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Original article

Exploring *Bacopa monnieri* phytochemicals as potential RSV inhibitors: An in-silico analysis of key compounds against fusion and glycoprotein targets

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ABSTRACT

Background: *Respiratory Syncytial Virus* (RSV), also known as Human Orthopneumovirus, is a significant cause of lower respiratory tract infections worldwide, particularly impacting children. Despite the urgent need, effective vaccines and treatments remain limited. Natural products, especially phytochemicals from medicinal plants, have recently gained attention as potential sources of antiviral agents. We investigated phytochemicals from *Bacopa monnieri*, an herb with a long-standing history in traditional medicine, for their antiviral potential against RSV. Using in-silico molecular docking, six key phytochemicals (Betulinic acid, Jujubogenin, Bacogenin-a1, Pseudojujubogenin, Bacosine, and Ebelin lactone) were selected based on Lipinski's rule and binding affinity toward RSV's essential proteins, the Fusion (F) and Glycoprotein (G). Docking results revealed that Jujubogenin exhibited the strongest binding affinity, with scores of -9.4 kcal/mol for the F protein and -8.7 kcal/mol for the G protein, suggesting its potential to inhibit RSV entry and infection processes. This study demonstrates that phytochemicals from *Bacopa monnieri* may offer a promising direction for developing natural therapeutics against RSV.

Introduction

Respiratory Syncytial Virus (RSV) which is also recognized as Human Orthopneumovirus is one of the deadliest viruses whose vaccines have still not been developed. RSV causes pulmonary infection which affects the lower respiratory system and are more prone to children [1]. RSV infection has become one of world's largest concern. The virus has caused huge death, with a mortality range between one lakh to two lakh children every year [2]. It leads to a chronic infection that causes inflammation of the airways in the lungs, resulting in pneumonia and bronchiolitis. Over time, this infection severely damages the lungs, causing the respiratory system to fail, which can ultimately lead to apnea and death [3,4]. So far, only a limited number of therapeutics have been developed, with Ribavirin and Palivizumab being the only two antiviral medications approved for infants [5]. Human RSV, a member of Paramyxoviridae family is an enveloped, single stranded RNA virus and have negative polarity. It is confirmed that the virus was isolated for the first time from chimpanzees in 1955 [6]. Later, it was accepted that the same strain of the virus causes infection in the humans. The human immune response to RSV infection involves a strong

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neutrophil response, followed by a pulmonary CD8+ T-cell response, which along with IFN plays a protective role.

However, only one serotype of RSV has been known causing infection in humans. This serotype has been classified into two strains A and B. These strains simply differ from each other on the basis of structural membrane protein. This structural membrane protein is combined with a lipid bilayer envelope which enclosed the ribonucleoprotein core. This lipid bilayer is attached to several different structural proteins which are used by the virus to bind to the host cell [7].

In recent years, natural products have garnered attention as a promising reservoir for novel antiviral agents [8, 9]. Phytochemicals derived from medicinal plants exhibit unique structural diversity and a broad array of biological activities [10, 11], which position them as potential candidates for antiviral drug discovery [12]. *Bacopa monnieri*, commonly known as Brahmi, has been used in Ayurveda and other traditional medicinal practices for centuries, primarily known for its cognitive-enhancing properties [13, 14]. This plant is characterized by a diverse range of bioactive phytochemicals, which have been associated with a wide range of medicinal effects, including antioxidant, anti-inflammatory and neuroprotective activities [15].

Current antiviral therapies for RSV are limited to drugs such as ribavirin and monoclonal antibodies such as palivizumab, which primarily target viral replication and immune modulation, respectively. Ribavirin inhibits viral RNA synthesis, while Palivizumab targets the F protein, preventing viral fusion. However, these treatments have limitations, including high cost, limited efficacy, and adverse effects [16]. *Bacopa monnieri*, traditionally used in Ayurvedic medicine, contains bioactive compounds such as jujubeogenin and bacogenin-A1 [17], which show promise in targeting RSV proteins involved in viral attachment (G protein) and fusion (F protein). Unlike standard drugs, phytochemicals offer the advantage of inhibiting multiple viral mechanisms simultaneously, potentially reducing resistance. Phytochemicals often exhibit favorable safety profiles following Lipinski's rule, which predicts good bioavailability and minimal toxicity. They are also more cost-effective and sustainable than monoclonal antibodies such as palivizumab. phytochemicals from Bacopa monnieri represent a promising direction for RSV therapeutics. Their multi-target capabilities, safety, and affordability position them as potential alternatives or adjuncts to standard drugs.

