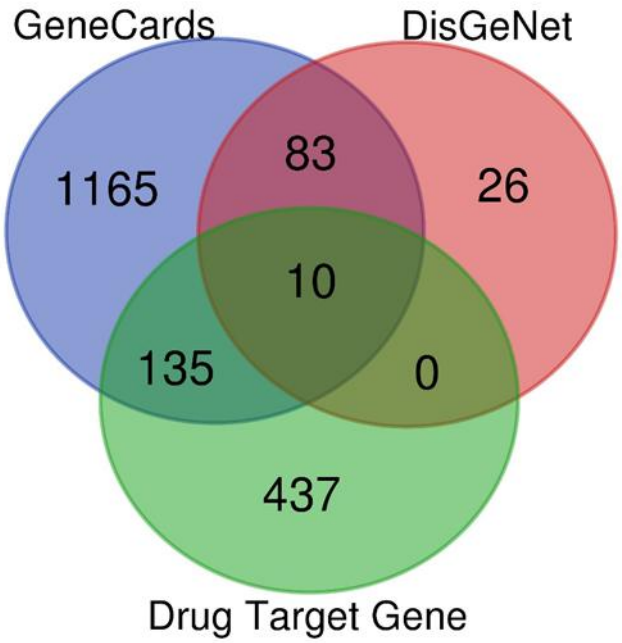


Figure 1.
VENN diagram showing common gene targets

GO and KEGG pathway enrichment analysis

DAVID Bioinformatics Resources was applied to examine the functional and pathway enrichment information for the potential genes. GO terms can be divided into three categories: biological process (BP), cellular component (CC), and molecular function (MF), as shown in Figure 2. The KEGG database contains adequate information about the known metabolic pathways and regulatory pathways; therefore, it accelerates the gene mapping to KEGG pathways to systematically analyse gene functions. This study focuses on discovering insights into the precise biological function and signalling pathways. In this regard, a list that carries information about genes with some degree of uniqueness was obtained. GO and KEGG pathway enrichment analysis was performed on the selected genes. The probability of selected genes randomly falling in a particular domain has been represented as a p-value. In general, the p-value has an inverse correlation with the significance of a term.

PPI network analysis

The PPI network was built using the 10 overlapped genes submitted to the STRING database. Nodes and

their interactions in the PPI network indicate the interrelationship between multiple targets during disease development (Figure 3).

Network of Compound-Targets

The interaction between the prospective targets and active compounds was analysed using Cytoscape to establish the relationship between the active compounds and the possible targets. A compound-target network was constructed using 7 possible target genes and 17 active components of the *Sophora* genus (Figure 4).

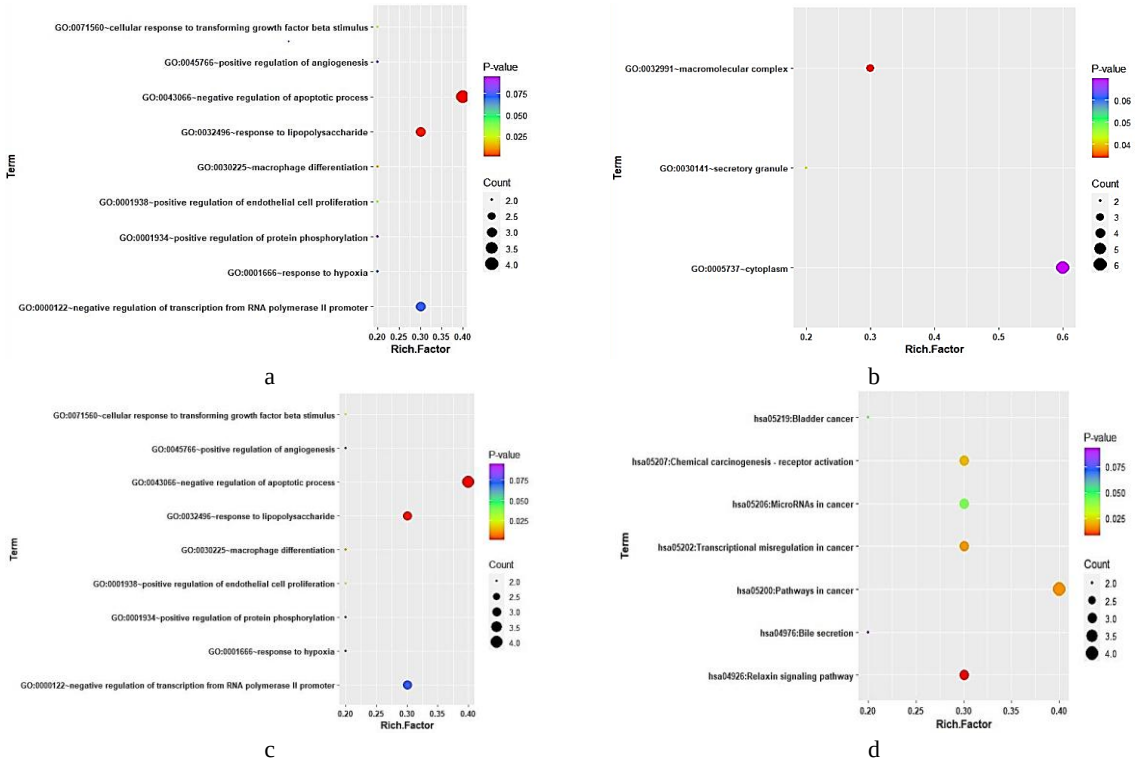


Figure 2.

The plot of the Go enrichment analysis of chronic bronchitis-related genes. Biological process (a), cellular component (b), molecular function (c) and KEGG pathway enrichment analysis (d).

