Bioactive Compounds from Natural Products as RHOA/ROCK and VEGFR1 Inhibitors: An In-silico Approach for Developing Therapeutics for ALI/ARDS

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Acute Respiratory Distress Syndrome (ARDS) is a dangerous lung condition characterised by non-cardiogenic pulmonary edoema caused by various factors, including inflammation and hypoxia. It is a more severe and evolved form of Acute Lung Injury (ALI) and requires the patient to be on mechanical ventilation for survival. Several medicinal plants, herbs, oils, and natural extracts have been studied for their anti-inflammatory properties and their targeted action on respiratory disorders. The target of the current study is to elaborate on the target-specific action of bioactive compounds from natural products by Molecular Docking and study their drug-likeness along with their other important pharmacokinetic properties. Bioactive compounds (total 71) from Zingiber officinale (ginger), Trifolium pratense (red clover), Curcuma longa (turmeric), Melaleuca alternifolia (tea tree), Ocimum tenuiflorum (Tulsi), Chlorophytum borivilianum (Safed Musli), Cinnamomum cassia (cinnamon), Elettaria cardamomum (cardamom), and Glycine max (soybean) were selected to be investigated and were screened against RhoA and VEGFR1. The ADMET properties and drug-likeness of the bioactive compounds were studied using Molinspiration and ADMETlab 2.0. Docking studies revealed that Hecogenin (-8.4 and -10.3 kcal/mol), Neotigogenin (-7.7 and -9.8 kcal/mol), and Neohecogenin (-7.6 and -9.7 kcal/mol) produced the best docking results, showing the lowest binding energies for RhoA and VEGFR1, respectively. These energies were found to be comparable to the standard ligands Fasudil (-7.3 kcal/mol for RhoA) and Pazopanib (-8.0 kcal/mol for VEGFR1) for the selected targets. Moreover, Stigmasterol (-7.6 kcal/mol) and Genistein (-8.4 kcal/mol) showed a good binding affinity with RhoA and VEGFR1, respectively. The ADME properties of these molecules were also studied. Thus, the best-docked ligands mentioned above can be used as potential novel compounds against these two targets to develop therapeutics against ARDS. Further in-vitro and in-vivo experiments are required to cement these claims and prepare next-generation natural therapeutics for ARDS.

Keywords: ARDS; Inflammation; Molecular Docking; Ligands; Targets; VEGFR1; RhoA.

Laennec first used the term "idiopathic pulmonary oedema" to define ARDS in 1821. Since Ashbaugh and his colleagues were the first to use the term "ARDS" in 1967, its definition has changed over time to include more symptoms that help us understand the disease.[1] Since then, controversies in defining ARDS have ranged from the "Murray Lung Injury Score" to the "The American-European Consensus (AECC)" definition to the "The Berlin definition". While the

Murray Lung Injury Score from 1988 incorporates the 'scoring' of hypoxemia, respiratory system compliance, chest radiographic findings, and level of end-expiratory positive pressure, the total score for the presence of ARDS in a patient must be above 2.5. [1,2] The AECC definition from 1994 defines ARDS as having (i) an acute and sudden onset of respiratory distress; (ii) a chest X-ray with bilateral infiltrates; (iii) the presence of non-elevated left atrial pressure; and (iv) severe hypoxemia d" 200 mm Hg based on the PaO_2/FiO_2 ratio (regardless of PEEP). [3,4] The 2012 Berlin definition considers patients suffering from ARDS under the following criteria: (i) the beginning of disease within one week of respiratory symptoms; (ii) acute respiratory failure along with unclear morbidities, including heart failure or oedema; (iii) a chest X-ray or CT scan result showing bilateral infiltrates and consistent pulmonary oedema; and (iv) severity defined according to oxygenation.[3,5]

ARDS is a potentially fatal lung condition characterised by fluid accumulation in the alveoli (alveolar oedema), which results in decreased oxygen transfer to the organs (hypoxia) and affects their normal functioning. It used to be called "Adult Respiratory Distress Syndrome," but it was changed to "Acute Respiratory Distress Syndrome" when it was seen in both children and adults. ARDS can be caused by polymorphisms in more than one gene, severe pneumonia, sepsis, inhaling dangerous substances (inhalation injury), or taking too much of a drug. [6] ARDS has a 30–40% mortality rate, with a mortality rate of 24% in patients aged 15–19 and 60% in patients aged 80 or more. Even though scientists have done a number of clinical studies, neither ventilatory support nor drug interventions have led to any improvements. Therefore, supportive therapies and treatment remain the primary approach for patients. Treatment is divided into pharmacologic and non-pharmacologic strategies to minimize the risk of harm from ventilation and gas exchange.[7,8] Drug treatments such as inhaled vasodilators and corticosteroids are considered the best treatments.

