Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(3): 087
http://doi.org/10.31083/j.fbl2703087

Original Research

Vitex negundo L. derived specialized molecules unveil the multi-targeted therapeutic avenues against COPD: a systems pharmacology approach

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Academic Editor: Marcello Iriti
Submitted: 20 October 2021 Revised: 9 February 2022 Accepted: 10 February 2022 Published: 8 March 2022

Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is an inflammatory disease caused by increasing breathing passage obstruction which completely disrupts human homeostasis. Some patients require lung transplantation or long-term oxygen therapy. COPD is one of the noxious diseases and its fourth leading cause of death around the globe. There is an immediate need for potential drug development to tackle this serious disease. Folk medicines are used to combat complex diseases that have shown effectiveness in the treatment of breathing diseases. *Vitex negundo* L. is an ethnobotanically important medicinal plant used for various ailments and modulates human cellular events. This shrub has diverse specialized metabolites and is being used as complementary medicine in various countries. Though systems-level understanding is there on the mode of action, the multi-target treatment strategy for COPD is still a bottleneck. **Methods**: In this investigation, systems pharmacology, cheminformatics, and molecular docking analyses were performed to unravel the multi-targeted mechanisms of *V. negundo* L. potential bioactives to combat COPD. **Results**: Cheminformatics analysis combined with the target mining process identified 86 specialized metabolites and their corresponding 1300 direct human receptors, which were further imputed and validated systematically. Furthermore, molecular docking approaches were employed to evaluate the potential activity of identified potential compounds. In addition, pharmacological features of these bioactives were compared with available COPD drugs to recognize potential compounds that were found to be more efficacious with higher bioactive scores. **Conclusions**: The present study unravels the druggable targets and identifies the bioactive compounds present in *V. negundo* L., that may be utilized for potential treatment against COPD. However, further *in vivo* analyses and clinical trials of these molecules are essential to deciphering their efficacy.

Keywords: COPD; cheminformatics; human health; Lamiaceae; specialized metabolites; systems pharmacology; *Vitex negundo* L.

1. Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the complex progressive disorders that affect the respiratory system [1] and causes chronic inflammation of the airways [2]. COPD is accountable for more than 3 million deaths annually, making it the third leading cause of mortality [3]. There are several causes for COPD, which provoke the Endoplasmic Reticulum (ER) stress including smoking, free radicals, carcinogens, and reactive oxygen species [4]. Since ER strives to reimpose the cellular homeostasis through unfolded protein response (UPR), it is evident that ER stress and UPR activation occur in patients with COPD [5]. The other major cause for COPD is breathing of polluted air and also the result of subjection to inhaled irritants [1,6]. COPD is frequently associated with non-small cell lung cancer (comorbidity) [7]. Factors such as age, bacterial colonization, cardiovascular diseases, usage of antibiotics and steroids, poor quality of life, and airway obstruction severity intensify the risk for COPD [8,9]. One of the uncharacterized risk factors of COPD is the longtime occupational exposure to irritants like organic and inorganic dust, chemical agents, and fumes [1]. These risk factors are being changed over time with new forms of smoking and also the location with the difference in air pollution [10]. COPD is commonly accompanied by exacerbations, which are marked with increased cough, excess sputum production, and phlegm [11]. It is deemed that among the exacerbations, 70–80% are prompted by bacterial or viral respiratory infections and the remaining 20–30% are due to environmental pollution exposure or unknown causation or origination [12]. In general, COPD is an escalating disease of the airways, the microvasculature, and the alveoli and is strongly associated with cardiovascular diseases [13]. Although COPD represents an increased

burden to the Healthcare system, it is not easy to diagnose and curate the disease due to its heterogenicity and complexity. Currently, this disease is treated by both Pharmacological and non-pharmacological approaches. The pharmacological intervention includes inhaled bronchodilators, antibiotics, corticosteroids, Oxygen therapy, and antioxidants. Based on the clinical conditions of the patient, adjunct therapies are also performed. Non-pharmacological intervention includes Chest percussion therapy, which is believed to improve sputum clearance. Ventilatory support should be the primary goal to reduce morbidity and mortality [12]. Currently, the prognosis of COPD is achieved by post-bronchodilator spirometry and by Pulse oximetry [1].

The Indian medical system is one of the ancient traditional health care systems in the globe and this system is predominantly based on herbal plants. Thus, repurposing Indian traditional practices can be employed in mining new options to treat deadly diseases. Among numerous plants mentioned in the Indian medical system, *Vitex negundo* L. commonly known as Nirgundi belongs to the family Lamiaceae [14] is one of the significant plants that was being used in the treatment for various disorders.

The plant contains various bioactive compounds extracted and concentrated from roots, leaves, and seeds in the form of iridoids, terpenes, volatile oils, lignans, steroids, and flavonoids. These different bioactive compounds exhibit various pharmacological properties including anti-inflammatory, antimicrobial, antioxidant, anti-ulcer, anti-diabetic properties, hepatoprotective properties [15]. The leaves have been used as a sedative, vermifuge, astringent, tonic, febrifuge, and also used in imparting the joint swellings from Acute Rheumatism. The dried fruit can be used as a Quinacrine and is used in the treatment of cough, cold, heart diseases, coronary thrombosis, rheumatic difficulties, etc. [16]. Jianpiyifei II granules (JPYF II) is one of the herbal medications used for COPD in China which comprises *V. negundo* L. is an important component [5,15]. Since we have a limited number of drugs and disease-modifying therapies to treat COPD, the unraveling of the genetic determinants of COPD provides the unbiased identification of molecular determinants which would pave the way to derive new insights into the pathogenesis of COPD leading to novel therapeutic interventions and preventive strategies.

Despite the significance of the Indian traditional treatment system, the functions of phytocompounds, their potential human targets, and their mode of actions are still indistinct. Several studies have shown the specific activity of these compounds, but the mode of action and multiple potentialities of these compounds are yet to be fully discovered. Hence, the present study aims to highlight the advances in the discovery of druggable targets of phytomolecules in association with COPD through systems pharmacology, cheminformatics, and molecular docking approaches. This study tries to address the primary queries regarding:

(i) The regulatory aspects of phytocompounds against COPD.

(ii) The biological processes mediated by plant molecules through human targets.

Cheminformatics approach was employed to segregate the phytochemicals with significant curative properties through target-compound interactions and molecular docking was performed to evaluate the interactions. The derived COPD immunological receptors were analyzed using bioinformatic databases to identify the pathways and mechanisms in which the bioactive compounds act. So, it is expected that these *in silico* systems approaches can aid in the investigation of the immunological significance of *V. negundo* L. derived specialized molecules and will significantly promote the development of new drugs for the treatment of COPD and other respiratory diseases in mere future.

