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INTERACTION BETWEEN DIHYDROMYRICETIN AND SPIKE GLYCOPROTEIN SARS-COV-2 AND ITS DELTA AND OMICRON VARIANTS - A MOLECULAR DOCKING STUDY[1]

INTERAÇÃO ENTRE DIHIDROMIRICETINA E GLICOPROTEÍNA SPIKE DO SARS-COV-2 E SUAS VARIANTES DELTA E ÔMICRON - UM ESTUDO MOLECULAR

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ABSTRACT

The SARS-CoV-2 virus has been responsible for a global pandemic which caused millions of deaths. Still, novel antiviral agents have been investigated against SARS-CoV-2. In this view, molecular docking simulations could be used for evaluating possible antiviral agents to be tested in further experimental runs. Hence, the aim of this research was to evaluate the interaction of dihydromyricetin DHM against Spike glycoproteins of pristine SARS-CoV-2 and its Delta and Omicron variants through molecular docking simulations. To validate such results, the same docking protocol was applied to a control structure (galangin, a major component of propolis extract) which was already able to interact with spike glycoprotein of SARS-CoV-2 both *in silico* and *in vitro*. Regarding the interaction of DHM/Spike glycoprotein, DHM presented higher spontaneity in binding to Delta (ΔG = -8.9 kcal.mol^{-1}) and Omicron (ΔG = -7.4 kcal.mol^{-1}) variants than SARS-CoV-2 (ΔG = -5.7 kcal.mol^{-1}). Furthermore, DHM/spike glycoprotein interactions for Delta and Omicron variants presented higher similarity to galangin/spike glycoprotein interaction (based on the aminoacid residues involved with the interactions with the polar/non-polar contacts), indicating that DHM and galangin could bind similarly to SARS-CoV-2 variants in the *in vitro* standpoint. Thus, it is suggested that DHM may be tested as a potential antiviral agent against SARS-CoV-2 through experimental runs.

Keywords: flavonoids, *in silico*, plant extract, nanoscience, COVID-19.

RESUMO

O vírus SARS-CoV-2 foi responsável por uma pandemia global que causou milhões de mortes. Ainda assim, novos agentes antivirais foram investigados contra o SARS-CoV-2. Diante disso, simulações de docking molecular podem ser utilizadas para avaliar possíveis agentes antivirais a serem testados em outros experimentos. Portanto, o objetivo desta pesquisa foi avaliar a interação da dihidromiricetina (DHM) contra as glicoproteínas Spike do SARS-CoV-2 primitivos e suas variantes Delta e Ômicron por meio de simulações de docking molecular. Para validar tais resultados, o mesmo protocolo de docking foi aplicado a uma estrutura controle (galangina, um dos principais componentes do extrato de própolis) que já era capaz de interagir com a glicoproteína Spike do SARS-CoV-2 tanto in silico quanto in vitro. Em relação à interação da glicoproteína DHM/Spike, o DHM apresentou maior espontaneidade na ligação às variantes Delta (ΔG = -8,9 kcal.mol^{-1})