On the other hand, some non-drug strategies include choosing PEEP, setting the tidal volume, and lying down. [9] Many molecular mechanisms have also been discovered, which have significantly increased our understanding of the causes of ARDS. However, these mechanisms have not yet become ARDS treatments. [10] New therapeutic opportunities, such as gene therapy, mesenchymal stem cell therapy, lung ultrasound, specific and sensitive biomarkers, and the recent development of RALE (Radiographic Assessment of Lung Edema) are expected to improve the outcomes of ARDS in the future. [11]

Pathogenesis and targets

So far, studies have focused on finding risk factors linked to genes that code for cellular defense, vascular permeability, alveolar integrity, cell development and growth, coagulation, and oxidative stress. [12,13,14] Even though candidate gene association has been questioned in the past due to its difficult reproducibility and understanding, it has helped find a few genes that are linked to ARDS susceptibility or outcome. [15,16] Researchers have looked at many genes, including interleukins (IL-6 and IL-10), interleukin-1 receptor antagonist (IL-1RN), VEGFA, angiotensin-converting enzyme (ACE), soluble mannose-binding lectin 2 (MBL2), and visfatin (NAMPT). [14] As of 2015, 68 different case studies on people of European descent had linked a total of 81 candidate genes to ARDS susceptibility or outcomes. [14]

VEGF Receptor 1 as a therapeutic target

Several preclinical studies have shown that the VEGF pathway is used for progressive signaling in ARDS. [17] Therefore, it can be assumed that VEGF signaling plays a role in the pathogenesis of ARDS. VEGF receptors are primarily expressed in cells, including alveolar type II epithelial cells, respiratory epithelial cells, and activated alveolar macrophages.[18] Under normal conditions, VEGF promotes the formation and proliferation of vascular endothelial cells. However, it can increase vascular permeability during pathogenic conditions and cause inflammation. During the first GWAS of sepsis-associated ARDS, Beatriz et al. (2020) looked at people with European ancestry (n = 1935) and found a common variant (rs9508032) of the FLT1 gene (which codes for VEGFR1) that makes people less likely to get sepsis-associated ARDS. [19] Previously, the variation was neither associated with the susceptibility nor the outcome of ARDS. Even though a link between the FLT1 gene and pulmonary complications has been found,[20] Hernadez-Pacheco and colleagues (2018) did a different study. Using information from rat models and ICU patients, an integrative multi-

omics analysis was done, and a risk variant in the FLT1 gene was found. [21] They concluded that altered levels of VEGF receptors can either protect against or increase the severity of ARDS. In ARDS patients, having the right amount of VEGF receptors can protect the lungs from damage by reducing VEGF activity and vascular permeability. [21,22] Patients on ventilators with high VEGF receptor levels are more likely to get sepsis, alveolar abnormalities, cytokine storms, and eventually die. [23,24] Therefore, it can be concluded that the novel locus present in the FLT1 gene may serve as a potential diagnostic biomarker and VEGFR1 as a therapeutic marker in the diagnosis of ARDS.[25]

RhoA/ROCK as a therapeutic target

RhoA/ROCK cascade activation is one of the primary events that leads to the rigorous inflammatory response in the type 1 pneumocytes and vascular endothelium, causing lung injury and eventually resulting in the life-threatening condition of ARDS. [26] Rho-kinase activity is responsible for the increased expression of several pro-inflammatory cytokines, including IL-8, IL-1â, IL-6, and a transcription factor called NF-êB as discussed earlier. These pro-inflammatory cytokines are responsible for alleviating lung injury and respiratory distress. It has been observed that inhibition of RhoA eventually leads to reduced expression of these cytokines and further decreases the risk of vascular injury.

Using a mouse model, the study found differential expression of IL-6 when treated with the standard Rho-kinase inhibitor Y-27632, which reduced inflammation, evidencing the above statement. [27] Another study found TNF-á and IL-1â to be involved in the inflammatory action in ventilator-induced ARDS, with Rho-kinase activity being the triggering factor. It was observed that the microvesicles shed from pulmonary cells during the disease progression were rich in TNF-á and IL-1â. The increased activity of Rho-kinase was the primary mediating factor. [28] Another study employed a standard Rho-A inhibitor, Fasudil, in studying the differential inflammatory response. Using a mouse as a model, researchers looked at how Fasudil was expressed in lung tissue. The results were confirmed using QT-PCR and western blotting. A significant decrease was observed in the inflammatory activity in the lung tissue when treated with the inhibitor.[29]

Another cytokine, TGF-â1, is known to exhibit increased expression in ALI. A study involving a Rho-A inhibitor has shown differential expression of TGF-â1, employing three *in-vitro* models. [30] These studies are essential to reinforce the hypothesis that Rho-kinase inhibitors have a significant regulatory immunomodulatory potential. These inhibitors can be further employed to curb inflammation and other comorbidities.