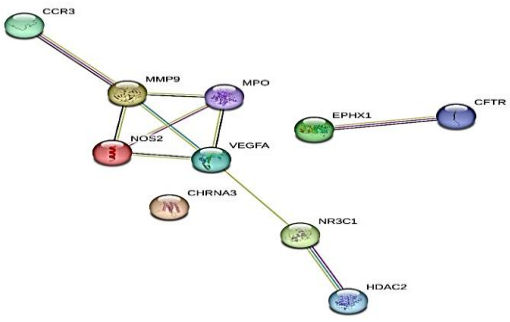


Figure 3.

Potential protein-protein interaction (PPI) network of common genes

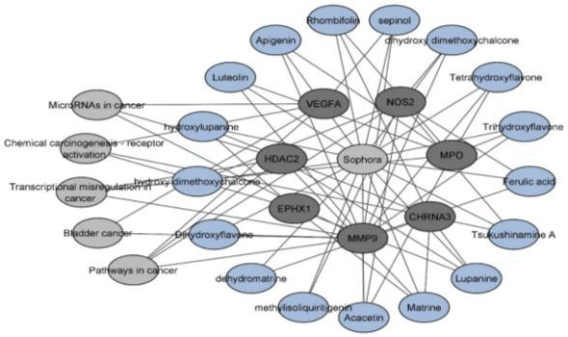


Figure 4.

Compound–target–pathway interaction network

The blue-coloured nodes in the network represent *Sophora* constituents, while the dark, grey-coloured nodes represent chronic bronchitis targets. Grey-coloured nodes represent the pathways in which these genes are involved. Cytohubba was performed, the degree of ten was applied, and the top seven active compounds were obtained (Figure 5). VEGFA has the highest rank, followed by MMP9, NOS2, MPO, NR3C1, CCR3, CFTR, EPHX1 and HDAC2. These nine targets were then used for molecular docking. The degree of ten was then applied, and the top ten genes and compounds were obtained (Figure 6), which were then further used in molecular docking.

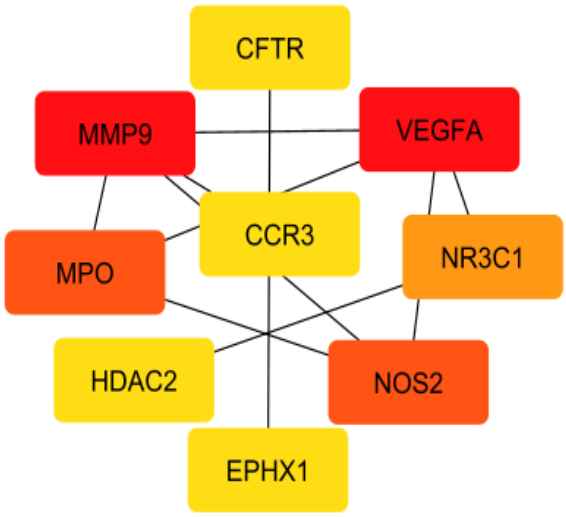


Figure 5.
Top 09 target genes for chronic bronchitis by Cytoscape

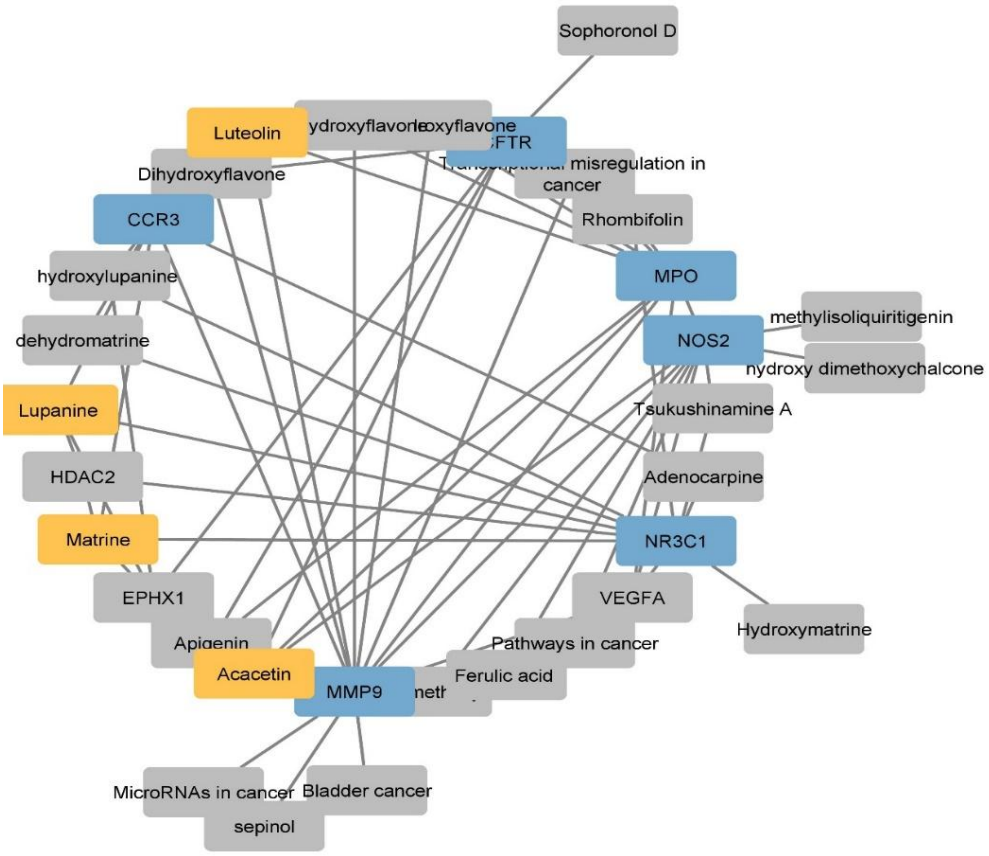


Figure 6.
Key gene target pathway analysis by CytoHubba. Blue nodes represent selected genes (CFTR, CCR3, MPO, NOS2, NR3C1) for docking analysis, yellow nodes indicate phytochemicals (Acacetin, Lupanine, Matrine, Luteolin), and grey nodes depict related biological pathways

Molecular Docking

Molecular docking was applied to identify potential targets of constituents that could lower the incidence of chronic bronchitis. The docking analysis was employed to estimate the binding energies of components and targets and the possible binding pockets in the target proteins. Based on comparing the hub genes with the KEGG analysis results, six target genes, NR3C1, CCR3, MPO, MMP9, CFTR and NOS2, and four active compounds of genus *Sophora*, were chosen

for molecular docking. In molecular docking, the lower (more negative) the binding energy, the higher the predicted affinity for binding the ligand to the target. The binding score of all the compounds and targets ranged from -6.9 to -9.5 kcal/mole (Table II). Docking complexes with the best docking scores are shown (Figures 7, 8, and 9). These three docking complexes were used for MD simulations.