2. Materials and methods

2.1 Retrieval of phytocompounds

Scrutinization of literature and web sources [17,18] revealed the presence of various bioactive compounds in *V. negundo* L. and were enlisted in Table 1. In total, 86 phytocompounds were procured and their Canonical SMILES were retrieved from the PubChem database [19].

2.2 Identification and mining of human targets

Canonical SMILES of these compounds were employed to identify the human targets by using the SwissTargetPrediction tool (http://www.swisstargetprediction.ch/). Thus, identified human targets were submitted to the Expression Atlas database (https://www.ebi.ac.uk/gxa/home), and their features such as UniProt ID, orthologs, and Chromosome number were collated.

2.3 Gene enrichment and ontology analysis

Gene target identifiers were subjected to Network Analyst (https://www.networkanalyst.ca/) [20] to acquire the information about Gene Ontology (GO) classified as molecular functions and biological processes against *Homo sapiens*. In addition, Gene enrichment networks were also procured through the Network Analyst plugin.

2.4 Network construction

Cytoscape v3.8.2 [21] was used for constructing Compound-Target-Network, which plays an important role in identifying and understanding the mechanism of compound activity. Additionally, the interaction between the target genes was visualized using gene mania (https://genemania.org/) [22]. Based on these studies, potential targets were identified.

The protein-protein interaction (PPI) of these potential proteins was performed using STRING v10.5 (https://string-db.org/) with a high confidence score of 0.7. This

Table 1. List of phytocompounds with their PubChem ID.

S.No.	Compounds	PubChem ID	Abb
1	Negundoside	9935561	NS
2	Agnuside	442416	AS
3	Thujene	520384	TJ
4	α-Pinene	6654	α-P
5	Camphene	6616	CP
6	α-Elemene	80048	α-E
7	δ- Elemene	12309449	δ-E
8	Sabinene	18818	SN
9	Friedelin	91472	FD
10	Vitamin-C	54670067	VC
11	Carotene	6419725	CT
12	Casticin	5315263	CC
13	Artemetin	5320351	ART
14	Terpinen-4-ol	11230	T-4-ol
15	Spathulenol	92231	STL
16	Caryophyllene epoxide	14350	CPE
17	Caryophyllenol	61125	CPL
18	Farnesol	445070	FN
19	β-Pinene	14896	β-P
20	Stearic acid	5281	STA
21	Behenic acid	8215	BHA
22	Myrcene	31253	MC
23	Δ^3-Carene	26049	Δ^3-C
24	Limonene	22311	LMN
25	β-Phellandrene	11142	β-PA
26	γ-Terpinene	7461	γ-TP
27	Dihydromyrcenol	29096	DHC
28	Sabinene hydrate	62367	SBH
29	Linalool	6549	LNL
30	Amyl isovalerate	95978	AIV
31	Nonanol	8914	NNL
32	4-Terpineol	11230	4-TP
33	α-Terpineol	17100	α-TP
34	Carveol	7438	CV
35	Eugenol	3314	EUG
36	β-Caryophyllene	5281515	β-CPL
37	Isocaryophyllene	5281522	ICP
38	Humulene	5281520	HML
39	Aromadendrene	91354	ADD
40	Viridiflorene	10910653	VDFE
41	δ-Cadinene	441005	δ-C
42	4,4″-Dimethoxy-trans-stilbene	20828	4,4″- DM-T-SB
43	Elemol	92138	EL
44	Caryophyllene oxide	1742210	CPO
45	Vitexicarpin	5315263	VTC
46	Terpinen-4-ol	11230	TP-4-ol
47	Viridiflorol	11996452	VDF
48	α-Copaene	442355	α-C
49	Camphor	2537	CMR
50	1,8-Cineol	2758	1,8- C
51	α-Guaiene	5317844	α- G
52	Neral	643779	NL
53	Geranial	638011	GRN
54	Bornyl acetate	6448	BA
55	Nerolidol	5284507	NLD
56	β-Elemene	6918391	β-E
57	*n*-Tritriacontane	12411	n-T
58	*n*-Hentriacontanol	68345	n-H
59	Epifriedelinol	119242	EFDL
60	Oleanolic acid	10494	OLNA
61	*n*-Nonacosane	12409	n-N
62	Vitedoin A	21574226	VDA
63	Vitedoamine A	11348702	VAA
64	Negundin A	10043572	NA
65	Negundin B	10473569	NB
66	Vitedoin B	11771639	VDB
67	β-Sitosterol	222284	β-ST
68	α-Selinene	10856614	α-S
69	Germacren-4-ol	6429375	GMC-4-ol
70	β-Eudesmol	91457	β-EDM
71	Acetyl oleanolic acid	151202	AOA
72	Sitosterol	222284	SS
73	(E)-Nerolidol	5281525	E- N
74	β-Selinene	28237	β-SN
75	α-Cedrene	608041	α-CD
76	Germacrene D	5317570	GD
77	Hexadecanoic acid	985	HDA
78	p-Cymene	7463	P-C
79	Valencene	9855795	VLC
80	β-Bisabolol	12300146	β-BB
81	Cedrol	65575	CD
82	γ-Eudesmol	6432005	γ-EM
83	Squalene	638072	SQL
84	Vitexin	5280441	VTN
85	Aucubin	91458	AUC
86	Isovitexin	162350	IVT

interactome was utilized to understand the regulatory aspects of potential targets.

2.5 Identification of properties of active compounds

Canonical SMILES of the corresponding phytocompounds were subjected to the Molinspiration tool (https://www.molinspiration.com/) to predict the properties such as the number of violations (nVio), GPCR ligand activity (GPCR), Enzymes and nuclear receptors (Ncr), Kinase in-