MATERIALS AND METHODS

Selection of receptors and ligands

The role of natural herbs in treating respiratory diseases has been well illustrated and deeply studied. The anti-inflammatory, anti-carcinogenic, and antioxidant properties of these herbs have been well exploited since medieval times. These properties can be attributed to several bioactive compounds present in these plants and their products. These compounds can act as promoters, inhibitors, or nutraceuticals, depending on their bioavailability and rate of absorption in the human system. Consumption of plant products, herbal extracts, and compounds extracted from plants has been known to exhibit therapeutic effects on affected tissues. This knowledge has been part of several ancient medical regimens worldwide. Moreover, these compounds have also been studied *in-vitro* and *in-vivo* for their pharmacological properties.

In this study, the authors have compiled a list of bioactive compounds from herbs that have been well explored for their anti-inflammatory effects. These herbs were selected from a vast research literature and database of pharmacological attributes. A library of compounds from these herbs was created, and their physical properties were examined. Compounds with a molecular weight of less than 500 were chosen. This is because compounds with a molecular weight of more than 500 can't pass through the blood-brain barrier (BBB). The 3-D structures of the herbs that met these criteria were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). All the downloaded structures were prepared using PyMol and further saved in .pdb format. These

compounds served as ligands for future molecular docking experiments. Also, the 3D structures of FDA-approved inhibitors for the chosen targets were downloaded and used as a standard for the docking experiment. Also, the 3D structures of FDA-approved inhibitors for the selected targets were downloaded and used as a standard for the docking experiment.

3D structures of selected targets (RhoA and VEGFR1) were also downloaded (PDB id: 1A2B and 3HNG) from the PDB (Protein Data Bank) (https://www.rcsb.org/). These targets were selected via a thorough literature search and analysis of several databases, including KEGG and Reactome. The pathways primarily affected by the disorder were selected for target preparation. The intermediate molecules, promoters, co-factors, competitive inhibitors, and terminators were all analyzed to select the most suitable target. The selected targets were further checked for research status, including *in-vitro, in-vivo,* and clinical trials. These targets were not well explored and therefore selected for this study.

Preparation of receptor and ligand

PyMol was used to get the structure of the target protein ready for molecular docking by getting rid of water molecules, adding hydrogen, and making the structure neutral. Further, the prepared receptor was subjected to molecular docking using AutoDock Vina v1.1.2. The screened compounds were chosen as test ligands for the experiment, and a docking procedure was performed to understand the receptor-ligand interaction. A total of 71 compounds from anti-inflammatory herbs were used as ligands in this study.

Molecular Docking

The CASTp 3.0 (Computes Atlas of Surface Topography of Proteins) server (http://sts.bioe.uic.edu/castp/) was used to find the active sites on the target proteins. This analysis provided the most suitable position for the most rigid target-drug interaction throughout the target protein.[31] The prepared receptor was selected as the macromolecule for the docking study. All the selected compounds were docked against the target protein to evaluate their binding energies using AutoDock Vina.[32] The grid dimensions were set along the lines of the CASTp evaluation. Docking

was performed on all of the test ligands, anti-diabetic drugs, and kinase inhibitors with a default exhaustiveness of 8. The binding energies were tabulated, and ligands exhibiting good binding affinity with the target were selected to evaluate detailed interactions with protein residues and ADMET analysis.

Drug-likeness analysis and ADMET evaluation of the selected phytocompounds

The drug-likeness attributes of the bioactive compounds showing good binding affinity with the target were studied using Molinspiration, which is an open-access web-based tool used for screening compounds based on several standard criteria (https://www.molinspiration.com/). Furthermore, the pharmacokinetic properties of the selected compounds were evaluated using ADMETlab 2.0 (https://admetmesh.scbdd.com/service/evaluation/index).

It calculates the drug-likeness of the compounds across the parameters of physicochemical properties, toxicity, medicinal chemistry, absorption, distribution, metabolism, and excretion.

Furthermore, two-dimensional visualization of the interaction between the target and the selected ligands was done using BIOVIA's Discovery Studio Visualizer. This step would provide detailed insight into the interactions between the amino acid residues and the ligand sub-particles, with hydrogen bond interactions being the most preferred kind.

RESULTS

Molecular Docking Results

The protein molecule was selected as the macromolecule, and the compounds were selected as ligands for the docking study. Firstly, both molecules were prepared for the experiment, and then the grid dimensions were set. The grid dimensions were set in accordance with the CASTp evaluations, and a config file was prepared to mention all the details as obtained in the grid box, as well as the energy range and exhaustiveness. Following that, a command for the docking process to start was executed, and results were available in 30–60 seconds.