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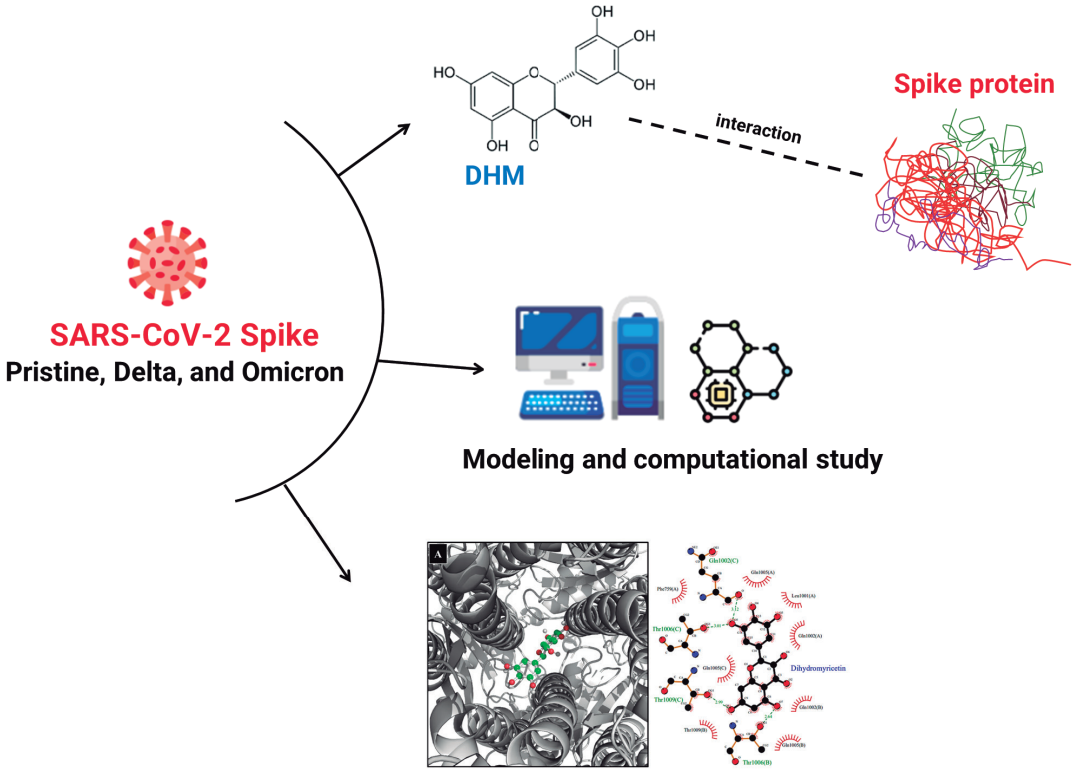
e Ômicron (ΔG = -7,4 kcal.mol^{-1}) do que o SARS-CoV-2 (ΔG = -5,7 kcal.mol^{-1}). Além disso, as interações DHM/glicoproteína Spike para variantes Delta e Ômicron apresentaram maior semelhança com a interação de glicoproteína Spike/galangina (com base nos resíduos de aminoácidos envolvidos na alterações com os contatos polares/não polares), indicando que DHM e galangina podem se ligar de forma semelhante ao SARS-CoV- 2 variantes do ponto de vista in vitro. Assim, sugere-se que a DHM possa ser testada como um potencial agente antiviral contra o SARS-CoV-2 por meio de execuções experimentais.
Palavras-chave: *flavonoides, in silico, extrato vegetal, nanociência, COVID-19.*

GRAPHICAL ABSTRACT



INTRODUCTION

The contamination for the novel COVID-19 generated a serious pandemic, which was responsible for millions of deaths worldwide between December 2019 and August 2020 (CHAN *et al.*, 2020; HUANG *et al.*, 2020). The SARS-CoV-2 virus belongs to the *Coronavidae* family, which are a group of viruses of single-strand positive-sense RNA, responsible for causing severe respiratory syndrome, being also capable of eliciting neurological, hepatic, and enteric injuries in human beings (CHAN *et al.*, 2013; HOLMES, 2003; ZUMLA *et al.*, 2016).

Structurally, the SARS-CoV-2 genome is enclosed by a capsid, which is composed of a nucleocapsid protein N that is surrounded by other three proteins: (*i*) the membrane protein, (*ii*) the envelope protein, and (*iii*) the spike glycoprotein (MARIANO *et al.*, 2020). Among these proteins,

spike glycoprotein plays a great role on hosting SARS-CoV-2 and host receptor binding mechanism (CAI *et al.*, 2020). It is worthy to mention that the nucleocapsid is embedded in a phospholipid bilayer and it is also surrounded by two kinds of spike glycoproteins: (*i*) one that works as an anchoring site, presented in other "CoV"s and (*ii*) as hemagglutinin-esterase, another type that is presented in only a few of "CoV"s (XIA *et al.*, 2020, ZHU *et al.*, 2021). Moreover, literature has been showing that the Spike glycoprotein is of great relevance to the receptors binding, being considered a key in demarcating host tropism and to transmission of SARS-CoV-2 (XIA *et al.*, 2020). Also, the way SARS-CoV-2 is harmful to the human body depends on the binding between the viral peak of spike glycoprotein and the receptors of angiotensin converting enzyme 2 (ACE2) receptors, in which the cleavage of ACE2 receptor is needed for the cell input by a type 2 transmembrane serine protease to activate the spike glycoprotein (LEUNG, 2021).