Table II

Binding energy of the docking complexes. A lower score indicates better binding ability

Compounds	**Gene target**	**Binding Energy** (kcal/mol)
Acacetin	NOS2	-8.9
	MPO	-9.2
	CFTR	-9.2
	MMP9	-9.1
Luteolin	CFTR	-7.3
	MMP9	-9.5
	MPO	-9.1
Lupanine	CCR3	-6.9
	NR3C1	-8.3
Matrine	NR3C1	-7.8

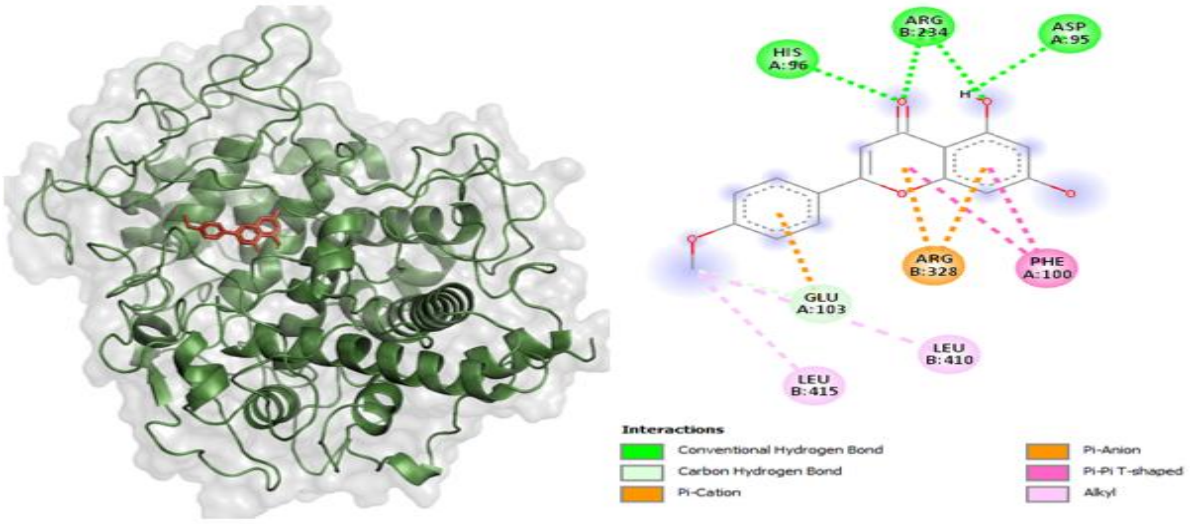


Figure 7.
3D and 2D diagram of MPO_Acacetin docking complex

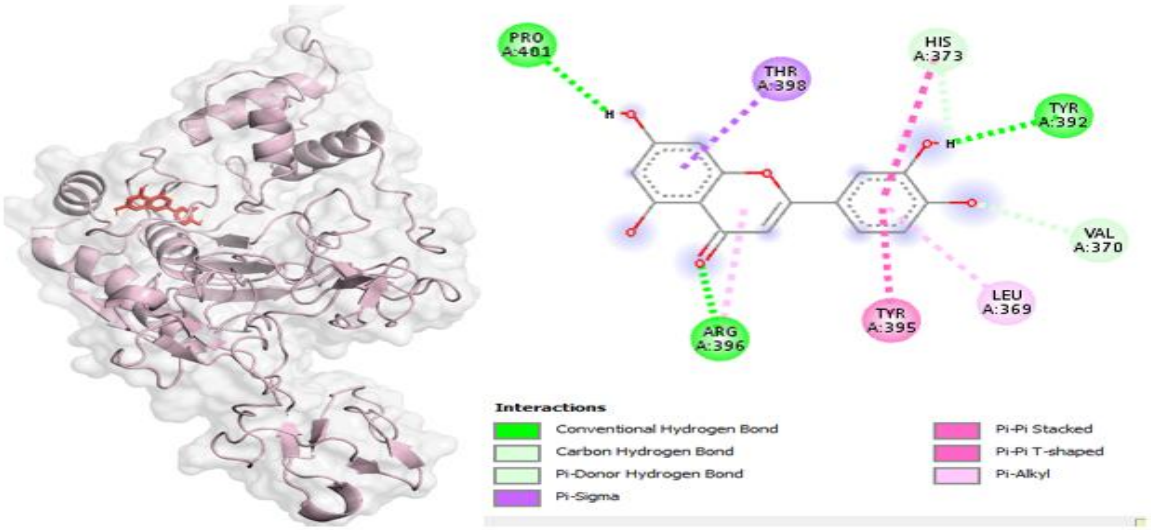


Figure 8.
3D and 2D diagram of MMP9_Luteolin Docking complex

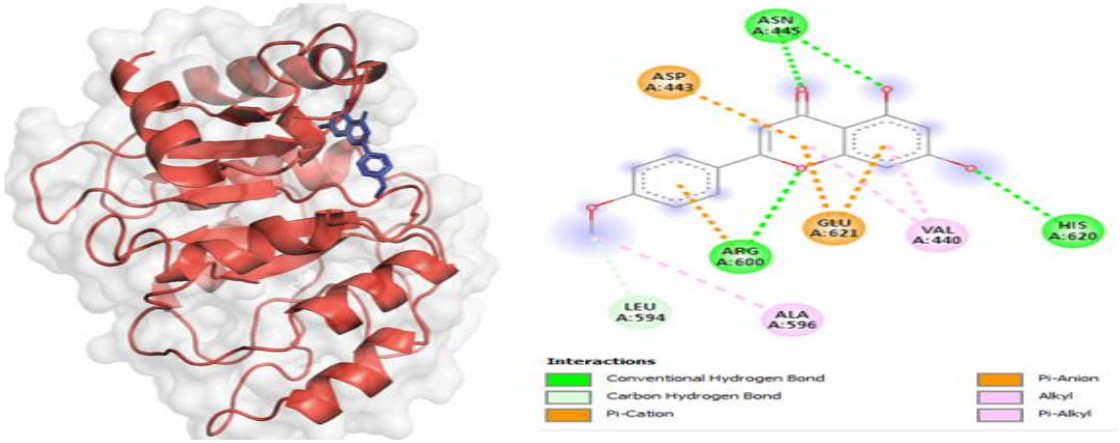


Figure 9.
3D and 2D diagram of CFTR_Acacetin Docking complex

Molecular Dynamics Simulation

The docking scores indicated significant interaction between the ligands and proteins. MD simulations are used to better understand the binding kinetics of ligands and proteins. GROMACS was used to simulate the molecular dynamics of the complexes acacetin-CFTR, luteolin-MMP9, and acacetin-MPO for 200 nanoseconds. The trajectories were analysed concerning interaction energies