Drug-Likeness and ADMET Properties of the Selected Phytocompounds

　　　　Binding energy provides an overview of the interaction between the macromolecule and the ligand. But there are several other criteria to evaluate the drug-likeness of any compound and further evaluate its potential *in-vitro*, *in-vivo* and in clinical trials. In this study, Molinspiration, a web-based platform to calculate molecular properties and bioactivity scores, was employed for different scoring criteria, including the number of hydrogen bond acceptors and donors (nON and nOHNH), number of atoms, partition coefficient (miLog P), number of violations to the Lipinski rule of five, and molecular volume. All the Lipinski parameters were calculated for the screened compounds and tabulated.

　　　　The majority of the compounds chosen met the Lipinski rule of five. For a compound to be therapeutic, the rule states that its molecular mass must be less than 500 Da, it must have less than five hydrogen bond donors, less than ten hydrogen bond

acceptors, and that the octanol-water coefficient must be less than five. Violation of more than two rules is not desirable for a therapeutic. Stigmasterol, Genistein, Apigenin, Tokorogenin, Hecogenin and Neotigogenin were the compounds with the best drug-likeness according to this criteria.

　　　　Furthermore, pharmacokinetic evaluation of the selected bioactive compounds was done using ADMETLAB 2.0. This web-based platform gives a detailed evaluation of the compounds based on all the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) criteria. Absorption is the most important part of a

Table 1. Molecular docking results showing the binding energies of best-docked compounds using AutoDock Vina