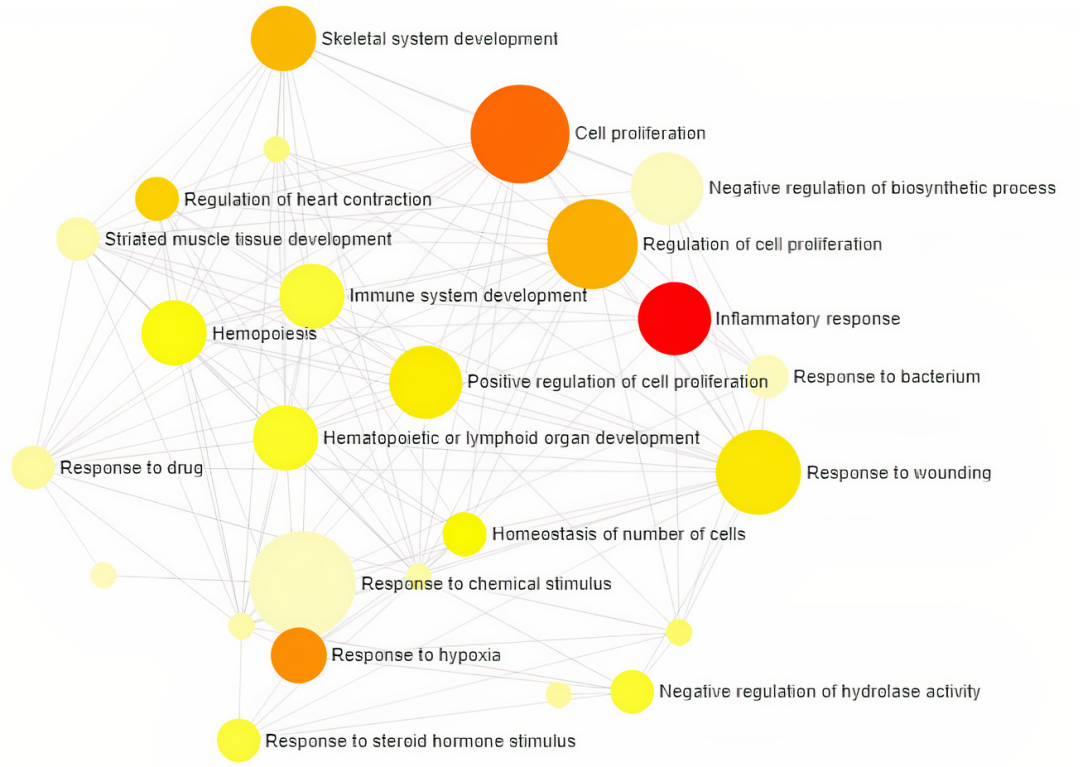

Fig. 1. Target genes involved in Biological Processes and the category are directly proportional to the node size. The nodes are color shaded according to the significance level (adjusted *p*-value < 0.05).

hibitory activity (Ki), Enzyme inhibitory activity (Ei) and Protease inhibitory activity (Pi).

2.6 Molecular docking

Molecular Docking (MD) was performed for the pharmacologically active compounds against the COPD responsible human targets to evaluate the potentials of pharmacologically active compounds.

2.7 Compound comparison

Commercial drugs available for COPD were retrieved from various sources and their bioactive properties including nVio, GPCR, Ncr, Ki, Ei, and Pi were compared with plant-derived molecules to identify the pharmacologically active compounds.

3. Results

3.1 Retrieval of phytocompounds

The canonical SMILES of all the 86 phytocompounds were procured from the PubChem database, which is further employed for systems pharmacological analysis.

3.2 Identification and mining of human targets

Results of SwissTargetPrediction reveal the Human receptors, targeted by phytocompounds. On whole, 86 phytocompounds targeting 1300 targets were identified in this study. A list of compounds along with their target receptors was enlisted in **Supplementary Table 1**. In addition, the UniProt ID, chromosome number, and orthologous information were also retrieved and tabulated (Table 2). This information can be employed for deeper analyses of molecular functions.

3.3 Gene enrichment and ontology analysis

Gene Ontology analysis using Network Analyst revealed the involvement of significant proteins in various biological processes including Response to hypoxia, Hemopoiesis, Cell proliferation, Wound and Inflammatory responses (Fig. 1), and molecular functions such as Enzyme binding, Neurotransmitter receptor activity, Growth Factor activity and cytokine receptor activity (Fig. 2). In addition, gene enrichment networking predicts the activity of these genes involved in various disorders like Inflammatory bowel disease (IBD), Leishmaniasis, Malaria, Amoebiasis, and also in pathways which include MAPK signaling pathway, HIF-1 signaling pathway, FoxO signaling pathway, and TGF-beta signaling pathway (Fig. 3). The activity of these phytocompounds on its targets may reduce the risk of COPD and its associated disorders.

3.4 Network construction

3.4.1 C-T-N analysis

The C-T-N was also constructed using the Cytoscape v3.8.2, which displays the interaction of 86 compounds

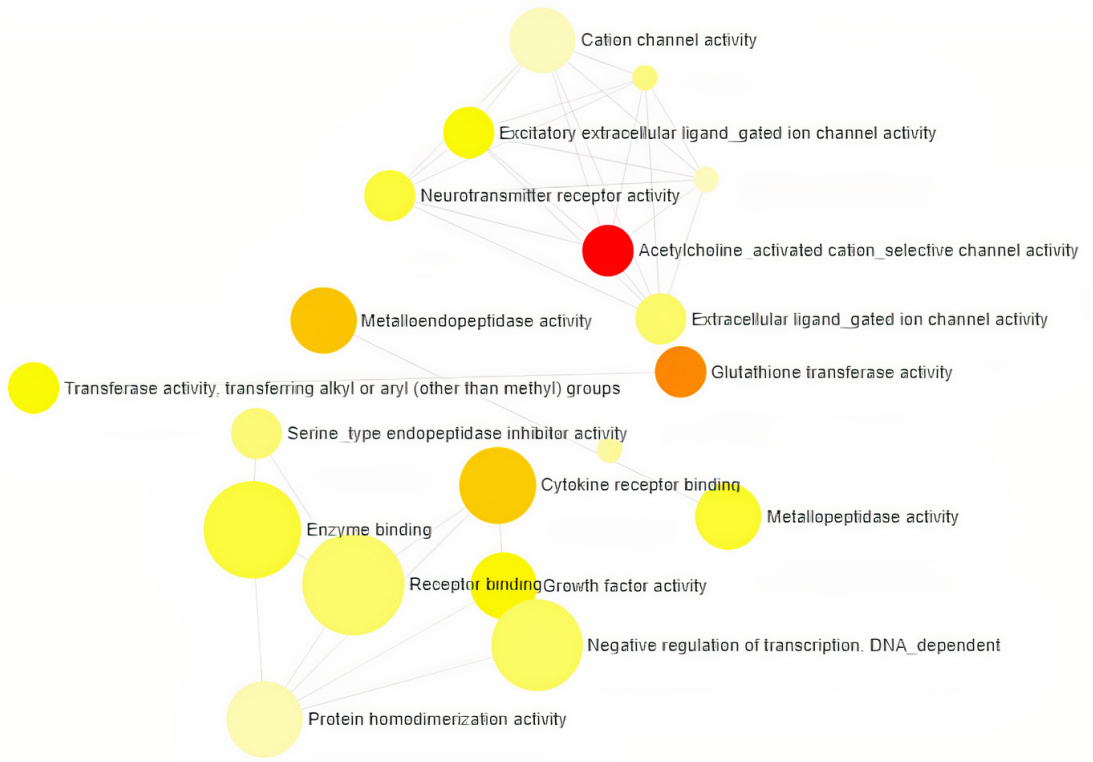

Fig. 2. Classification of Human Targets with encoding Molecular Functions and the category is directly proportional to the node size. The nodes are color shaded according to the significance level (adjusted p-value < 0.05).