At the end of 2020, the rapid development of SARS-CoV-2 was accomplished with the introduction of some variants, classified as "variants of concern" (VOC) by the World Health Organization (WHO), as they can modify viral properties of SARS-CoV-2 due to the change on the human immunological composition ("Evaluation of the Effect of D614g, N501y and S477n Mutation in Sars-Cov-2 through Computational Approach | EuropePMC; 2020. | EuropePMC", [S.d.]). Furthermore, the VOC concept used by WHO refers to SARS-CoV-2 variants with mutations in their spike protein receptor binding domain (RBD), what enhances the RBD-hACE2 binding affinity and causes a fast virus dissemination (KUMAR *et al.*, 2022). Said that, the higher the virus replication, higher is the probability of formation of SARS-CoV-2 mutations (DUDAS *et al.*, 2021).

The Delta variant (B.1.617.2), which was discovered at the end of 2020 in India, was present in more than 163 territories until August 2021, what led WHO to declare it the most prevalent strain around the world and which transmission rate is 40-60% higher than Alpha variant (B.1.1.7), with a higher risk of hospitalization, being dangerous to people that did not completed vaccination (BIAN *et al.*, 2021, MAHASE, 2021). Likewise, the Omicron variant (B.1.1.529), which was discovered in samples collected at Botswana and South Africa on November 2021, was also associated with high transmission rates, with increased reinfection cases and possible inefficiency of vaccination, what turned it more transmissible and serious than Delta variant (KUMAR *et al.*, 2022).

Based on an overview of SARS-CoV-2 virology and its variants, some basis could be established for prevention and specific treatments of COVID-19 disease (ZHU *et al.*, 2021). Nowadays, some commercial drugs possess known pharmaco-kinetical properties as well as pharmaco-dynamic characteristics, however, they do not have specific anti-SARS-CoV-2 effect and might be associated to diverse serious side-effects (CINATL *et al.*, 2003).

The use of natural substances has been often explored as an alternative therapy to both prevent or reduce some viral replications (XIAO *et al.*, 2021). Dihydromyricetin (DHM) is a flavonoid found on *Hovenia dulcis* (*Rhamnaceae*) leaves, which is commonly known as Japanese grape originated

from east Asia (Japan, Korea and China mainly) (YANG *et al.*, 2019). The interest of scientific Community on DHM relies on its wide bioactive properties, which includes potential antioxidant, antiviral and anti-inflammatory activity (SOLNIER, FLADERER, 2021).

In silico investigations are insightful approaches that convey to the 3R principle, that is, replacement, reduction, and refinement (JEAN-QUARTIER *et al.*, 2018). Molecular docking is an *in silico* tool that have been using in virtual screening and the proposal of novel drugs, such as antiviral agents (FADLALLA *et al.*, 2022; PINZI, RASTELLI, 2019. Basically, molecular docking simulations focus on studying possible interactions between large receptors (e.g., macromolecules such as proteins) and small chemical ligands (PRIETO-MARTÍNEZ *et al.*, 2018).

Molecular docking studies involving SARS-CoV-2 have been investigated in the search of possible antiviral agents. These studies aimed at interacting antiviral candidates with spike glycoprotein or nucleoprotein of SARS-CoV-2. Regarding spike glycoprotein, interaction with natural substances such as key components of propolis (GÜLER *et al.*, 2021; HARISNA *et al.*, 2021) and curcumin (NOGUEIRA *et al.*, 2021; SURAVAJHALA, *et al.*, 2021) were already investigated through molecular docking.