S. No.	Compounds	Binding energy (kcal/mol)	
		RhoA (1A2B)	VEGFR1 (3HNG)
1	Stigmasterol	-7.6	-7.6
2	Beta-Sitosterol	-7.3	-7.2
3	Hecogenin	-8.4	-10.3
4	Genistein	-6.0	-8.4
5	9,10-Anthracenedione	-7.1	-9.0
6	Neotigogenin	-7.7	-9.8
7	Quercetin	-7.0	-7.0
8	Apigenin	-7.2	-7.9
9	Equol	-6.6	-8.0
10	Neohecogenin	-7.6	-9.7
11	Tokorogenin	-7.5	-9.2
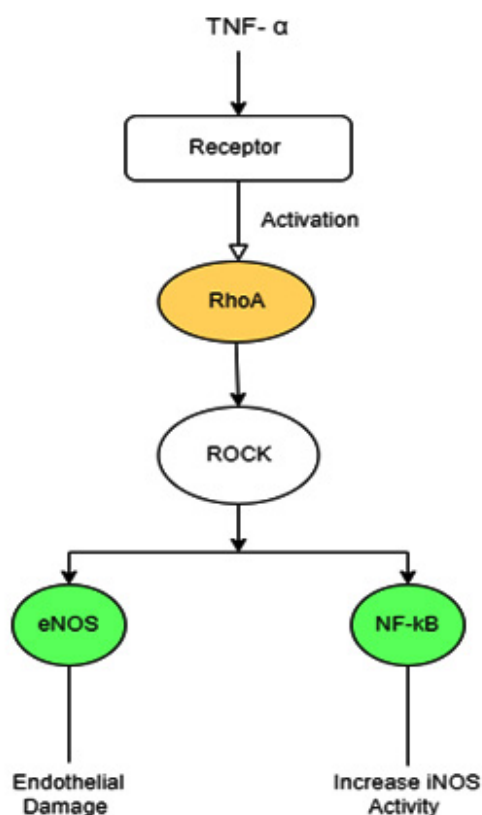
Fig. 1. TNF-α mediated activation of RhoA
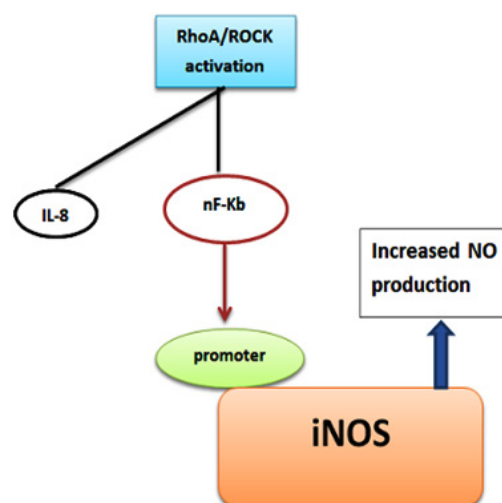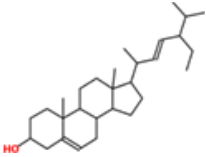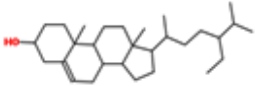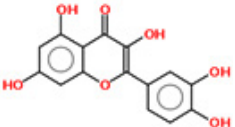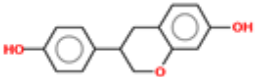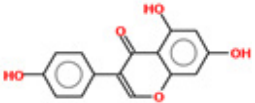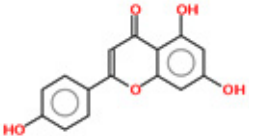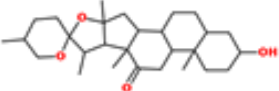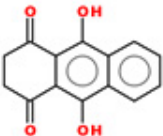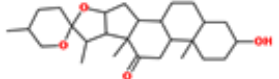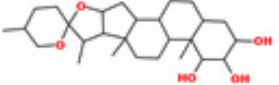
Fig. 2. Rho-A as a regulator of NO release and activity
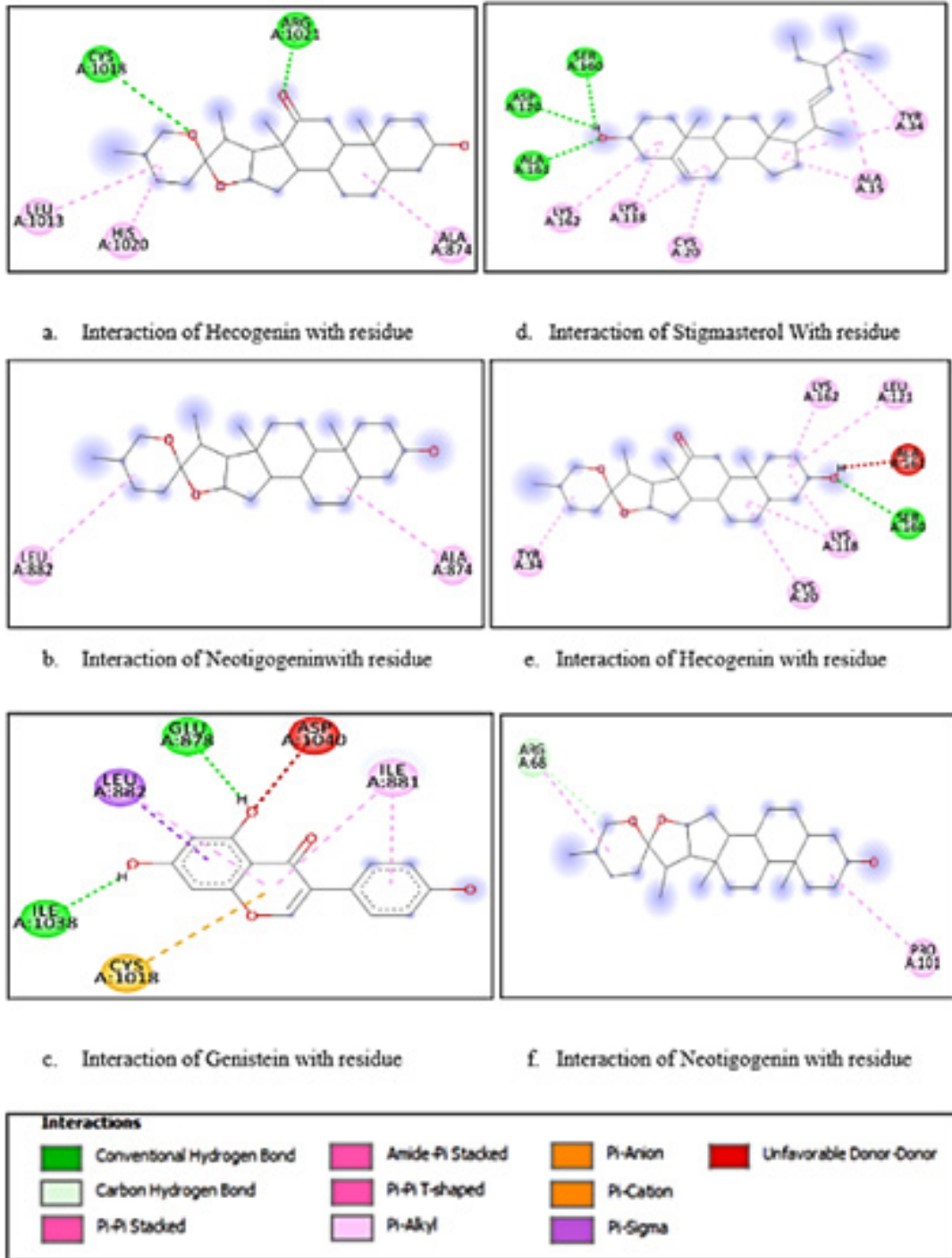
Fig. 3. 2D protein-ligand interactions of best docked compounds using Biovia Discovery Studio (Figure 3a,3b and3c: Interaction of given compound with VEGFR1. Figure 3d,3e and 3f: Interaction of given compound with RhoA)

Table 2. Evaluation of drug-likeness of the best docked compounds using Molinspiration

