NATURAL TERPENES PROMOTE STRUTURAL STABILIZATION TO THE SPIKE GLYCOPROTEIN OF SARS-COV-2

HIGHLIGHTS:

- **Antiviral Potential of Terpenes Against SARS-CoV-2**: This study explores the antiviral activity of four natural terpenes — α-bisabolol, citral, cis-jasmone, and eucalyptol — using molecular docking simulations, focusing on their interaction with the SARS-CoV-2 spike glycoprotein.
- **Terpene-Spike Glycoprotein Interaction and Molecular Stabilization:** The results indicate that the terpenes form stable complexes with the spike glycoprotein, suggesting a potential mechanism for blocking viral entry by disrupting the interaction between the virus and host cell receptors.
- **Implications for Antiviral Therapeutic Development**: These findings provide valuable insights for the design of novel antiviral agents, highlighting the potential of terpenes as viral entry inhibitors and promising candidates for therapeutic strategies against COVID-19.

ABSTRACT

Compounds exhibiting potent antiviral activity against the novel coronavirus remain insufficiently understood, posing significant challenges in the development of effective therapeutic interventions. Among various classes of bioactive molecules, terpenes represent a promising group of natural

compounds with diverse and extensive antiviral properties. This study aims to explore, through advanced in silico approaches, the antiviral potential of four specific terpenes—α-bisabolol, citral, cis-jasmone, and eucalyptol—against the spike glycoprotein of the SARS-CoV-2 virus. To this end, molecular docking simulations were employed to assess the interaction between these terpenes and the spike protein, with the goal of determining the capacity of these compounds to form stable complexes that might interfere with the viral entry process. The results from this computational analysis, encompassing chemical, spatial, and energetic data, indicate that these four terpenes possess the ability to bind effectively to the spike glycoprotein, thereby potentially stabilizing this crucial protein. Such stabilization could impair the virus's ability to interact with host cell receptors, providing a molecular mechanism by which these terpenes may hinder viral entry. These findings support the hypothesis that the complexation of terpenes with the spike glycoprotein serves as a promising strategy to obstruct the virus's capacity to invade host cells, offering valuable insights for the design of novel antiviral agents targeting SARS-CoV-2.

Keywords*:* SARS-CoV-2; COVID19; Terpenes; Spike-glycoprotein; docking.

1. INTRODUCTION

Nidovirus is a large order of RNA viruses that consists of many genera and species, including the Coronaviridae family (UCCELLINI et al., 2014). These viruses are known to infect mainly mammals, but the infection in birds (coronavirus) was already detected (PASTERNAK et al., 2006). Coronaviruses (CoVs) are positive-enveloped viruses and possess a single-stranded RNA.

These viruses cause a variety of diseases, ranging from asymptomatic cases to a fatal infection (PASTERNAK et al., 2006).

The first cases of the new coronavirus pneumonia (COVID-19) occurred in Wuhan, China, in December 2019 (LI et al., 2020a) and the number of COVID-19 cases confirmed in the worldwide has exceeded 166,352,00 with 3 449,189 deaths, according to Weekly epidemiological update (WHO et al., 2021). The SARS-CoV-2 is highly contagious and the transmission occurs presumably via airborne droplets and fecal-oral route (DU et al., 2020).

The SARS-CoV-2 spike protein (S) is the main molecule present at the surface of the virion (WRAPP et al., 2020). This large glycoprotein assembles in trimers that form a structure crown-like on the envelope, which gives the name to this virus family (SIGRIST et al., 2020). The SARS-CoV-2 spike glycoprotein RGD (tripeptide of Arginine, Glycine, and Aspartate) lies in the receptor binding domain (amino acids 319 to 541) at the border of the subdomain (amino acids 437 to 508) that is specifically involved in the binding to human ACE2 (LI et al., 2020b; XIAO et al., 2003).

Although the SARS-CoV-2 antiviral activity is still poorly understood, terpenes are an interesting group of natural agents with specific and far-reaching antiviral activities that can be used to improve the therapeutic effectiveness of standard antiviral therapy (PADUCH et al., 2007).

At the moment there is no treatment and no vaccine available for the population, only studies involving the possible benefit of chloroquine, a broadly used antimalarial drug (COLSON et al., 2020; GAO et al., 2020). Therefore, our research objective is to identify the antiviral activity of (-)-α-bisabolol, citral, *cis*-

jasmone and eucalyptol against coronavirus main protein: SARS-CoV-2 spike protein.