such as Columbic short-range (Coul-SR) hydrogen bonding and Lennard-Jones short-range (LJ-SR) were calculated as the -34.54 ± 8.8 and -118.02 ± 12 in the MPO-Acacetin (Figure 10a), -79.77 ± 1.8 and -175.95 ± 1.2 in the MMP9_luteolin (Figure 11a) while -58.87 ± 6 and -132.14 ± 2.5 in the CFTR_acacetin (Figure 12a). The RMSD values lie between 0.5 and 0.6nm, 0.6 and 0.9nm and 0.4 and 0.6 nm in MPO_acacetin, MMP9_luteolin and CFTR_acacetin complexes, as shown in Figure 10b, 11b and 12b, respectively. The H-bonding lies within 0-4, 0-6 and 0-5 in MPO_acacetin, MMP9_luteolin and CFTR_acacetin complexes, respectively, as shown in Figures 10c, 11c and 12c. Together, these results confirm the accuracy of the docking results. All the graphs shown in Figures 10, 11 and 12 were generated using the Matplotlib library of the Python programming language.

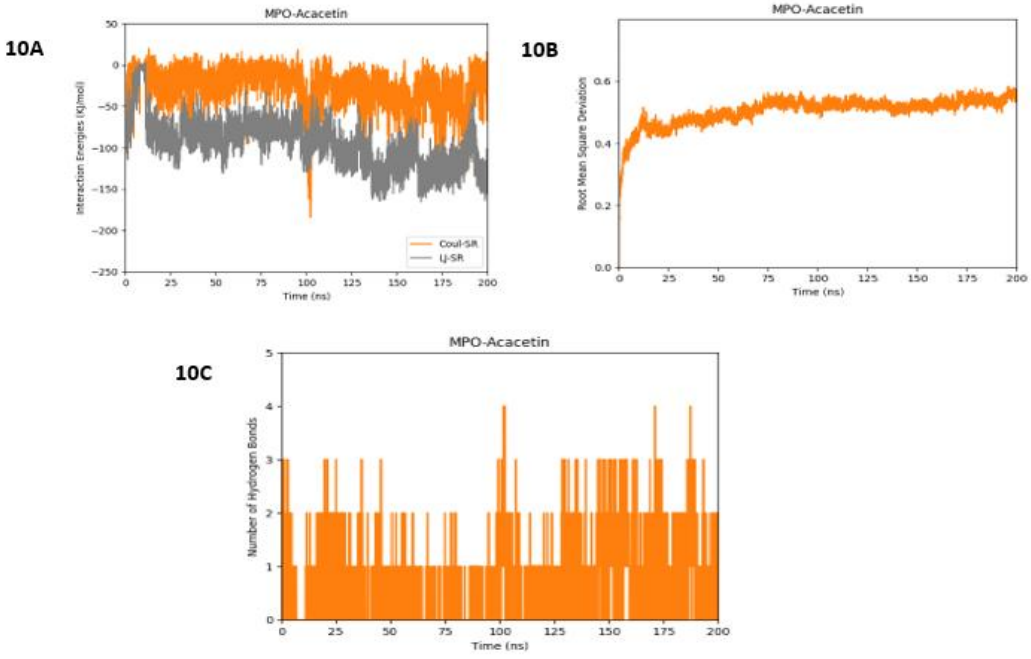


Figure 10.
MD simulation of the MPO_Acacetin complex: (A) Interaction energy plot, (B) RMSD plot, (C) Hydrogen bond count