Ligand	Molecular Structure	Molecular weight (g/mol)	LogP	Hydrogen bond donor count	Hydrogen bond acceptor count
Stigmasterol		412.7	7.80	1	1
Beta-Sitosterol		414.72	8.02	1	1
Quercetin		302.24	1.68	7	5
Equol		242.27	3.07	3	2
Genistein		270.24	2.27	5	3
Apigenin		270.24	2.46	5	3
Neohecogenin		444.66	5.46	4	1
Neotigogenin		416.65	6.12	3	1
9,10-Anthracenedione		242.23	1.91	4	2
Hecogenin		430.63	5.01	4	1
Tokorogenin		448.64	4.50	5	3

Table 3. Pharmacokinetic scoring of selected bioactive compounds on their absorption parameters using ADMETlab 2.0

Phytocompounds	Caco-2 permeability	MDCK permeability (cm/s)	PGP-inhibitor	Pgp-substrate	HIA	$F_{30\%}$
Stigmasterol	High	High	High	High	High	High
Quercetin	Low	High	High	High	High	Low
Equol	High	High	High	High	High	Low
Genistein	High	High	High	Moderate	High	Low
Apigenin	High	High	High	Low	High	Low
Neohecogenin	High	High	Low	High	High	Moderate
Neotigogenin	High	High	Moderate	High	High	Moderate
9,10-Anthracenedione	High	High	High	High	Low	Low
Hecogenin	High	High	Low	High	High	Moderate
Tokorogenin	High	High	High	High	High	Moderate
Beta-sitosterol	High	High	Moderate	High	High	High

Table 4. Pharmacokinetic scoring of selected bioactive compounds on their Distribution and Metabolism parameters using ADMETlab 2.0

Phytocompounds	PPB (%)	VD (L/kg)	BBB penetration	CYP inhibitors	CYP Substrates
Stigmasterol	Low	High	High	-	2C19, 2D6
Quercetin	Low	High	High	1A2	-
Equol	Low	High	High	1A2, 2C19, 2C9, 2D6	1A2, 2C9, 2D6
Genistein	Low	High	High	1A2, 2D6, 3A4	2C9, 2D6
Apigenin	Low	High	High	1A2, 2D6, 3A4	2C9, 2D6
Neohecogenin	Low	High	Low	-	2C19, 2D6
Neotigogenin	Low	High	Low	-	2C19, 2D6
Anthracenedione	Low	High	High	1A2	2C9
Hecogenin	Low	High	Low	-	2D6, 2C19
Tokorogenin	Low	High	Moderate	-	2D6, 2C19
Beta-Sitosterol	Low	High	High	-	2C19, 2D6

Table 5. Pharmacokinetic scoring of selected bioactive compounds on their Excretion and Toxicity parameters using ADMETlab 2.0

Phytocompounds	Clearance (mL/min/kg)	$T_{1/2}$	hERG blockers	H-HT	Carcinogenicity	Respiratory Toxicity
Stigmasterol	High	High	High	High	High	High
Quercetin	High	Long	High	High	High	High
Equol	High	Long	High	High	Low	High
Genistein	High	Long	High	High	Moderate	High
Apigenin	High	Long	High	High	High	High
Neohecogenin	High	Long	High	Moderate	High	Low
Neotigogenin	High	Long	High	High	High	Moderate
Anthracenedione	High	Long	High	High	Low	Low
Hecogenin	High	Long	High	High	High	Low
Tokorogenin	High	Long	High	High	High	Low
Beta-sitosterol	High	High	High	High	High	High

compound's bioavailability. A drug can be given in many ways, and its target areas can be reached through systemic circulation. So, the first step in pharmacokinetic evaluation is to study the parameters of how compounds are absorbed. Caco-2 and MDCK permeability, PGP interactions, HIA, and F30% scores provide a detailed account of compounds' initial oral bioavailability and cellular surface adsorption.

The drug's distribution and metabolism in the body are important pharmacokinetic criteria for understanding how it works and how the interactions between ligands and the target moiety lead to the formation of new complexes. The drug's ability to pass through the blood-brain barrier (BBB) and how well it binds to plasma proteins (PPB) are important to its distribution. The interaction with CYP isoforms also shows the rate of activation and deactivation, which is important for how the drug in question is broken down. Rapid metabolism and inactivation show a higher degree of drug activity in the host system.

The analysis of all the ADMET properties (Absorption, Distribution, Metabolism, Excretion and Toxicity) put forward the pharmacokinetic front of the study. Stigmasterol, Hecogenin and Neotigogenin were the compounds with the best ADMET results and were considered for further molecular evaluation. These results also show that these molecules have excellent pharmacological potential and could be used as potential ligands in any other drug development study for their respective targets.