2. MATERIAL AND METHODS

The interaction between 4 terpenes and SARS-CoV-2 S protein was analyzed *in silico* using molecular docking simulation. Redocking of the cocrystallized ligand (spike glycoprotein-ACE2 complex) was realized to validate the docking protocol (YAN et al., 2020).

The docking was performed using AutoDock Vina code (version 1.1.2), using 3-way multithreading and Lamarkian Genetic Algorithm (TROTT et al., 2019). Centralized throughout the receptor, the grid box was defined with parameters of 100 Å x 100 Å x 100 Å, seeking all the possible binding sites based on the energy of association between terpenes and the SARS-CoV-2 S protein in certain positions.

The three-dimensional structures of 4 terpenes: (-)-α-bisabolol, citral, *cis*-jasmone and eucalyptol and SARS-CoV-2 S protein were obtained in PubChem and Protein Data Bank (1549992, 638011, 1549018, 2758 and 6VSB, respectively). The provided data was analyzed using PyMol v1.4.7 (DELANO et al., 2014), which allows a detailed investigation of the complexes formed: energy of association, chemical binding, amino acid residues involved and conformational nuances.

3. RESULTS AND DISCUSSION

SARS-CoV-2 S protein endures viral activity which contributes in the infection mechanism in human cells. Total 10 docked were performed for each

terpene on the whole protein 3-D structure of SARS-CoV-2 S protein which was considered only the lowest free energy of docked complex with hydrogen bonds. The most energetic cluster was investigated about its specificity in the interaction (reproducibility), affinity (quantity of chemical bonds), stability (spatial compatibility) and the structural stabilization capacity (complexing energy).

Based on the data obtained with the molecular docking of the 4 terpenes [(-)-α-bisabolol, citral, *cis*-jasmone and eucalyptol] with the SARS-CoV-2 S protein, it was observed that all had affinity for the viral protein, specifically in two distinct domains: S1/S2 (protease cleavage site) and RBD (receptor binding domain) down promoter (WRAPP et al., 2020). Interestingly the S1/S2 processing site exhibits different motifs among coronaviruses, with many of them displaying cleavage after a basic residue. Therefore, the priming process is likely to be ensured by different host cell proteases depending on the sequence of the S1/S2 cleavage site (SIGRIST et al., 2020). The most favorable interaction for each ligand occurred mainly in secondary alpha-helix structures and without spatial impediments (Figure 1).

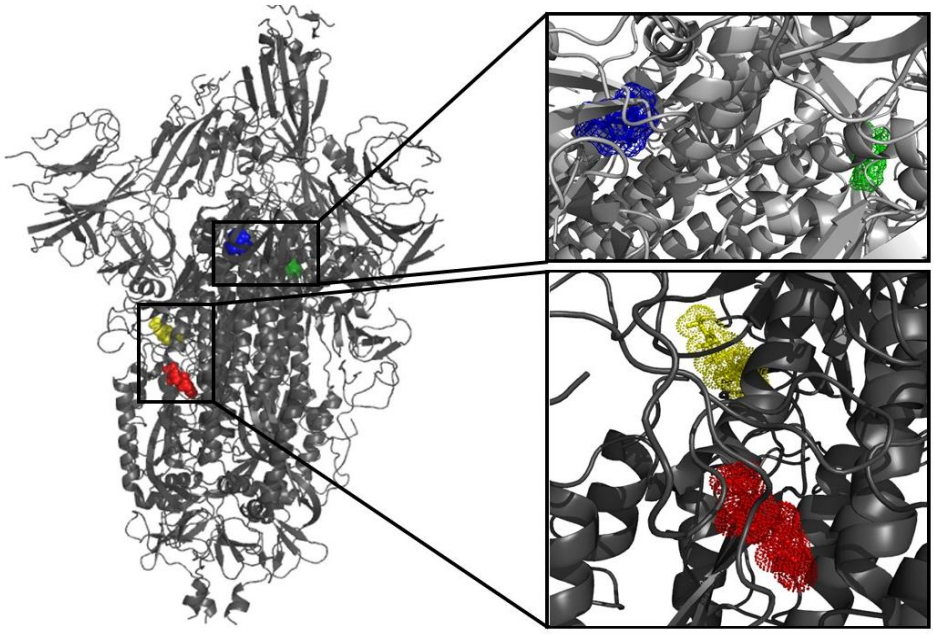

Figure 1: Side view of Protein S of SARS-Cov-2 after complexing 4 terpenes - (-)-α-bisabolol (yellow), citral (red), *cis*-jasmone (green) and eucalyptol (blue) indicating affinity of the ligands to a responsible domain for protease cleavage (S2) and another responsible for binding to receptors (RBD) - each domain was classified according to Wrapp et al. (2020).