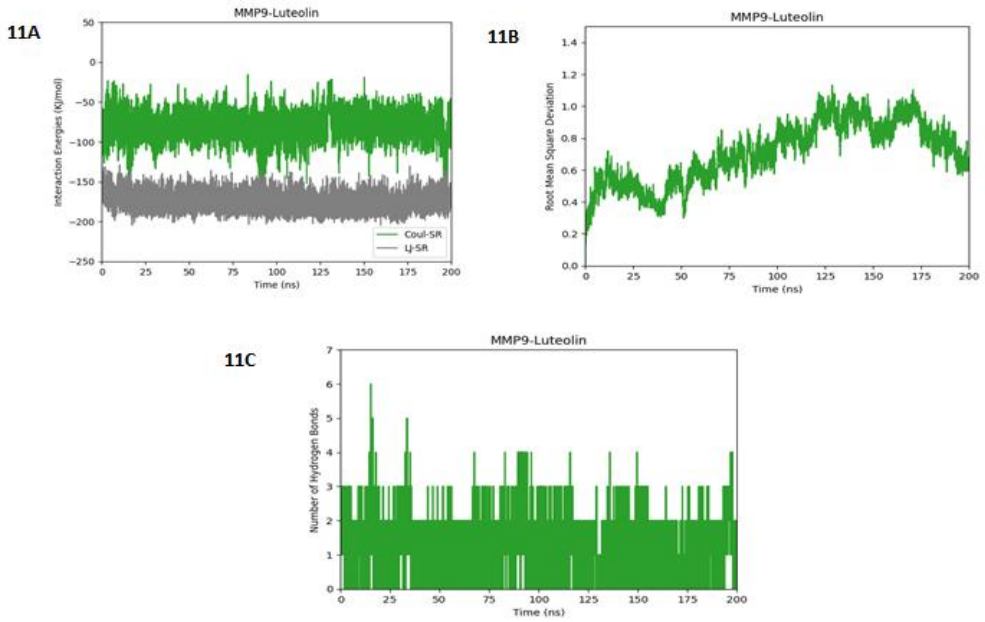


Figure 11.
MD simulation of the MMP9_Luteolin complex: (a) Interaction energy plot, (b) RMSD plot, (c) Hydrogen bond count

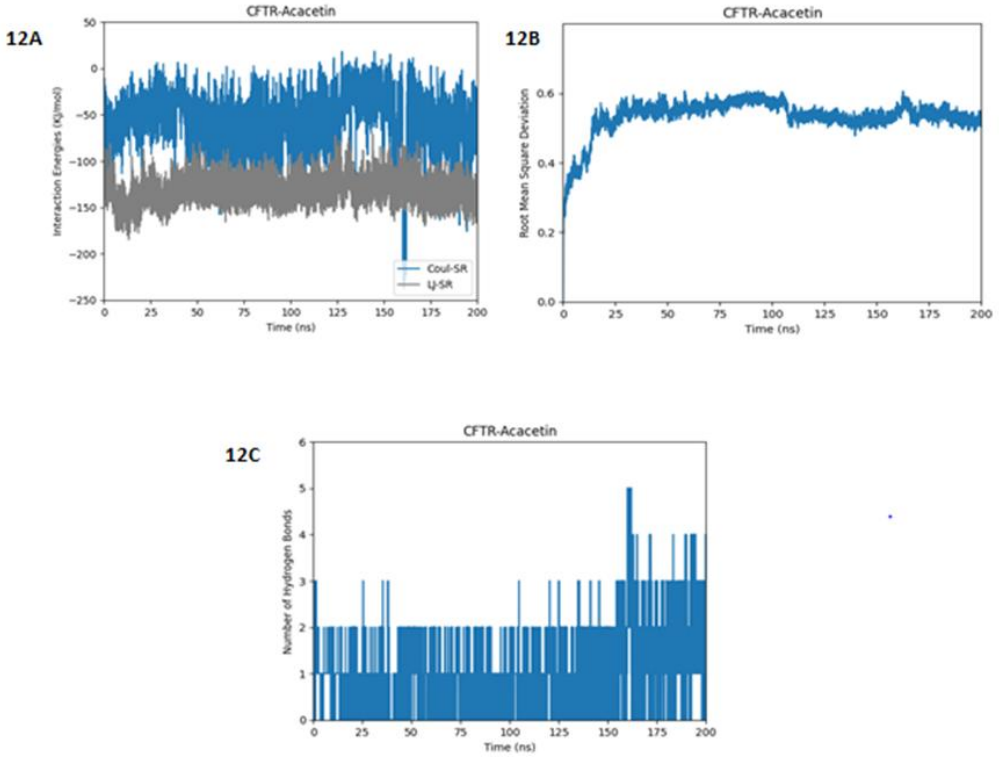


Figure 12.
MD simulation of the CFTR_Acacetin complex: (a) Interaction energy plot, (b) RMSD plot, (c) Hydrogen bond count

Binding free energy calculation by MM-PBSA:
The MM-PBSA method was applied to determine the free energy of binding. A △G value of 16.2 ± 5.07 kcal/mol was observed for MPO-Acacetin. CFTR-Acacetin exhibited a △G value of -6.61 ± 4.19 kcal/mol, whereas -21.43 ± 2.69 kcal/mol was observed for MMP9_Luteolin, as depicted in Figure 13. The T△S value of each complex is positive.

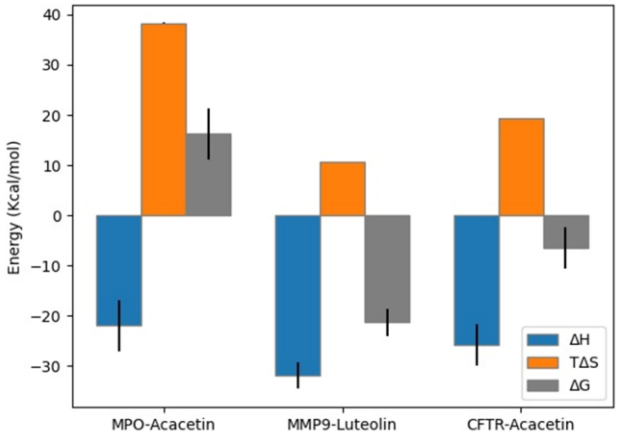


Figure 13.
Binding energies of MPO-Acacetin, MMP9_Luteolin and CFTR-Acacetin, respectively

Chronic bronchitis is the most serious form of the disease, with a high prevalence rate. The condition is caused by goblet cells' excessive production and secretion of mucus. Despite the influence of CB on morbidity and mortality, there has not been much success in creating efficient treatments [32-36]. The most effective plant-based medicinal target against chronic bronchitis was identified using *in silico* techniques like network pharmacology, molecular docking, and molecular dynamics simulations. The results show that the selected plants' active ingredients can target important proteins connected to several biological processes and signalling cascades implicated in chronic bronchitis. However, more *in vivo* studies and clinical trials are required to fully understand these effects.