This study aims to investigate *Bacopa monnieri* phytochemicals as potential RSV inhibitors using in-silico methods. By focusing on key viral proteins involved in RSV entry and pathogenesis, this research intends to identify compounds within *Bacopa monnieri* that show promising binding affinities to these targets. In-silico screening offers a rapid, cost-effective approach to early-stage drug discovery, allowing for efficient identification of promising candidates for further study. The findings from this study may ultimately contribute to the development of novel, natural therapeutics against RSV.

Methods

Selection of Phytochemicals:

Phytochemicals are bioactive compounds in plants that play an important role in health promotion and disease prevention [18]. There are many different sources of phytochemicals, including whole grains, vegetables, fruits, nuts, and herbs. To date, over a thousand phytochemicals have been identified. Dietary fibres, carotenoids, polyphenols, isoprenoids, phytosterols, saponins, and certain polysaccharides are a few of the important phytochemicals. Strong antioxidant properties and antibacterial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral properties are all displayed by these phytochemicals [19]. We used IMPPAT database [20] to identify 47 phytochemicals from *Bacopa monnieri*. From these, we selected six best ligands (Betulinic acid, Jujubogenin, Bacogenin-A1, Pseudojujubogenin, Bacosine, and Ebelin lactone), based on Lipinski's rule acceptance and their strong receptor-ligand binding affinities. Lipinski's Rule of Five analysis was conducted using tools ADMETlab 3.0 [21] plays an important role in evaluating the drug-likeness of phytochemicals. ADMET helps the researchers to evaluate the kinetics and toxicity of potential drug candidates, allowing molecules to be screened for their drug-like properties before moving into the experimental stages**.** It also facilitates the assessment of bioavailability, drug-likeness and potential toxicity, thereby enhancing the decision-making process in drug development

[22]. To do this we have given inputs of smiles of phytochemicals and run it to get the result whether the molecule is toxic or non-toxic. These selected phytochemicals exhibited promising bioactive profiles and were non-toxic, that is why they are included in the study. Figure 1 shows the chemical structures of the six selected molecules.

Selection of Target Proteins:

To select key RSV proteins for analysis, we utilized the NCBI databases [23] to obtain gene sequences and protein annotations. RSV's 15.2 kb genome encodes 11 proteins, among which the Fusion (F) protein and Glycoprotein (G) are critical for viral entry and pathogenesis [24]. These proteins were chosen due to their established roles in viral infection mechanisms and potential as therapeutic targets. High-resolution structures (Figure 2) were then obtained from the Protein Data Bank (PDB) [25] to facilitate precise in-silico docking simulations. This integration of genomic data from NCBI and structural data from the PDB enables a comprehensive computational approach to assess potential antiviral compounds targeting these essential RSV proteins.

F Protein (PDB ID: 3QQ9)

The F protein is highly conserved among the Paramyxoviridae family where the fusion of the viral envelop and the formation of syncytium both processes need the F protein and are enhanced by the G protein. The F protein synthesizes as 67 kDa precursor [21, 26] which go through the proteolytic cleavage to produce two disulphide linked polypeptides which is F1 and F2, from the region of C and N termini [27]. The entry of portal for the F protein to the cell membrane is via the N terminus region of the F1 polypeptide where trans membrane segment is located closely to the C terminus [28]. There are several functions of F protein which includes mediating virus entry into target cell, promotes virus fusion, brings macropinocytosis of RSV, increases air wa4s mucous secretion and promotes the adhesion of neutrophils and eosinophils to the airways [29].