The terpenes used have low molecular weight: (-)-α-bisabolol (222.37 g/mol), citral (152.23 g/mol), *cis*-jasmone (164.24 g/mol) and eucalyptol (154.25 g/mol), which favors the formation of several chemical bonds. The association of terpenes in different domains shows that small conformational nuances can be determinant in the interaction between ligand-protein, allowing smaller ligands to be able to: suffer less impediments, recruit a greater number of amino acids and consequently establish more chemical bonds (Figure 2).

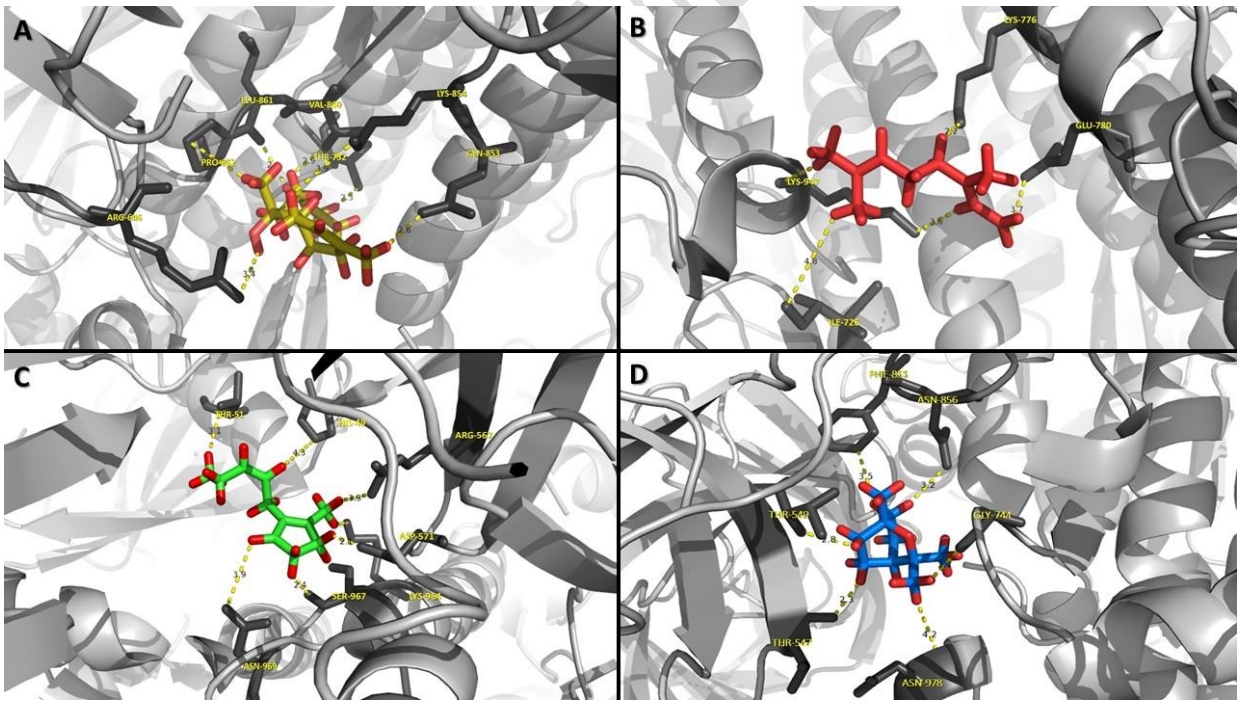

Figure 2: Complex (-)-α-bisabolol-SARS-CoV-2 S protein, in which 6 amino acid residues (Arg646, Thr782, Gln853, Lys854, Val860, Leu861, Pro862) were recruited, with chemical bonds of 2.6 to 3.9 angstroms (A) ; Citral-SARS-CoV-2 S protein complex, in which 4 amino acid residues (Ile726, Lys776, Glu780 and Lys947) were recruited, with chemical bonds of 2.2 to 4.8 angstroms (B); *cis*-jasmone-SARS-CoV-2 S protein complex, in which 7 amino acid residues were recruited (His49, Thr51, Arg567, Asp571, Lys964, Ser967 and Asn969), with chemical bonds of 2.1 to 4.3 angstroms (C); Eucalyptol-SARS-CoV-2 S protein complex, in which 6 amino acid residues (Thr547, Gly744, Thr549, Phe855, Asn856 and Asn978) were

recruited, with chemical bonds of 2.3 to 4.2 angstroms (D). Chemical interactions are indicated by yellow, dashed lines.