The GeneCard and DisGeNet databases provided 1392 and 118 findings for possible chronic bronchitis targets, respectively. Twenty-two chemical compounds were used to generate 2000 probable targets using the Swiss Target Prediction database. Targets for chronic bronchitis discovered in databases and from 22 phytochemicals were possibly mapped, and they produce 10 common genes. Through Cytoscape, a network of chosen phytochemicals and gene targets was created. The top nine hub genes identified by cytohubba with the most significant degree values were VEGFA, MMP9, NOS2, MPO, NR3C1, CCR3, CFTR, EPHX1 and HDAC2 (Table III).

Table III
Functions and pathological consequences of key biomarkers involved in bronchitis

Biomarker	Function in Bronchitis	Pathological Consequences
VEGFA (vascular endothelial growth factor A)	Increases vascular permeability, promotes angiogenesis, and recruits inflammatory cells	Oedema, airway wall thickening, and increased inflammatory infiltration
MMP9 (matrix metalloproteinase-9)	Degrades extracellular matrix, promotes tissue remodelling, and disrupts lung architecture	Airway remodelling, emphysema-like changes, and lung tissue damage
NOS2 (nitric oxide synthase 2)	Produces nitric oxide, induces oxidative stress, and contributes to airway inflammation	Oxidative stress, airway hyperresponsiveness, and increased inflammatory cytokine production
MPO (myeloperoxidase)	Released from neutrophils, generates oxidative stress and leads to mucus hypersecretion	Neutrophilic inflammation, oxidative damage, and excessive mucus production
NR3C1 (glucocorticoid receptor)	Mediates anti-inflammatory effects, regulates immune responses, and controls glucocorticoid action	Steroid resistance, impaired inflammation control, and chronic airway inflammation
CCR3 (C-C motif chemokine receptor 3)	Regulates eosinophilic recruitment and inflammatory cell migration in airway inflammation	Eosinophilic inflammation, airway remodelling, and bronchial hyperresponsiveness
CFTR (cystic fibrosis transmembrane conductance regulator)	Maintains airway hydration and mucus clearance; dysfunction leads to thickened mucus secretion	Defective ion transport, thick mucus accumulation, and impaired mucociliary clearance
EPHX1 (epoxide hydrolase 1)	Involved in the detoxification of environmental pollutants and oxidative stress in bronchial tissues	Increased susceptibility to environmental pollutants, DNA damage, and oxidative stress
HDAC2 (histone deacetylase 2)	Regulates histone modifications and inflammation control; reduced expression linked to steroid resistance	Steroid resistance, chronic inflammation, and epigenetic dysregulation in bronchitis

The KEGG enrichment analysis results showed that numerous signalling pathways are involved in the onset and progression of chronic bronchitis. Nine biological processes (BP) were identified because of the GO analysis. These processes include the response to lipopolysaccharide, regulation of apoptotic and angiogenesis processes, and protein phosphorylation. Active compounds and crucial targets that had been assessed underwent molecular docking to validate the results of network pharmacology.

The molecular docking technique is a predictive method used for interaction studies of the drug and target proteins. Docking results show that MPO with Acacetin, MMP9 with leuteolin and CFTR with acacetin have good binding energies as mentioned in Table II. MMP9 protein plays a significant role in chronic bronchitis. It breaks down and remodels the lung extracellular matrix. Chronic bronchitis inflammation increases MMP9 synthesis [35,37,38]. This increased MMP9 activity degrades airway wall structural proteins, modifying and narrowing the airway. It also recruits and activates immune cells, worsening inflammation. Chronic bronchitis progresses and causes airway dysfunction due to elevated MMP9 levels [39]. Cystic fibrosis transmembrane conductance regulator (CFTR) is important in chronic bronchitis. CFTR is a chloride channel located on the surface of epithelial cells in various tissues, including the respiratory tract [40]. The absence of the CFTR protein may be caused by genetic factors in chronic bronchitis. Transport of refrigerants across cell membranes, atmospheric surface water, and ion balance contribute to this imbalance. This causes the mucus to swell, dry up the fluid, impede flow, and block the airways. Bacteria grow in the thick mucus and die, causing recurrent infections and chronic inflammation in the bronchi [41]. The protein myeloperoxidase (MPO) is present mainly in neutrophils that fight diseases. Chronic bronchitis causes respiratory tract inflammation, recruiting neutrophils and increasing MPO [42]. MPO causes tissue damage by creating reactive oxygen species and oxidative stress. It also increases airway inflammation by releasing inflammatory mediators. The presence of MPO in chronic bronchitis supports its role in disease development and therapeutic potential [43].

Luteolin, a flavonoid compound, has shown efficacy in treating chronic bronchitis. Studies have shown that luteolin has anti-inflammatory properties that can modulate immune responses [44]. In chronic bronchitis, the main feature of which is constant inflammation in the airways, luteolin has been found to prevent the release of inflammatory mediators and prevent the activation of inflammatory cells. The role of acetin, a flavonoid compound in certain plants, in chronic bronchitis has been investigated. Acetin has been found to have antioxidant effects, prevent excessive mucus production, and improve airways, potentially benefiting people with chronic bronchitis [45].