In fact, DHM was already evaluated against Mpro (a main protease of SARS-CoV-2) by molecular docking in the study of Xiao and contributors (XIAO *et al.*, 2021)as a flavonol, also has antiviral and anti-inflammatory potential. However, the inhibition of dihydromyricetin on SARS-CoV-2 Mpro and the protective effect of dihydromyricetin on pulmonary inflammation and fibrosis have not been proved and explained. Purpose: The coronavirus main protease (Mpro. The authors aimed at attenuating pulmonary inflammation/fibrosis by evaluating the possible inhibition of SARS-CoV Mpro and DHM, which was confirmed by molecular docking and *in vitro* methods.

In brief, DHM was already investigate for tyrosinase inhibition (CHEN *et al.*, 2018), and for diabetic neuropathic pain relieving agent (GUAN *et al.*, 2019), by using molecular docking. Still and to the best of our knowledge, the interaction of DHM against SARS-CoV-2 spike glycoprotein and its Delta and Omicron variants was not evaluated from the molecular docking viewpoint. Hence, the aim of this research was investigating the interaction between DMH and spike proteins of pristine and variant forms (Omicron and Delta) of SARS-CoV-2 through molecular docking simulations, focusing on antiviral properties of DHM.

METHODS

The interaction between DHM and SARS-CoV-2 spike proteins was carried out by molecular docking, performed in Auto Dock Vina. The *docking* protocol proposed by Forli and colleagues (FORLI, HUEY, *et al.*, 2016) was used with adaptations. Such adaptions were using the *eBoxSize* script to calculate the grid box dimensions according to the ligand size, as it enhances docking

simulations (FEINSTEIN, BRYLINSKI, 2015, LOPREIATO *et al.*, 2021). The following SARS-Cov-2 spike glycoproteins were obtained from Protein Data Bank (PDB): 6VYB (pristine form), 7W92 (Delta variant), and 7TGW (Omicron variant) (WALLS *et al.*, 2020; WANG *et al.*, 2022; YE *et al.*, 2022). The dihydromyricetin ($C_{15}H_{12}O_8$) ligand was obtained from PubChem database (CID: 161557). The tridimensional and bidimensional visualization of docking interactions were produced through PyMol and LigPlus, respectively. The binding affinity was approximated by the free energy of binding (FEB, expressed in kcal.mol^{-1}) according to Equation 1, in which more negative values indicate higher spontaneity of receptor-ligand binding.

$$\Delta G \approx FEB = \Delta G_{vdW} + \Delta G_H + \Delta G_e + \Delta G_{int} \quad (1)$$

Where:

ΔG: receptor-ligand complex docking binding affinity.

ΔG_{vdW}: chemical potentials related to Van der Walls interactions.

ΔG_H: chemical potentials related to hydrogen bonding.

ΔG_e: chemical potentials related to electrostatic interactions.

ΔG_{int}: chemical potentials related to intramolecular interactions.

The Root Mean Squared Deviation (RMSD), an important docking metric, was evaluated together with the binding affinity to analyze the results. The RMSD is given by Equation 2. Also, it were considered as favorable *docking* poses the runs in which RMSD < 2.0 Å and RMSD ≠ 0 Å, serving as a validating approach (SCHWEIKER, LEVONIS, 2020, TROTT, OLSON, 2009).

$$RMSD(pose_{ilig}, pose_{irec}) = \sqrt{\frac{\sum_n (atom_{(lig)} - atom_{irec})^2}{n}} \quad (2)$$

Where:

$atom_{(ilig)}$: mean atomic position of ligand.

$atom(_{irec})$: mean atomic position of receptor.

n: mean atomic position of the system.