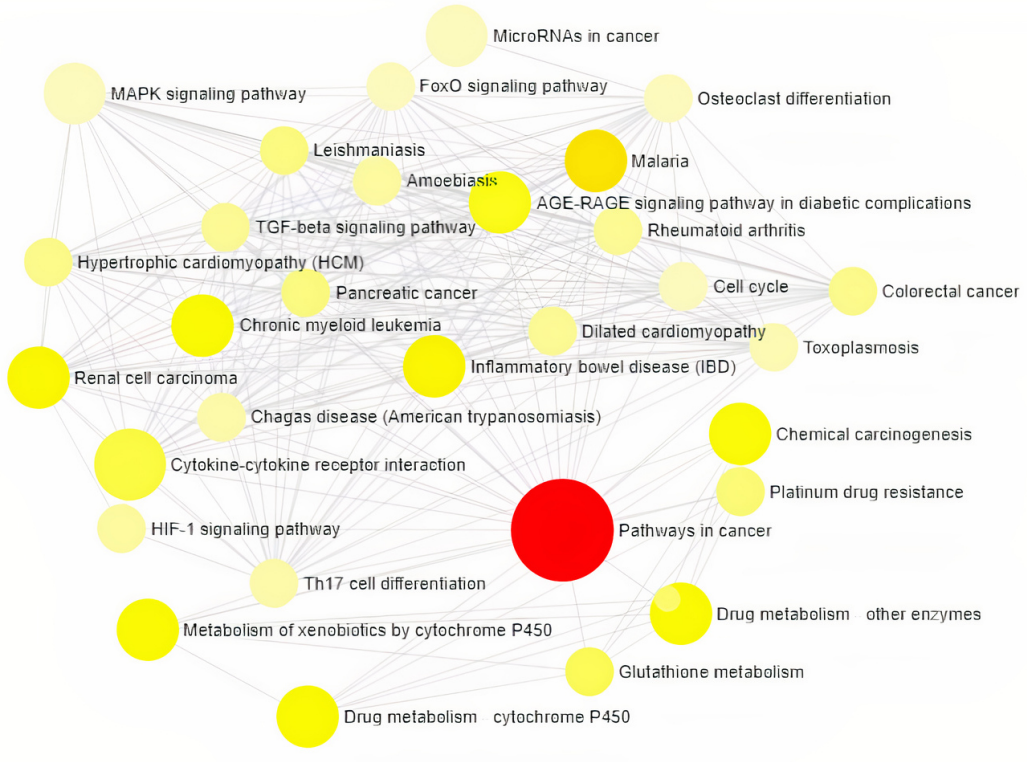

Fig. 3. Visualization of Network-based Pathway enrichment Analysis is directly proportional to the size of the node. The nodes are color shaded according to the significance level (adjusted p-value < 0.05).

Table 2. Features of human active receptors.

S.No.	Compound	Target	UniProt ID	Chr. No.	Orthologs
1	Negundoside	HSP90AA1	H0YJF5	14	HSP90AA1 (*Equus caballus*)
2	Agnuside	HSP90AA1	H0YJF5	14	HSP90AA1 (*Equus caballus*)
3	Thujene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
4	α-Pinene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
5	Camphene	HSD11B1	P28845	1	Hsd11b1 (*Mus musculus*)
6	α-Elemene	ADORA1	A0A087X173	1	Adora1 (*Rattus norvegicus*)
7	δ-Elemene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
8	Sabinene	HSD11B1	P28845	1	Hsd11b1 (*Mus musculus*)
9	Friedelin	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
10	Vitamin-C	GSK3B	A0A3B3ITW1	3	GSK3B (*Sus scrofa*)
11	Carotene	ADORA1	A0A087X173	1	Adora1 (*Rattus norvegicus*)
12	Casticin	AKR1B1	P15121	7	AAD14 (*Saccharomyces cerevisiae*)
13	Artemetin	ABCG2	Q9UNQ0	4	SNQ2 (*Saccharomyces cerevisiae*)
14	Terpinen-4-ol	AR	E9PEG3	X	AR (*Papio anubis*)
15	Spathulenol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
16	Caryophyllene epoxide	SQLE	Q14534	8	Sqle *(Rattus rattus)*
17	Caryophyllenol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
18	Farnesol	SQLE	Q14534	8	Sqle *(Rattus rattus)*
19	β-Pinene	SLC5A7	Q9GZV3	2	Slc5a7 (*Mus musculus)*
20	Stearic acid	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
21	Behenic acid	FABP4	P15090	8	Fabp4 (*Mus musculus)*
22	Myrcene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
23	Δ^3-Carene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
24	Limonene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
25	β-Phellandrene	AR	E9PEG3	X	AR (*Papio anubis*)
26	γ-Terpinene	TRPV1	I3L1R6	17	TRPV1 (*Gallus gallus*)
27	Dihydromyrcenol	HSD11B1	P28845	1	Hsd11b1 (*Mus musculus*)
28	Sabinene hydrate	TRPM8	Q7Z2W7	2	Trpm8 (*Mus musculus*)
29	Linalool	TRPM8	Q7Z2W7	2	Trpm8 (*Mus musculus*)
30	Amyl isovalerate	CA1	E5RHP7	8	CA1 (*Pan troglodytes*)
31	Nonanol	TRPM8	Q7Z2W7	2	Trpm8 (*Mus musculus*)
32	4-Terpineol	AR	E9PEG3	X	AR (*Papio anubis*)
33	α-Terpineol	AR	E9PEG3	X	AR (*Papio anubis*)
34	Carveol	AR	E9PEG3	X	AR (*Papio anubis*)
35	Eugenol	ADORA1	A0A087X173	1	Adora1 (*Rattus norvegicus*)
36	β-Caryophyllene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
37	Isocaryophyllene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
38	Humulene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
39	Aromadendrene	HSD11B1	P28845	1	Hsd11b1 (*Mus musculus*)
40	Viridiflorene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
41	δ-Cadinene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
42	4,4″-Dimethoxy-trans-stilbene	RELA	E9PSE4	11	RELA (*Pan troglodytes*)
43	Elemol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
44	Caryophyllene oxide	SQLE	Q14534	8	Sqle*(Rattus rattus)*
45	Vitexicarpin	AKR1B1	P15121	7	AAD14 (*Saccharomyces cerevisiae*)
46	Terpinen-4-ol	AR	E9PEG3	X	AR (*Papio anubis*)
47	Viridiflorol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)

Table 2. Continued.