Based on the docking results, docking complexes with good binding energies were selected for molecular dynamics simulations. Molecular dynamics simulations can reveal dynamic interactions between drugs and target molecules, providing information on binding mechanisms, stability, and interaction patterns [29,46]. Protein-ligand interaction energies such as columbic hydrogen bond (Coul-SR) and Lennard-Jones (LJ-SR) values were -79.77 ± 1.8 and -175.95 ± 1.2 for MMP9 and luteolin, and -58.87 with acacetin. Strong interaction with CFTR and (± 6 and -132.14 ± 2.5) and MPO with acacetin (-34.54 ± 8.8 and -118.02 ± 12). MMPBSA is a post-processing technique for MD approaches to determine the binding free energy, the main energy involved in binding. The net binding free energy, calculated by subtracting 5000 frames selected from the last 50 ns of the MD trajectories, was calculated as MMP9_Luteolin (-21.43 ± 2.69 kcal/mol) > CFTR_Acacetin (-6.61 ± 4.19 kcal/mol) > MPO (4.19 ± 5.07 kcal/mol). The results confirmed the stability of MMP9_luteolin compared to the other two complexes. The results show that the main energy contribution is the electronic energy involved in hydrogen bonds, which is consistent with the hydrogen bond analysis shown in Figure 11C. Overall, this study indicates that herbal remedies are highly effective in treating chronic bronchitis and may provide an alternative to conventional treatments with a lower risk of side effects.

Conclusions

The findings of this study provided the foundation for new chronic bronchitis targets, pharmacological regimes with multiple targets, and representations of the efficacy of various medications. A combination of network pharmacology and molecular docking techniques was used to determine the molecular mechanism of different plant species for the treatment of chronic bronchitis. According to the network analysis's findings, these plants contain compounds with a wide range of targets and operate on various chronic bronchitis-related pathways, which may assist in lessening the severity of chronic bronchitis' negative consequences. According to our research, the genes MMP9, CFTR, and MPO are effective therapeutic targets for the prevention and management of chronic bronchitis and Luteolin and acacetin are indicated as potential therapeutic agents to target these genes.

Acknowledgement

The authors are grateful to the University of the Punjab, Lahore, Pakistan, for supporting this study

and would also like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2025R350), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare no conflict of interest.

References

1. Prasad B, Chronic Obstructive Pulmonary Disease (COPD). *Int J Pharm Res Techn.*, 2023; 10(1): 67-71.
2. Crețu A, Ghiciuc CM, Mitrofan EC, Lupușoru RV, Hancianu M, Ciubotariu D, Mitrofan C, Onofrei IM, Antibiotic-related adverse events in hospitalized chronic obstructive pulmonary disease patients with frequent exacerbation: a retrospective study. *Farmacia*, 2023; 71(4): 848-855.
3. Antus B, Pharmacotherapy of Chronic Obstructive Pulmonary Disease: A Clinical Review. *Int Scholar Res Notices*, 2013; 2013(582807): 1-11.
4. Alharbi KS, Fuloria NK, Fuloria S, Rahman SB, Al-Malki WH, Javed Shaikh MA, Thangavelu L, Singh SK, Rama Raju Allam VS, Jha NK, Chellappan DK, Dua K, Gupta G, Nuclear factor-kappa B and its role in inflammatory lung disease. *Chem Biol Interact.*, 2021; 345: 109568.
5. Favrat B, Burnier M, Nussberger J, Lecomte JM, Brouard R, Waeber B, Brunner HR, Neutral endopeptidase versus angiotensin converting enzyme inhibition in essential hypertension. *J Hypertens.*, 1995; 13(7): 797-804.
6. Burgel PR, Nesme-Meyer P, Chanez P, Caillaud D, Carre P, Perez T, Roche N, Cough and sputum production are associated with frequent exacerbations and hospitalizations in COPD subjects. *Chest.*, 2009; 135(4): 975-982.
7. Valipour A, Fernandez-Bussy S, Ing AJ, Steinfort DP, Snell GI, Williamson JP, Saghaie T, Irving LB, Dabscheck EJ, Krimsky WS, Waldstreicher J, Bronchial Rheoplasty for Treatment of Chronic Bronchitis. Twelve-Month Results from a Multicenter Clinical Trial. *Am J Respir Crit Care Med.*, 2020; 202(5): 681-689.
8. Garnock-Jones KP, Roflumilast: A Review in COPD. *Drugs*, 2015; 75(14): 1645-1656.
9. Arnold MT, Dolezal BA, Cooper CB, Pulmonary Rehabilitation for Chronic Obstructive Pulmonary Disease: Highly Effective but Often Overlooked. *Tuberc Respir Dis.*, 2020; 83(4): 257-267.
10. Beutler JA, Natural Products as a Foundation for Drug Discovery. *Curr Protoc Pharmacol.*, 2019; 86(1): e67.
11. Hopkins AL, Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol.*, 2008; 4(11): 682-690.
12. Gogoi B, Saikia SP, Virtual Screening and Network Pharmacology-Based Study to Explore the Pharmacological Mechanism of *Clerodendrum* Species for Anticancer Treatment. *Evid Based Complement Alternat Med.*, 2022; 2022: 3106363.
13. Ma J, Huang J, Hua S, Zhang Y, Zhang Y, Li T, Dong L, Gao Q, Fu X, The ethnopharmacology, phytochemistry and pharmacology of *Angelica biserrata* – A review. *J Ethnopharmacol.*, 2019; 231: 152-169.
14. Sander T, Freyss J, von Korff M, Rufener C, DataWarrior: an open-source program for chemistry aware data visualization and analysis. *J Chem Inf Model.*, 2015; 55(2): 460-473.
15. Daina A, Michielin O, Zoete V, SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.*, 2019; 47(W1): W357-W364
16. Daina A, Michielin O, Zoete V, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.*, 2017; 7: 42717.
17. Toutain PL, Bousquet-Melou A, Bioavailability and its assessment. *J Vet Pharmacol Ther.*, 2004; 27(6): 455-466.
18. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D, The GeneCards Suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinformatics*, 2016; 54: 1.30.1-1.30.33.
19. Pinero J, Bravo A, Queralt-Rosinach N, Gutierrez-Sacristan A, Deu-Pons J, Centeno E, Garcia-Garcia J, Sanz F, Furlong LI, DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res.*, 2017; 45(D1): D833-D839.
20. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA, DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol.*, 2003; 4(5): P3.
21. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.*, 2000; 25(1): 25-29.
22. Bonnot T, Gillard M, Nagel D, A simple protocol for informative visualization of enriched gene ontology terms. *Bio-protocol*, 2019; 2019: e3429-e3429.
23. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P, Jensen LJ, von Mering C, The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. *Nucleic Acids Res.*, 2011; 39(Database issue): D561-D568.
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T, Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.*, 2003; 13(11): 2498-2504.
25. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY, cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol.*, 2014; 8 Suppl 4(Suppl 4): S11.
26. Rahman MA, Shorobi FM, Uddin MN, Saha S, Hossain MA, Quercetin attenuates viral infections by interacting with target proteins and linked genes in chemicobiological models. *In Silico Pharmacol.*, 2022; 10(1): 17.