Table 6. Amino acid residues interacting with ligands; visualised using Biovia Discovery Studio

Target	Compound	Interacting AA residues	No. of H-bonds	No. of Other interactions
RhoA	Hecogenin	Cys 1018 Arg 1021 Leu 1013 His 1020 Ala 874	2	3
	Neotigogenin	Leu 882 Ala 874	0	2
	Genistein	Glu 878 Asp 1040 Ile 881 Cys 1018 Ile 1038 Leu 882	2	4
VEGFR1	Hecogenin	Lys 162 Leu121 Ala 161 Ser 160 Lys 118 Cys 20 Tyr 34	1	6
	Neotigogenin	Apg 68 Pro 101	0	2
	Stigmasterol	Ser 160 Asp 120 Ala 161 Lys 162 Lys 118 Cys 20 Ala 15 Tyr 34	2	6

Interaction of Selected Phytochemicals with Target Proteins

Studying the interaction of ligand molecules with amino acid moieties present on the active site of the protein structure gives us insight into the nature and strength of the interaction between them. For studying the inhibitory nature of these phytochemicals, we need to measure their binding affinity with the target along with the probable interaction. These parameters are comparable to the natural ligands in the human system and the pre-existing standard inhibitors. If the research yields positive results, i.e., if the compound exhibits good binding and the nature of the interaction is strong and favorable, such compounds are selected for further research and drug development procedures.

The nature of interactions between ligands and targets was studied using a 2D model in Biovia Discovery Studio. There can be a number of interactions between these two moieties, including hydrogen bonds, ionic interactions, and C-H bonds, which are considered important for the purpose of drug discovery. Table 6 shows the results of the 2D analysis of the interactions obtained using Discovery Studio.

DISCUSSION

Ashbaugh and his colleagues first defined Acute Respiratory Distress Syndrome (ARDS) in 1967 based on a case report they wrote about. The patients included both children and adults who had symptoms such as increased lung stiffness, pulmonary edema, breathing difficulties, and hypoxaemia. Besides these symptoms, doctors also saw signs of sepsis, trauma, pneumonia, and aspiration. [1] In 1992, AECC came up with some criteria for diagnosing ARDS. [4] In 2012, these criteria were updated to become "The Berlin Definition." [5] Clinical disorders associated with ARDS vary according to geographical location and healthcare systems in developing countries. For 50 years, there has been improvement in understanding the disease, its epidemiology, and its pathophysiology. It provides researchers with the knowledge to improve the treatment of the disease. Also, the treatment of ARDS has gotten better because of the many randomized trials that have been done to reduce the use of mechanical ventilation and fluid therapy. [33]

There are still challenges with screening ARDS patients because there is no strong evidence or agreement. Also, the disease only happens in a small number of people who have risk factors, which makes screening hard. [34] Moreover, the development of the disease is quickly leading to patients with such severity getting hospitalized within 12–14 hours. Therefore, clinical scores have been created to predict the patients at a high risk of developing the disease, such as the Lung Injury Prediction Score (LIPS).[35,36,37] LIPS synthesise clinical data such as comorbidities, risk factors, and acute physiological variables. All these data generate a score, where a high score is an indication that the patient is at high risk of developing the disease. [38]

Another potential predictive instrument for ARDS is biomarkers. A study was conducted by Rubin et al. (1990). He came to the conclusion that VWF, a biomarker for endothelial damage, was high in people with non-pulmonary sepsis who were in the early stages of ARDS. [39] In the same way, Ang-2 and IL-8 were also found to be higher during the early development of ARDS. So, finding the ARDS biomarkers has helped a lot with treating the disease in a more personalised way. [35]

In-silico molecular docking experiments, drug-likeness analysis, and drug-induced gene expression analysis of the selected bioactive compounds provide the computational background for the drug development process. In this study, 71 phytochemicals from a variety of sources were selected and analyzed for their therapeutic potential against sepsis-associated ARDS. Stigmasterol, Genistein, Beta-sitosterol, Apigenin, Hecogenin, Neo-hecogenin, Neotigogenin, 9,10-Anthrocenedione, Tokorogenin, Equol, and Quercetin were among the 11 compounds that demonstrated excellent binding affinity to the targets.

Stigmasterol, a phytosterol derived from soybean oil, demonstrated excellent binding energy with both targets and yielded promising results in protein-ligand interaction studies. The anti-inflammatory potential of stigmasterol has been well studied and documented. It reportedly suppressed the expression of pro-inflammatory

cytokines, including TNF-á, IL-6, iNOS, and COX-2, and along with that increased the release of the pro-inflammatory cytokine IL-10 in a collagen- induced arthritic mice model, in a study demonstrating the inhibitory effects of stigmasterol on inflammatory factors and its overall impact on arthritis progression. [40] Another *in-silico* molecular docking study provided proof that stigmasterol is a down regulator of iNOS expression. iNOS is a crucial regulator of NO levels in the epithelial cells, which regulate the level of ALI (Acute Lung Injury).[41] Another study provided evidence for its therapeutic role in cell-mediated lung injury by suppressing the VCAM-1 (vascular cell adhesion molecule-1) levels in ovalbumin induced asthmatic Guinea pigs. VCAM-1 is released by cytokine activated cells and its free circulatory forms lead to increased inflammatory response.[42] Also, stigmasterol's effect on the two targets chosen for this study has never been seen before, so it could act as a potential inhibitor against them.