S.No.	Compound	Target	UniProt ID	Chr. No.	Orthologs
48	α-Copaene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
49	Camphor	NR1I3	Q6GZ72	1	NR1I3 (*Sus scrofa*)
50	1,8-Cineol	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
51	α-Guaiene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)
52	Neral	ALDH1A1	P00352	9	Aldh1a1 (*Mus musculus*)
53	Geranial	ALDH1A1	P00352	9	Aldh1a1 (*Mus musculus*)
54	Bornyl acetate	ACHE	F8WD34	7	ACHE (*Chlorocebus sabaeus*)
55	Nerolidol	SQLE	Q14534	8	Sqle*(Rattus rattus)*
56	β-Elemene	CXCR3	P49682	X	CXCR3 (*Bos taurus*)
57	*n*-Tritriacontane	SHBG	B0FWH6	17	SHBG (*Ovis aries*)
58	*n*-Hentriacontanol	TRPM8	Q7Z2W7	2	Trpm8 (*Mus musculus*)
59	Epifriedelinol	TRPM8	Q7Z2W7	2	Trpm8 (*Mus musculus*)
60	Oleanolic acid	PTPN1	B4DSN5	20	Ptpn1 (*Mus musculus*)
61	*n*-Nonacosane	SHBG	B0FWH6	17	SHBG (*Ovis aries*)
62	Vitedoin A	SHBG	B0FWH6	17	SHBG (*Ovis aries*)
63	Vitedoamine A	NQO2	Q5TD07	6	NQO2 (*Pan troglodytes*)
64	Negundin A	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
65	Negundin B	FLT3	E7ER61	13	FLT3 (*Equus caballus*)
66	Vitedoin B	PTPN1	B4DSN5	20	Ptpn1 (*Mus musculus*)
67	β-Sitosterol	AR	E9PEG3	X	AR (*Papio anubis*)
68	α-Selinene	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
69	Germacren-4-ol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
70	β-Eudesmol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
71	Acetyl oleanolic acid	PTPN1	B4DSN5	20	Ptpn1 (*Mus musculus*)
72	Sitosterol	AR	E9PEG3	X	AR (*Papio anubis*)
73	(E)-Nerolidol	SQLE	Q14534	8	Sqle*(Rattus rattus)*
74	β-Selinene	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
75	α-Cedrene	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
76	Germacrene D	PPARA	B0QYX1	22	Ppara (*Mus musculus*)3
77	Hexadecanoic acid	PPARA	B0QYX1	22	Ppara (*Mus musculus*)3
78	*p*-Cymene	CYP2A6	M0R2Z4	19	Cyp2a12 (*Mus musculus*)
79	Valencene	PPARA	B0QYX1	22	Ppara (*Mus musculus*)3
80	β-Bisabolol	AR	E9PEG3	X	AR (*Papio anubis*)
81	Cedrol	UGT2B7	A0A087X084	4	Ugt49C1 (*Drosophila melanogaster*)
82	γ-Eudesmol	CYP19A1	H0YLS2	15	Cyp19a1 (*Rattus norvegicus*)
83	Squalene	CNR2	P34972	1	CNR2 (*Macaca mulatta*)
84	Vitexin	AKR1B1	P15121	7	AAD14 (*Saccharomyces cerevisiae*)
85	Aucubin	CDA	P32320	1	CDA (*Oryctolagus cuniculus*)
86	Isovitexin	AKR1B1	P15121	7	AAD14 (*Saccharomyces cerevisiae*)

with 1300 target genes (Fig. 4). These interactions revealed the multi-target properties of compounds, thus increasing the creditability of these compounds as a potent therapeutical drug for treating COPD.

3.4.2 Gene cross-talks

Interactions between the gene identifiers revealed the multiple interactions between the human targets and were visualized using Gene Mania (Fig. 5).

3.4.3 Gene interaction network

Unique genes involved in COPD prognosis namely *AAT*, *CHRNA3*, *CHRNA5*, *IREB2*, *MMP12*, *SOX5*, and *TGFB1* were identified by combinations of network pharmacology and cheminformatics. The interaction network of these genes has 27 nodes and 133 edges. These interactions have an average of 9.85 nodal degrees within the neighbor proteins. Protein-protein interaction of COPD unique genes enrichment *p*-value was $< 1.0 \times 10^{-16}$, which indi-

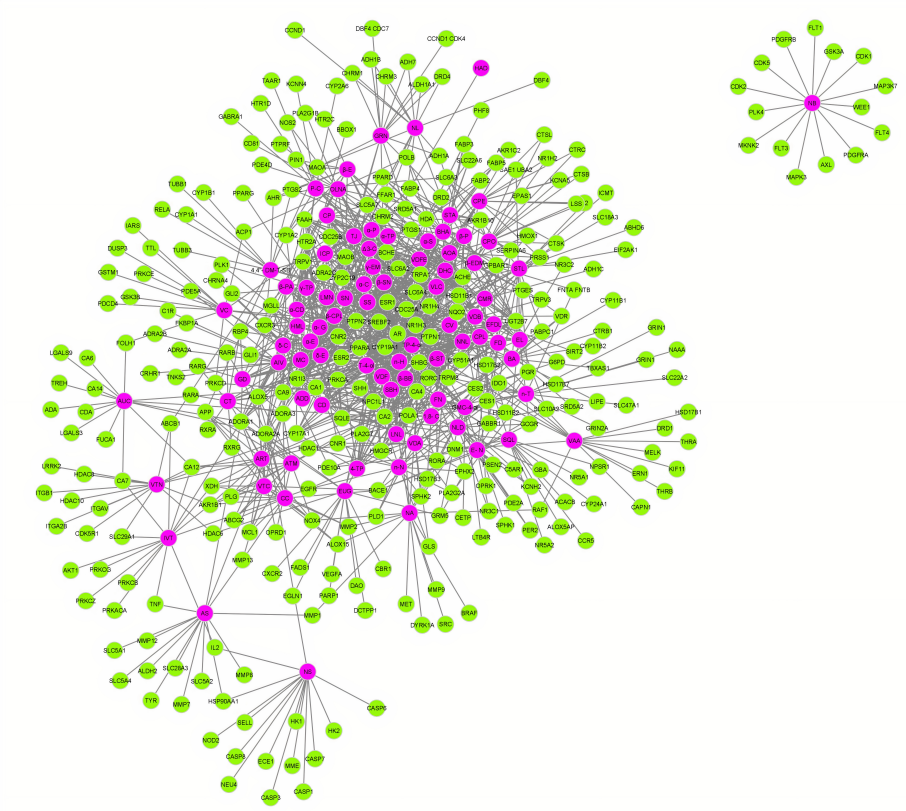

Fig. 4. Compound-Target-Network (C-T-N). The violet color indicates compounds and the green color represents human COPD targets.