27. Li Z, Li S, Wei X, Peng X, Zhao Q, Recovering the missing regions in crystal structures from the nuclear magnetic resonance measurement data using matrix completion method. *J Comput Biol.*, 2020; 27(5): 709-717.
28. Rajendran P, Rathinasabapathy R, Kishore SC, Bellucci S, Computational-simulation-based behavioral analysis of chemical compounds. *J Compos Sci.*, 2023; 7(5): 196.
29. Hollingsworth SA, Dror RO, Molecular dynamics simulation for all. *Neuron.*, 2018; 99(6): 1129-1143.
30. Vanommeslaeghe K, Hatcher E, Acharya C, Kundu S, Zhong S, Shim J, Darian E, Guvench O, Lopes P, Vorobyov I, Mackerell AD Jr, CHARMM general force field: a force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *J Comput Chem.*, 2010; 31(4): 671-690.
31. Valdes-Tresanco MS, Valdes-Tresanco ME, Valiente PA, Moreno E, gmx_MMPBSA: A new tool to perform end-state free energy calculations with GROMACS. *J Chem Theory Comput.*, 2021; 17(10): 6281-6291.
32. Kim V, Criner GJ, Chronic bronchitis and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.*, 2013; 187(3): 228-237.
33. Hassan N, Amin F, Bashir K, Irshad M, Jamil S, Munawar N, Haqqani H, Shabir H, Khan M, Antiviral response of drugs used against HBV patients of Khyber Pakhtunkhwa, Pakistan. *Bull Biolog Allied Sci Res.*, 2023; 2023: 49.
34. Nawaz K, Khan S, Bibi A, Insights into scabies prevalence and risk factors. *Bull Biolog Allied Sci Res.*, 2024; 2024: 68.
35. Ullah A, Bibi A, Ullah I, Kayani R, Asim M, Munawar N, Amjad M, Siraj M, Gohar M, Khan MA, An overview of hepatitis C virus and liver cirrhosis in Pakistan. *Bull Biolog Allied Sci Res.*, 2024; 2024: 64.
36. Ullah I, Ullah A, Rehman S, Ullah S, Ullah H, Haqqni S, Amir M, Gul F, Bashir K, Prevalence and risk factors of *Helicobacter pylori* infection among individuals with tobacco consumption habits in district Peshawar: a cross-sectional study. *Bull Biolog Allied Sci Res.*, 2023; 2023: 42.
37. Grzela K, Litwiniuk M, Zagorska W, Grzela T, Airway remodeling in chronic obstructive pulmonary disease and asthma: the role of matrix metalloproteinase-9. *Arch Immunol Ther Exp.*, 2016; 64(1): 47-55.
38. Pervaiz B, Hassan N, Fazal M, Mohammad Z, Munawar N, Ullah Z, Haqqani S, Zia T, Ullah A, Comparative analysis of different pathogenic microbes collected from mammalian milk. *Bull Biolog Allied Sci Res.*, 2021; 2021: 32.
39. Brajer B, Batura-Gabryel H, Nowicka A, Kuznar-Kaminska B, Szczepanik A, Concentration of matrix metalloproteinase-9 in serum of patients with chronic obstructive pulmonary disease and a degree of airway obstruction and disease progression. *J Physiol Pharmacol.*, 2008; 59(Suppl 6): 145-152.
40. Cantin AM, Cystic fibrosis transmembrane conductance regulator. Implications in cystic fibrosis and chronic obstructive pulmonary disease. *Ann Am Thorac Soc.*, 2016; 13(Suppl 2): S150-S155.
41. Dransfield M, Rowe S, Vogelmeier CF, Wedzicha J, Criner GJ, Han MK, Martinez FJ, Calverley P, Cystic fibrosis transmembrane conductance regulator: roles in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.*, 2022; 205(6): 631-640.
42. Aratani Y, Myeloperoxidase: its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys.*, 2018; 640: 47-52.
43. Goud PT, Bai D, Abu-Soud HM, A multiple-hit hypothesis involving reactive oxygen species and myeloperoxidase explains clinical deterioration and fatality in COVID-19. *Int J Biol Sci.*, 2021; 17(1): 62-72.
44. Li J, Zhao P, Li Y, Tian Y, Wang Y, Systems pharmacology-based dissection of mechanisms of Chinese medicinal formula Bufei Yishen as an effective treatment for chronic obstructive pulmonary disease. *Sci Rep.*, 2015; 5: 15290.
45. Singh RP, Agrawal P, Yim D, Agarwal C, Agarwal R, Acacetin inhibits cell growth and cell cycle progression, and induces apoptosis in human prostate cancer cells: structure-activity relationship with linarin and linarin acetate. *Carcinogenesis*, 2005; 26(4): 845-854.
46. Ganesan A, Coote ML, Barakat K, Molecular dynamics-driven drug discovery: leaping forward with confidence. *Drug Discov Today*, 2017; 22(2): 249-269.