G Protein (PDB ID: 6UVO):

G protein which stands for G glycoprotein is one of the major proteins of the RSV virus and the role of this protein is to help in the attachment to the host cell [30]. The protein is glycosylate type II trans membrane protein having the N terminal cytoplasm domain with the hydrophobic region and an ectodomain region made with mucin which is extremely diverse in nature consisting of serine, threonine, proline and is highly glycosylated with N and O linked sugar compounds [31]. The G protein has the major function in diminishing the immune system of our body. This soluble G protein functions to behave as body's own antigenic material, mimics to the host immune cells and starts the infection [32]. The G protein is identified as a changeable protein among the RSV. This change has introduced two strains of RSV which is now termed as RSV A and RSV B. This changeable viral G protein has made many changes in RSV genotype and is the main cause of forming different strains in past years [33]. The different strains of G protein differ in the size of nucleotide bases and includes insertion and deletion in its bases.

Molecular docking

Molecular docking is a computational technique used to predict the binding affinity and interactions between small molecules, such as phytochemicals, and target proteins [34]. We used SeamDock [35] a high-throughput docking tool, to evaluate the binding affinities of six selected phytochemicals from *Bacopa monnieri* (Betulinic acid, Jujubogenin, Bacogenin-a1, Pseudojujubogenin, Bacosine, and Ebelin lactone) against two critical Respiratory Syncytial Virus (RSV) proteins: the Fusion (F) protein and the Glycoprotein (G). SeamDock allowed us to conduct precise docking simulations, optimizing the binding poses and calculating binding affinities, making it possible to identify promising lead compounds for RSV inhibition

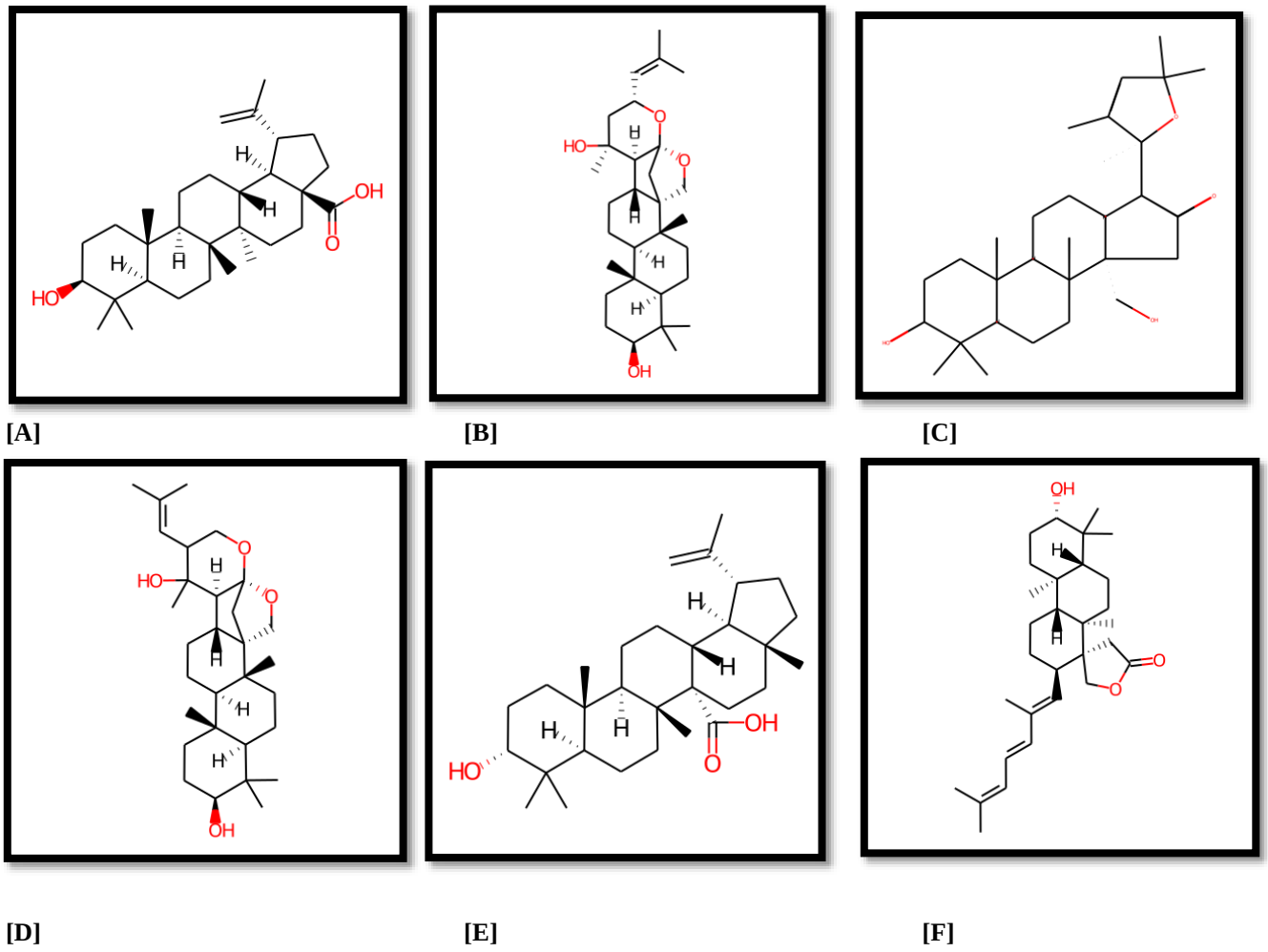
Results and discussion:

In this study, we employed in-silico docking techniques to evaluate the binding affinities of six phytochemicals from *Bacopa monnieri* against two critical proteins of Respiratory Syncytial Virus (RSV)—the F protein and the G protein (Table 1). These proteins are essential in the viral entry process: the F protein facilitates the fusion of the viral envelope with the host cell membrane, while the G protein plays a crucial role in viral attachment to host cells. Among the compounds tested, Jujubogenin emerged as the most promising candidate, demonstrating strong binding affinities for both the F and G proteins.

The molecular docking analysis (Figure 3) revealed that Jujubogenin exhibited a binding affinity of -9.4 kcal/mol with the F protein. This

result is particularly significant because the F protein is central to the fusion of the viral envelope with host cell membranes, a critical step for viral entry and subsequent infection. The strong binding affinity suggests that Jujubogenin may inhibit the fusion process, preventing RSV from entering host cells. In addition to its strong affinity for the F protein, Jujubogenin demonstrated a docking score of -8.7 kcal/mol against the G protein. The G protein is involved in the initial attachment of RSV to host cell receptors, making it an attractive target for therapeutic intervention. The strong binding affinity of Jujubogenin for this protein suggests that it could interfere with the virus's attachment to host cells, thereby inhibiting the early stages of infection. This dual-targeting approach, which impacts both fusion and attachment, distinguishes Jujubogenin from other antiviral candidates that may only target one of these processes [36]. Compounds that target multiple stages of the viral lifecycle have shown enhanced efficacy and reduced likelihood of resistance, making Jujubogenin a promising candidate for further investigation. Dual-target strategies have been successfully employed in the development of antiviral drugs for other viruses, such as HIV [37] and influenza [38], demonstrating their potential to overcome viral escape mechanisms.