RESULTS AND DISCUSSION

Table 1 shows the results for the best poses of molecular docking involving DHM and spike protein of pristine SARS-CoV-2 and its Delta and Omicron variants. As observed, DHM presented lower values for ΔG when interacting with spike glycoproteins of Delta and Omicron variants in comparison with pristine of SARS-CoV-2. That, suggest the interaction of DHM and spike proteins might occur more spontaneously for Delta and Omicron variants. Noteworthy, all docking complexes interacted with spike proteins by forming both polar and non-polar contacts with aminoacid residues

of each receptor. It is worthy pointing out that the interaction of DHM and the pristine SARS-CoV-2 spike was still interesting, as there were 9 contacts formed with aminoacid residues of the receptor, in which three were polar while 6 were non-polar in nature.

It is important to note that such docking simulation results were validated through a control structure before further analysis. It is known that phytochemicals and plant extracts were already proposed (either *in silico* or *in vitro*) in literature as potential antiviral agents against SARS-CoV-2 (BASU *et al.*, 2020), in which standardized propolis ethanolic extracts have displayed ability to inhibit SARS-CoV-2 proliferation (SBERNA *et al.*, 2022). Such potential antiviral activity might be due to the phenolic content of propolis extracts, which are manly composed by galangin, a polyphenol that had already demonstrated binding affinity to SARS-CoV-2 spike glycoprotein through molecular docking (GÜLER *et al.*, 2021, SBERNA *et al.*, 2022). In view of that, the molecular docking protocol was applied to galangin (obtained from PubChem, CID 5281616) to validate the obtained results for DHM. Such validation results are shown in Table 2. It is important to note that such docking simulation results were validated through a control structure (galangin, CID 5281616) before further analysis.

Table 1 - Best poses obtained through molecular docking of dihydromyricetin against SARS-CoV-2 spike proteins.

Protein	ΔG (kcal.mol^{-1})	Contact	Aminoacid residues
Pristine SARS-CoV-2 Spike	-5.7	Polar	Gln1010, Thr1006, Thr1006
		Apolar	Gln1005, Gln1010, Ile1013, Leu763, Leu1012, Thr1009
SARS-CoV-2 Delta Spike	-8.9	Polar	Gln1002, Thr1006, Thr1006, Thr1009
		Apolar	Gln1002, Gln1005, Leu1001, Phe759, Thr1009
SARS-CoV-2 Omicron Spike	-7.4	Polar	Thr995
		Apolar	Gln999, Gly996, Leu998, Phe756, Thr995, Tyr753

Table 2 - Validation of molecular docking.

Protein	ΔG (kcal.mol^{-1})	Contact	Aminoacid residues	Residues involved in the interaction with DMH	% Interactions in common*
Pristine SARS-CoV-2 Spike	-7.6	Polar	Gln1002, Thr1006, Thr1009	Thr1006	56%
		Apolar	Gln1002, Gln1005, Gln1010, Leu763, Thr1006, Thr1009	Gln1005, Gln1010, Leu763, Thr1009	
SARS-CoV-2 Delta Spike	-8.7	Polar	Thr1006, Thr1009	Thr1009	71%
		Apolar	Gln1002, Gln1005, Leu1001, Thr1006, Thr1009	Gln1002, Gln1005, Leu1001, Thr1009	
SARS-CoV-2 Omicron Spike	-6.9	Polar	Thr995	Thr995	80%
		Apolar	Gln999, Leu998, Phe756, Thr995	Gln999, Leu998, Thr995	

*Galangin versus DHM (considering the amount of aminoacid residues with similar contact nature).

According to the results of Table 1 and Table 2, the ΔG value for DHM/pristine spike glycoprotein complex was lower than that of galangin/pristine spike glycoprotein complex, indicating that DHM has lower spontaneity on binding the spike glycoprotein of pristine SARS-CoV-2. Also, the aminoacid residues involved in the interaction of galangin/spike protein complex were all present in DHM/spike protein complex, but there were differences regarding the contact nature. These differences play a great role in the ΔG value, as docking binding affinity is expressed as a combination of polar and non-polar contacts, as seen in Equation 1. Despite, ΔG value for DMH/spike glycoprotein interaction was found similar to other compounds with potential antiviral properties against other viruses (GÜLER, AY ŞAL, *et al.*, 2021, HARISNA, NURDIANSYAH, *et al.*, 2021, NOGUEIRA, VERZA, *et al.*, 2021, SURAVAJHALA, PARASHAR, *et al.*, 2021). On the other hand, the higher ΔG values for the interaction of DHM against the spike glycoproteins of Delta and Omicron variants of SARS-CoV-2 indicated that DHM might be a promising antiviral agent against SARS-CoV-2 proliferation. Such finding is corroborated by the percentage of common interactions between the ligand and aminoacid residues located at the target site of the proteins, which was greater for variant spike glycoproteins. Furthermore, such percentage seemed to be directly proportional to the ΔG value, which may indicate that DHM interacts similar to galangin against the spike glycoproteins of Delta and Omicron variant of SARS-CoV-2.