The complexation between (-)-α-bisabolol and SARS-CoV-2 S protein involved 6 amino acid residues (Arg646, Thr782, Gln853, Lys854, Val860, Leu861, Pro862) with chemical bonds of 2.6 to 3.9 angstroms. In the citral-SARS-CoV-2 S protein complex, 4 amino acid residues (Ile726, Lys776, Glu780 and Lys947) were recruited, with chemical bonds of 2.2 to 4.8 angstroms. *cis*-jasmone-SARS-CoV-2 S protein complex recruited 7 amino acid residues (His49, Thr51, Arg567, Asp571, Lys964, Ser967 and Asn969), with chemical bonds of 2.1 to 4.3 angstroms and eucalyptol-SARS-CoV-2 S protein complex recruited 6 amino acid residues (Thr547, Gly744, Thr549, Phe855, Asn856 and Asn978), with chemical bonds of 2.3 to 4.2 angstroms. None of the terpenes showed spatial compatibility with the main RBD (receptor binding domains) of the viral protein, which includes amino acid residues between 319-541 of the protein sequence (SIGRIST et al., 2020), but were compatible with other domains that are also important in viral activity.

The association of terpenes in each interaction site, when recruiting more than 4 amino acid residues, suggests that conformational stabilization (energy loss) in that region can compromises the 3D folding and the flexibility of the domain. It promotes efficiency in viral activity, which makes (-)-α-bisabolol (-212.53 kcal/mol) and citral (-178.47 kcal/mol) two chemical agents that can be associated with therapeutic strategies involving the SARS-CoV-2 S protein's ability to cleave proteases. The same stabilization makes *cis*-jasmone (-192.95 kcal/mol) and eucalyptol (-132.56 kcal/mol) possible interference agents in the three-dimensional ability to recognize receptors, by compromising more than 6

amino acid residues of the SARS-CoV-2 S protein. This structural change can be confirmed by looking at the complexing energy of each terpene when interacting with SARS-CoV-2 S protein (Table 1).

Table 1. Binding energies between terpenes and SARS-CoV-2 S Protein (Etotal – Kcal/mol).

Cluster of SARS-CoV-2 spike glycoprotein	**Etotal (Kcal/mol)**			
	(-)-α-bisabolol	**citral**	***cis*-jasmone**	**eucalyptol**
01	- 212.53	- 178.47	- 192.95	- 132.56
02	- 190.94	- 137.38	- 190.94	- 121.35
03	- 170.35	- 128.95	- 170.35	- 119.47
04	- 168.31	- 125.38	- 168.31	- 116.86
05	- 162.84	- 122.84	- 162.84	- 114.57
06	- 162.12	- 120.68	- 162.12	- 112.82
07	- 161.50	- 119.03	- 161.50	- 110.38
08	- 158.65	- 117.62	- 158.65	- 109.88
09	- 157.85	- 116.31	- 157.85	- 105.59
10	- 157.64	- 114.98	- 157.61	- 105.01

4. CONCLUSION

Collectively, the chemical, spatial, and energetic evidence derived from this study strongly suggests that the interaction of terpenes with the spike glycoprotein of the SARS-CoV-2 virus may significantly inhibit the virus's ability to enter host cells. These findings provide compelling support for the hypothesis that the four terpenes under investigation—α-bisabolol, citral, cis-jasmone, and eucalyptol—hold considerable promise as potential therapeutic agents. Given their capacity to form stable complexes with the spike protein, these compounds emerge as robust candidates for further investigation in both in vitro and in vivo experimental models prior to any clinical trials. This is particularly encouraging, as these natural substances are not only affordable and easily accessible, but they also offer a potential avenue for combating the ongoing global COVID-19 pandemic caused by the SARS-CoV-2 virus. In the face of this worldwide public health emergency, the low cost and widespread availability of these terpenes render them highly attractive options for the rapid development of antiviral interventions. Should subsequent studies confirm their efficacy, these terpenes could represent a valuable, scalable addition to the arsenal of tools needed to mitigate the impact of this acute viral disease.

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