Hecogenin is another compound that exhibited good binding affinity with the two targets and has been reported to have therapeutic effects on a number of disorders and long-term illnesses. Its anti-inflammatory potential has been demonstrated in a study with arthritic rats, where oral administration of this compound inhibited the production of pro-inflammatory cytokines like TNF-á and IL-12, both of which play important roles in the progression of rheumatoid arthritis and are also associated with alveolar damage that leads to ARDS. [43] Furthermore, it has been reported to reduce NO levels in ulcerative colitis-ridden mice in a dose-dependent manner, further solidifying its status as a potential drug candidate for sepsis-associated ARDS. [43]

Genistein, an isoflavone, and a phytoestrogen made from red clover have also shown good binding energy with the targets, especially VEGFR1. Its anti-inflammatory role is well established. TNF-á-mediated NF-êB activity in epidermal keratinocyte cells is inhibited by genistein in a dose-dependent manner. [44] This pathway is responsible for the increased iNOS activity in vascular epithelial cells, further causing alveolar damage, as discussed in previous sections. Genistein has also modulated the production of pro-inflammatory cytokines, including IL-1â and IL-6, to curb VEGF-A-mediated inflammation. [45]

Y. Q. He et al. (2021) compiled the action of 156 herbal bioactive compounds and tabulated their therapeutic potential. The compilation provided a multi-targeted *in-vivo* and *in-vitro* action of these compounds. [46] Interestingly, natural inhibitors for VEGFR1 and RhoA have not been explored yet; this is one of the first studies focusing on *in-silico* drug development against these two targets. After thorough research, the best-docked compounds and those that pass the pharmacokinetic criteria can be used as novel inhibitors for these two targets.

CONCLUSION

In this study, 71 bioactive compounds from natural sources were investigated for their activity against VEGFR1 and RhoA. 11 out of the 71 compounds were found to exhibit excellent binding energy against the targets. These compounds include Stigmasterol, Quercetin, Equol, Genistein, Apigenin, Neohecogenin, Neotigogenin, 9,10-Anthracenedione, Hecogenin, Tokorogenin and Beta-sitosterol. Three compounds, in particular, Hecogenin, Neotigogenin and Stigmasterol, showed the best *in-silico* results for both targets, and Genistein exhibited excellent affinity for VEGFR1. Well-established literature evidence and web-based database platforms have reinforced the hypothesis. Moreover, the herbs that comprise these compounds have been known for their therapeutic benefits. These compounds were then evaluated using a variety of criteria for pharmacokinetic evaluation and drug-likeness. Their interaction with the target moiety was also studied on a molecular level, and all the forces of attraction were well visualised and scored.

This is one of the first studies investigating natural products for these two targets. Best-performing ligands can be further tested *in-vitro* and *in-vivo*, and further clinical trials would cement these compounds as novel inhibitors for these targets.

Future Prospects

This study is unique in its focus on developing therapeutics for sepsis-associated disorders, specifically acute respiratory distress syndrome (ARDS), through the exploration of two targets and their mechanisms. These targets have not been extensively studied before, making

this study a significant contribution to the field. Additionally, the results of this study are highly promising and warrant further investigation through wet experiments. The most stable ligands identified in this study should be used in the development of natural product-based therapeutics for ARDS.

In-vitro studies on cell lines like A549 and HUVEC would further solidify the potential of these ligands as pharmacological inhibitors for their targets. Different aspects of growing cells, like their viability, expression of target proteins, and NO levels in cells, can be monitored in order to fully cement the functionality of these compounds as potential inhibitors. Also, *in-vivo* experiments with animal models would help learn more about how these compounds are absorbed and broken down in the host system. Lastly, well-planned clinical trials with these compounds would finally help analyze their activity in the human system and would be the final step in a drug development regimen.

The use of herbal therapeutics and natural compounds in medication strategies has a rich history in various cultures worldwide, and it has become an increasingly popular area of scientific research in recent years. Hence, studies like the one mentioned in this context are vital to understanding the mechanism of action of these compounds as inhibitors. Additionally, the growing scientific interest in this field makes it a promising sector for the future, with potential for job opportunities in the field of herbal therapeutics.

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Conflict of Interests

The authors do not have any conflict of interest.

Data Availability

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

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