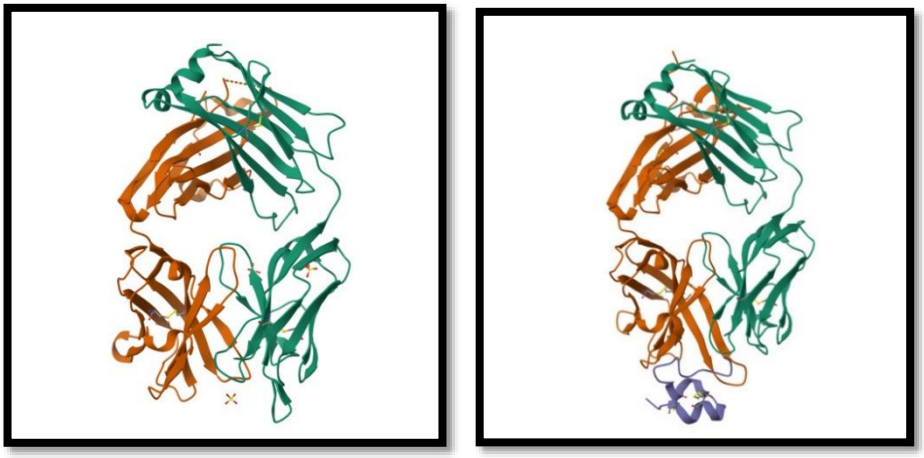
Figure 1. Chemical structure of selected phytochemicals (From A to F - Betulinic acid, Jujubogenin, Bacogenin-A1, Pseudojujubogenin, Bacosine, and Ebelin lactone respectively)



[A] **[B]** **[C]**

[D] **[E]** **[F]**

Figure 2. 3D structure of RSV proteins



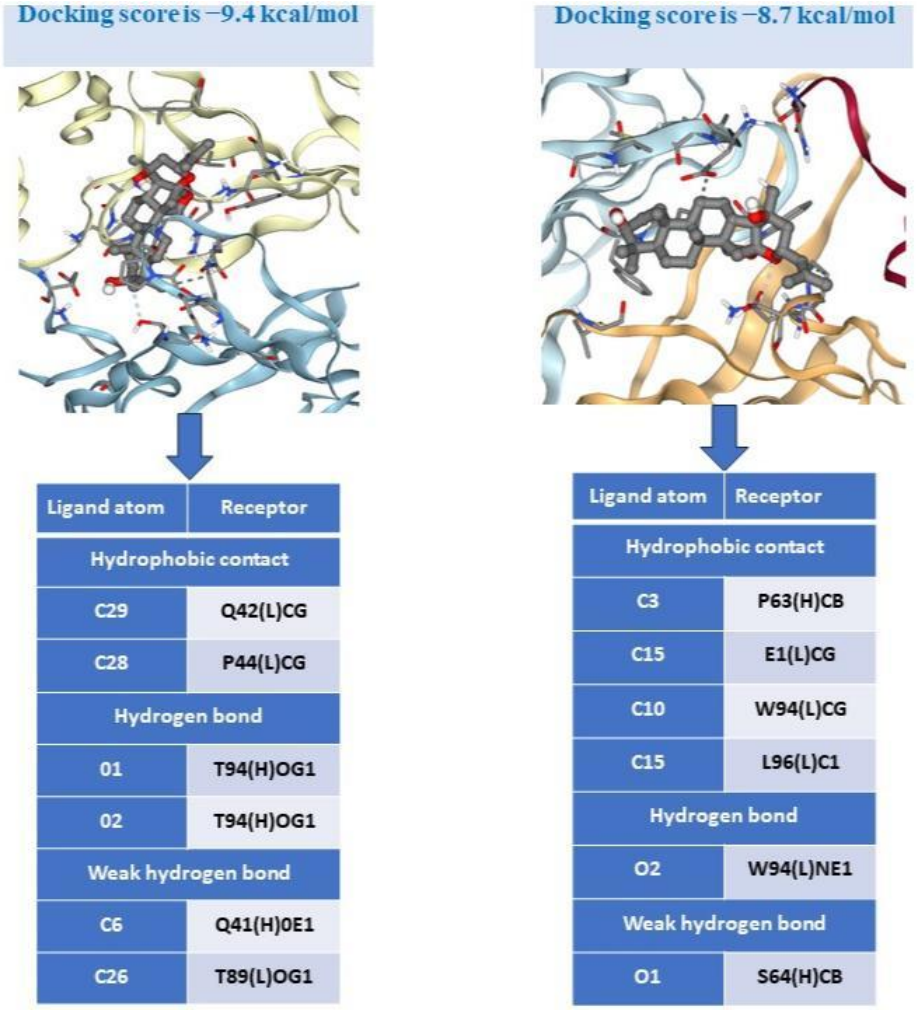
F Protein(3QQ9) **G Protein(6UVO)**

Table 1. Phytochemicals showing best binding affinity.