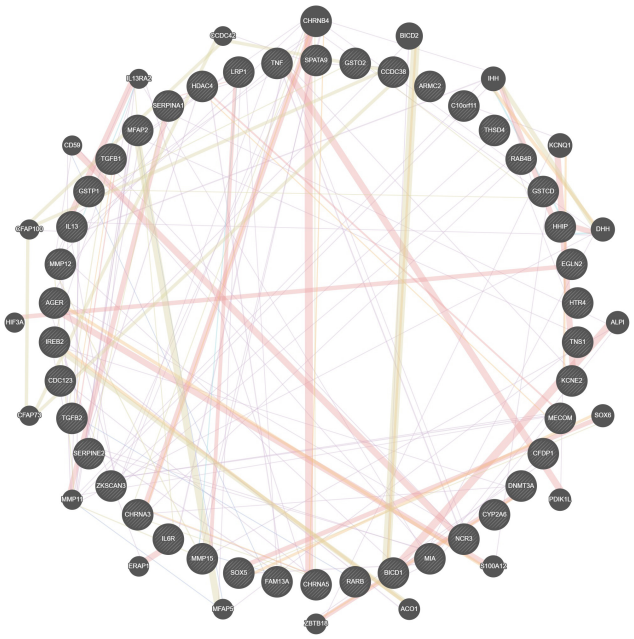

Fig. 5. Visualization of Gene Cross-talks.

cates biological connectivity among the interacting groups (Fig. 6). This molecular interaction revealed the complexity of COPD's unique genes and thus proves its multi-genic nature.

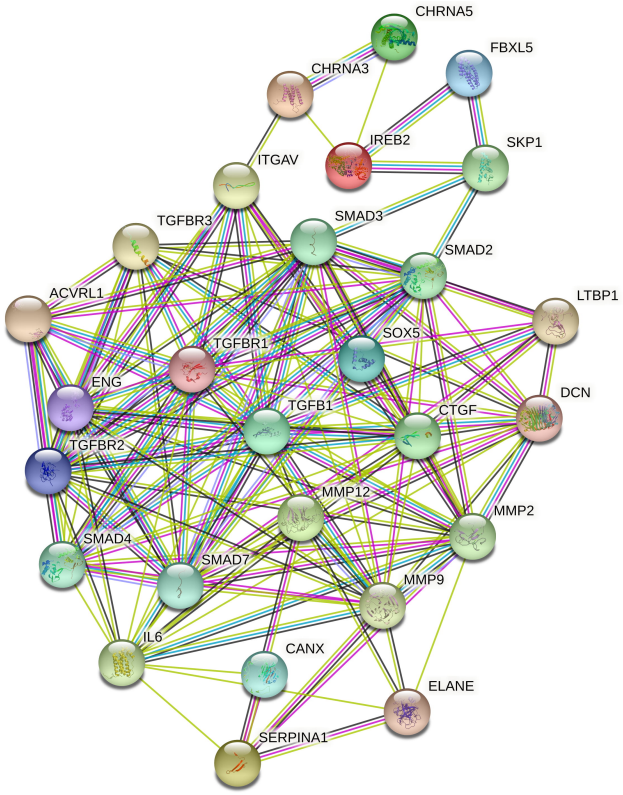

Fig. 6. COPD corresponding protein-protein interactions.

3.5 Identification of properties of active compounds

The properties of active compounds like GPCR, Ki, Pi, Ei, Ncr, and nVio were fetched from molinspiration tool. Compounds with zero nVio and with enzyme inhibitory scores above 0.5 were considered to be highly significant (Table 3). Thus, Caryophyllene Epoxide, Caryophyllene Oxide, Elemol, and Cedrol were found to be significant phytocompounds.

3.6 Molecular docking

Primary studies have identified four phytocompounds of *V. negundo* L. as pharmacologically significant. Molecular docking was performed using these compounds against the COPD responsible human targets: AAT, CHRNA3, CHRNA5, IREB2, MMP12, SOX5, and TGFB1 (**Supplementary Fig. 1**). The binding score of docking were provided in Table 4.

3.7 Compound comparison

The FDA-approved drugs used in the treatment of COPD were collated and the pharmacological properties of the active ingredient of these drugs were computed using the Molinspiration tool. Based on these properties, phytocompounds and commercially available drugs were compared, which leads to the identification of highly potent compounds which combat COPD. The details of potential bioactives are listed in Table 5.

4. Discussion

COPD is a systemic disorder [23] that involves structural alterations in both lung parenchyma and respiratory airways [24], which requires intensive research for the development of proper treatment methods. Since the allopathic formulations are not effective at present, exploration of alternative solutions has become necessary. More than 50% of the world's population has started the usage of alternative medicines, where the Indian ancient medicinal knowledge become highly significant [25]. India had a well-established ayurvedic system since ancient times [26], with more than 1500 well-studied plants are officially incorporated in ayurvedic formulations [27]. But the exact mode and mechanism of these medicinal plants remain unknown [26]. Hence, in this study, we have integrated the pharmacology, cheminformatics, and molecular docking approaches to identify the highly potent compounds present in *V. negundo* L. and to reveal its mode of action in treating COPD at the molecular level.

In this investigation, 86 bio-active molecules were identified with the help of cheminformatics. All these 86 molecules have strong interactions with more than 1300 human targets directly. These compounds have been hypothesized to be involved in various molecular and biological processes against COPD. Followingly, Cytoscape 3.8.2 was used to obtain the molecular interactions and C-T-N. These results predict the mechanism of drug compounds involved

Table 3. Features of active compounds.

Compound	GPCR lg	Ki	Ncr	Pi	Ei	nVio
Caryophyllene Epoxide	**–0.08**	**–0.86**	**0.62**	**0.00**	**0.57**	**0**
Caryophyllene Oxide	**–0.08**	**–0.86**	**0.62**	**0.00**	**0.57**	**0**
Elemol	**–0.10**	**–0.84**	**0.80**	**–0.01**	**0.52**	**0**
Cedrol	**–0.15**	**–0.94**	**0.03**	**–0.52**	**0.50**	**0**
β-Eudesmol	–0.02	–0.62	0.60	–0.10	0.48	0
Vitedoamine A	0.28	0.65	0.08	0.04	0.46	0
Germacren-4-Ol	0.05	–0.55	0.58	–0.28	0.41	0
Vitedoin B	–0.04	–0.33	0.55	–0.08	0.40	0
γ-Eudesmol	–0.29	–0.81	0.53	–0.32	0.40	0
β-Bisabolol	–0.20	–0.88	0.10	–0.52	0.36	0
Negundin A	0.10	0.12	0.20	–0.14	0.34	0
Carveol	–0.55	–1.40	0.25	–0.89	0.23	0
Vitamin-C	–0.53	–1.09	–1.01	–0.81	0.20	0
α-Terpineol	–0.51	–1.45	–0.02	–0.78	0.14	0
Vitedoin A	0.04	–0.19	0.07	–0.04	0.14	0
Casticin	–0.14	0.13	0.01	–0.34	0.12	0
Vitexicarpin	–0.14	0.13	0.01	–0.34	0.12	0
Artemetin	–0.15	0.12	0.00	–0.32	0.11	0
Linalool	–0.73	–1.26	–0.06	–0.94	0.07	0
Negundin B	0.05	–0.14	0.02	–0.13	0.07	0
Terpinen-4-Ol	–0.56	–1.68	–0.20	–0.92	0.06	0
Spathulenol	–0.42	–0.68	0.28	–0.36	0.06	0
4-Terpineol	–0.56	–1.68	–0.20	–0.92	0.06	0
Terpinen-4-Ol	–0.56	–1.68	–0.20	–0.92	0.06	0
Caryophyllenol	–0.06	–0.81	–0.25	–0.28	0.04	0
Neral	–0.86	–1.29	–0.42	–0.57	0.02	0
Geranial	–0.86	–1.29	–0.42	–0.57	0.02	0
Myrcene	–1.11	–1.51	–0.45	–1.31	–0.07	0
γ-Terpinene	–0.90	–1.37	–0.33	–1.55	–0.07	0
Dihydromyrcenol	–0.66	–1.18	–0.12	–0.71	–0.07	0
4,4″-Dimethoxy-Trans-Stilbene	–0.25	–0.23	–0.18	–0.40	–0.09	0
Bornyl Acetate	–0.32	–1.33	–0.59	–0.44	–0.12	0
Viridiflorol	–0.50	–0.82	–0.22	–0.48	–0.13	0
α-Guaiene	–0.49	–1.27	–0.01	–0.57	–0.14	0
1,8-Cineol	–0.93	–1.60	–1.07	–0.90	–0.15	0
Limonene	–0.91	–2.01	–0.34	–1.38	–0.21	0
Sabinene Hydrate	–0.59	–1.22	–0.31	–0.43	–0.25	0
Viridiflorene	–0.96	–1.08	–0.33	–0.61	–0.26	0
β-Phellandrene	–0.99	–1.55	–0.28	–1.31	–0.27	0
Amyl Isovalerate	–0.67	–1.19	–0.79	–0.58	–0.28	0
Aromadendrene	–0.67	–0.98	–0.21	–0.67	–0.30	0
α-Pinene	–0.48	–1.50	–0.62	–0.85	–0.34	0
β-Pinene	–0.53	–1.45	–0.50	–0.80	–0.34	0
Nonanol	–0.89	–1.13	–0.94	–0.98	–0.37	0
Eugenol	–0.86	–1.14	–0.78	–1.29	–0.41	0
Camphor	–0.79	–2.12	–1.21	–0.95	–0.52	0

Table 3. Continued.

Compound	GPCR lg	Ki	Ncr	Pi	Ei	nVio
Δ^3-Carene	–1.29	–1.51	–1.28	–1.28	–0.53	0
Thujene	–0.96	–1.79	–1.13	–1.02	–0.58	0
Sabinene	–1.15	–1.79	–0.69	–0.78	–0.60	0
p-Cymene	–1.18	–1.40	–1.21	–1.42	–0.78	0
Camphene	–1.02	–1.85	–1.15	–1.40	–0.82	0
α-Elemene	–0.55	–0.86	0.49	–0.64	0.26	1
Δ-Elemene	–0.36	–0.69	0.63	–0.60	0.46	1
Friedelin	0.02	–0.39	0.39	0.02	0.21	1
Farnesol	–0.13	–0.60	0.20	–0.43	0.42	1
Stearic Acid	0.11	–0.20	0.17	0.06	0.20	1
Behenic Acid	0.17	–0.10	0.23	0.17	0.17	1
-Caryophyllene	–0.34	–0.78	0.13	–0.60	0.19	1
Isocaryophyllene	–0.34	–0.78	0.13	–0.60	0.19	1
Humulene	–0.14	–0.93	0.34	–0.67	0.31	1
Δ-Cadinene	–0.58	–0.75	0.00	–0.68	0.19	1
α-Copaene	–0.33	–0.79	0.02	–0.49	0.10	1
Nerolidol	–0.17	–0.64	0.42	–0.43	0.39	1
β-Elemene	–0.36	–1.02	0.43	–0.38	0.30	1
n-Tritriacontane	0.03	–0.03	0.03	0.03	0.02	1
n-Hentriacontanol	0.06	0.01	0.10	0.08	0.08	1
Epifriedelinol	0.17	–0.18	0.41	0.17	0.31	1
Oleanolic Acid	0.28	–0.40	0.77	0.15	0.65	1
n-Nonacosane	0.04	–0.04	0.04	0.03	0.02	1
β-Sitosterol	0.14	–0.51	0.73	0.07	0.51	1
α-Selinene	–0.24	–0.97	0.34	–0.51	0.28	1
Acetyl Oleanolic Acid	0.18	–0.46	0.67	0.12	0.58	1
Sitosterol	0.14	–0.51	0.73	0.07	0.51	1
(E)-Nerolidol	–0.17	–0.64	0.42	–0.43	0.39	1
β-Selinene	–0.26	–0.94	0.35	–0.48	0.29	1
α-Cedrene	–0.24	–1.07	0.01	–0.70	0.41	1
Germacrene D	–0.30	–0.81	0.32	–0.67	0.26	1
Hexadecanoic Acid	0.02	–0.33	0.08	–0.04	0.18	1
Valencene	–0.31	–1.28	0.41	–0.79	0.26	1
Squalene	0.04	–0.10	0.19	–0.03	0.16	1
Vitexin	0.13	0.19	0.23	0.03	0.46	1
Aucubin	0.23	0.03	0.10	0.35	0.55	1
Isovitexin	0.12	0.15	0.23	0.04	0.47	1
Negundoside	0.24	–0.22	0.35	0.14	0.43	2
Agnuside	0.07	–0.10	0.15	0.19	0.33	2
Carotene	0.02	–0.14	0.49	–0.12	0.27	2

The bold format represents essential bioactive compounds to combat COPD.

in various biological processes to achieve therapeutic potential.

As highlighted by previous reports, the binding of compounds to only a single direct target does not yield relevant results [28]. From a network point of view, the receptors or genes that are needed to be targeted have shifted from

Table 4. Results of molecular docking using pharmacologically active plant compounds against COPD responsible human targets.