Phytochemical name	**Smiles**	**Lipinski Rules**	**Docking results (affinity) (F-protein)**	**(G - protein)**
Jujubogenin	CC(=C[C@@H]1O[C@@]23OC[C@@]4(C2)[C@@H]([C@H]3[C@@](C1)(C)O)CC[C@H]1[C@@]4(C)CC[C@@H]2[C@]1(C)CC[C@@H](C2(C)C)O)C	**Accepted**	**-9.4**	**-8.7**
Bacogenin-a1	OC[C@@]12CC(=O)[C@@H]([C@H]1CC[C@H]1[C@@]2(C)CC[C@@H]2[C@]1(C)CC[C@@H](C2(C)C)O)[C@]1(C)OC(C=C1C)(C)C	**Accepted**	**-9.1**	**-8.4**
Pseudojujubogenin	CC(=CC1CO[C@@]23[C@H](C1(C)O)[C@H]1CC[C@H]4[C@@]([C@@]1(C3)CO2)(C)CC[C@@H]1[C@]4(C)CC[C@@H](C1(C)C)O)C	**Accepted**	**1.** **2.** **3. -8.8**	**-8.5**
Bacosine	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@@]1(CC2)C(=O)O)(C)CC[C@@H]1[C@]3(C)CC[C@H](C1(C)C)O)C	**Accepted**	**-8.6**	**-7.9**

Ebelin lactone	O=C1OC[C@@]2(C1)[C@H](CC[C@H]1[C@@]2(C)CC[C@@H]2[C@]1(C)CC[C@@H](C2(C)C)O)/C=C(/C=C/C=C(C)C)C	**Accepted**	**-8.4**	**-8.4**
Betulinic acid	CC(=C)[C@@H]1CC[C@]2([C@H]1[C@H]1CC[C@H]3[C@@]([C@]1(C)CC2)(C)CC[C@@H]1[C@]3(C)CC[C@@H](C1(C)C)O)C(=O)O	**Accepted**	**-8.2**	**-7.8**

Figure 3. Molecular docking results of Jujubogenin with the two target proteins – [A] with F protein and [B] with G protein.



[A] **[B]**

While the in-silico docking results for Jujubogenin are promising, further experimental studies are needed to validate these findings. In-vitro assays could be conducted to test the compound's ability to inhibit RSV infection in cultured cells.

Additionally, animal models would provide critical insights into the therapeutic potential and safety profile of Jujubogenin. Furthermore, molecular dynamics simulations could offer a deeper understanding of the stability of Jujubogenin-protein complexes and its binding mechanism at the atomic level.

Moreover, future studies should explore the synergistic effects of Jujubogenin with other antiviral agents, as combination therapies often yield improved results and reduce the risk of resistance. Pharmacokinetic and toxicological profiling will be essential to evaluate whether Jujubogenin can be developed into an effective therapeutic for RSV, a viral infection that remains a major health burden, especially in children and immunocompromised individuals**.**

Conclusion

We explored *Bacopa monnieri* phytochemicals as potential RSV inhibitors using molecular docking simulations. Among the selected compounds, Jujubogenin emerged as the most promising candidate, exhibiting notable binding affinities with RSV's F and G proteins, which are essential for viral entry. Its binding scores of -9.4 kcal/mol and -8.7 kcal/mol for the F and G proteins, respectively, suggest a strong potential to inhibit RSV's infection mechanism. These findings encourage further investigation of Jujubogenin's mechanisms in preclinical models to validate its efficacy. Overall, this study underscores the potential of natural compounds as a resource for antiviral drug discovery, contributing to ongoing efforts in developing plant-based treatments for RSV.

Conflict of Interest

The authors declare that there is no conflict of interest related to this work.

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