S.No.	Target	Compound	Binding energy (kcal/mol)
1	AAT (Alpha–1 antitrypsin)	CPE	–6.3
		CPO	–6.6
		CD	–6.8
		EL	–5.8
2	CHRNA3 (Cholinegic Receptor Nicotinic Alpha 3 Subunit)	CPE	–5.7
		CPO	–5.2
		CD	–6
		EL	–5
3	CHRNA5 (Cholinegic Receptor Nicotinic Alpha 5 Subunit)	CPE	–6
		CPO	–5.7
		CD	–6.7
		EL	–5.5
4	IREB2 (Iron Responsive Element Binding Protein 2)	CPE	–7.1
		CPO	–6.9
		CD	–7.5
		EL	–5.9
5	MMP12 (Matrix Metallopeptidase 12)	CPE	–5.9
		CPO	–6.1
		CD	–6.3
		EL	–5.5
6	SOX5 (SRY–Box Transcription Factor 5)	CPE	–5.9
		CPO	–5.7
		CD	–5.8
		EL	–5.5
7	TGFB1 (Transforming Growth Factor Beta 1)	CPE	–5.6
		CPO	–5.5
		CD	–5.8
		EL	–5.1

unique proteins to a whole molecular network of genes involved in causing disease [29]. These multiple interactions of drugs with various targets increase the efficiency of the drugs and the probability of effective treatment [26]. Results from Swiss target prediction and C-T-N have shown that each potential compound can act on multiple targets, that are involved in the development of COPD. Since various gene regulations are intricated in causing COPD, multi-target effects of these compounds can be used to achieve superior results.

Subsequently, molecular docking analysis was performed to verify the potentials of these compounds. Results of docking display the significant binding energies between the plant compounds and the respective human targets. The genes AAT and IREB2 play a significant role in causing lung inflammation [30,31] genes CHRNA3 and CHRNA 5 play a crucial role in lung cancer development [32], and

Table 5. Comparison of commercially available drugs and Phytocompounds.

Drug for COPD	GPCR lg	Ki	Ncr	Pi	Ei	nVio
Tudorza Pressair	0.46	–0.44	–0.21	–0.21	0.09	0
Seebri Neohaler	0.58	–0.35	–0.21	–0.06	0.41	0
Atrovent	0.56	–0.29	–0.37	–0.07	0.17	0
Spiriva	0.63	–0.39	–0.15	0	0.15	0
Incruse Ellipta	0.42	–0.29	–0.19	0.04	0.27	0
Brovana	0.42	–0.16	–0.02	0.18	0.22	0
Foradil	0.42	–0.16	–0.02	0.18	0.22	0
Arcapta Neohaler	0.38	0.08	0.15	0.11	0.22	0
Serevent	0.38	0.08	0.15	0.29	0.27	0
Striverdi Respimat	0.24	–0.25	–0.31	0.02	–0.04	0
Pulmicort	0.21	–0.64	1.27	0.27	0.67	0
Aerobid	0.08	–0.48	1.49	0.45	0.7	0
Qvar	–0.11	–0.83	0.93	0.3	0.42	1
Flovent	0.15	–0.69	1.83	0.95	0.81	1
Asmanex	–0.30	–0.82	0.87	0.05	0.25	1
Alvesco	–0.03	–0.74	0.78	0.11	0.33	2
Bioactive Compounds	GPCR lg	Ki	Ncr	Pi	Ei	nVio
Caryophyllene Epoxide	**–0.08**	**–0.86**	**0.62**	**0**	**0.57**	**0**
Caryophyllene Oxide	**–0.08**	**–0.86**	**0.62**	**0**	**0.57**	**0**
Elemol	**–0.10**	**–0.84**	**0.8**	**–0.01**	**0.52**	**0**
Cedrol	**–0.15**	**–0.94**	**0.03**	**–0.52**	**0.5**	**0**

The bold format represents essential bioactive compounds to combat COPD.

genes MMP12, SOX5, and TGFB1 involve in the prognosis of emphysema [33–35]. These medical conditions namely lung inflammation, lung cancer, and emphysema are the hallmarks of COPD and these seven genes can be regarded as candidate genes for COPD. Targeting these genes will pave way for the treatment of COPD. Hence, molecular docking was performed against these candidate gene products. Among the phytocompounds, CD interacts with the highest binding affinities of –7.5 and –6.8 kcal/mol with IREB2 and AAT respectively. In general, CD exhibits the highest affinity with almost all the seven targets followed by CPE, CPO, and EL. These results revealed that *V. negundo* L. compounds possess significant regulatory actions on COPD target receptors through dynamic interactions.

Finally, highly potent molecules were identified by comparing the properties of phytocompounds with commercially available drugs, which shows the curative potential of *V. negundo* L. and also the proof for the Indian medical system as a highly potent approach for treating diseases, when modern treatment methods cannot yield desired outcomes.

6. Conclusions

In brief, among the 1300 genes targeted by phytocompounds present in *V. negundo* L., seven targets were predicted to be responsible for causing COPD using the combined analyses. Among 86 phytocompounds, four compounds were predicted to be highly potent phytocompounds, which can be employed for treating COPD after laboratory trials and validation.

The present study revealed that the *V. negundo* L. showed diverse immune–stimulants to treat the airflow obstruction and enhance the breathing capabilities of COPD cases. Biopharmaceutical research on *V. negundo* L. is still a bottleneck, interestingly our results on potentially specialized molecules and their pharmacological features, active human targets, enrichment analyses, and signaling networks have enabled the floodgates of research with the integration of Ayurveda to the era of modern medicine. This is the first investigation that identified the pivotal aspects of host immune responses to COPD. This study also conjectures that *V. negundo* L. phytomolecules and their combination with other biomolecules as is recommended by the Ayurvedic and modern medicine system—may result in combined effects and further studies are required.

Hence, we hypothesize that the identified potential compounds from this herb can be used to regulate the gene-targeted pathways, which can ultimately result in the cure of COPD. Yet, *in vivo* evaluation is required to further validate this hypothesis. Furthermore, it is suggested that obtained interaction can be useful to design competitive human immune targets antagonist which will pave the way to combat COPD.

Abbreviations

COPD, Chronic Obstructive Pulmonary Disease; ER, Endoplasmic Reticulum; GO, Gene Ontology; C-T-N, Compound-Target-Network; nVio, Number of Violations; GPCR, GPCR Ligand Activity; Ncr, Enzymes, and Nuclear Receptors; Ki, Kinase Activity; Ei, Enzyme Inhibition Activity; Pi, Protease Inhibitory Activity.

Author contributions

Conceptualization—SA, PM and MR; Data curation—RJ, and MAL; Investigation—SA, PM, RJ, MAL and SKP; Supervision—J-TC, MR; Validation—RS, HS, and J-TC; Writing - original draft—SA and PM; Writing - review & editing—RS, SKP, HS, J-TC, and MR. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBL/27/3/10.31083/j.fbl2703087.

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