Table 3 shows the descriptive statistics for the aminoacid residues involved in molecular docking interactions. In all cases, there were a great number of aminoacid residues involved in the interactions of spike glycoproteins and DHM. Moreover, the mode (most frequently interacting aminoacid residue) changed according to the variant of spike glycoprotein. That result illustrates that each variant of SARS-CoV-2 interacts quite different against DMH.

Table 3 - Descriptive statistics for the aminoacid residues involved in molecular docking.

Protein	Total number of residues*	Unique residues	Mode	Frequency
Pristine SARS-CoV-2 Spike	29	9	Gln1005	6
SARS-CoV-2 Delta Spike	26	6	Gln1002	5
SARS-CoV-2 Omicron Spike	20	7	Thr995	6

**Based on the raw data by considering at least 3 favorable docking runs (N=3)*

Figure 1 shows DMH interacting with SARS-CoV-2 Spike glycoprotein in qualitative way. It was noted that polar contacts presented a bond length ranging from 2.8-3.2 Å, what fallen in the range of intermolecular interactions. Likewise, Figure 2 shows the interactions of DHM against Delta SARS-CoV-2 Spike glycoprotein and Omicron SARS-CoV-2 Spike glycoprotein. It was observed that the polar contacts in DHM/Delta SARS-CoV-2 Spike glycoprotein complex ranged from 2.6-3.1 Å. In the case of DHM/Delta SARS-CoV-2 Spike glycoprotein complex, it displayed a single polar contact with bond length of 2.7 Å. Therefore, these results proved that DHM can be promising

substance with antiviral potential, once can interact with the pristine spike glycoprotein complex of the pristine SARS-CoV-2 and its variants Delta and Omega. Moreover, these finding corroborated the studies developed by of Xiao et al. (), in the antioxidant activity of DHM is also evaluated by molecular docking.

Figure 1 - SARS-CoV-2 Spike glycoprotein/DHM complexes. Left: 3D visualization. Right: 2D visualization.

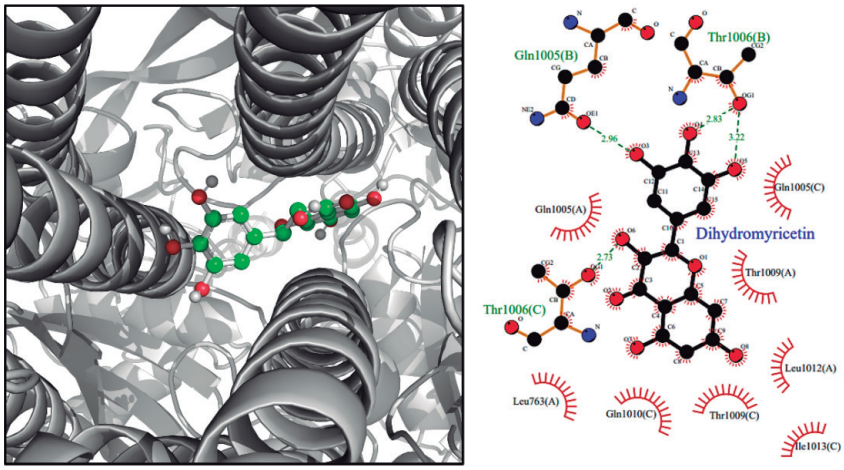
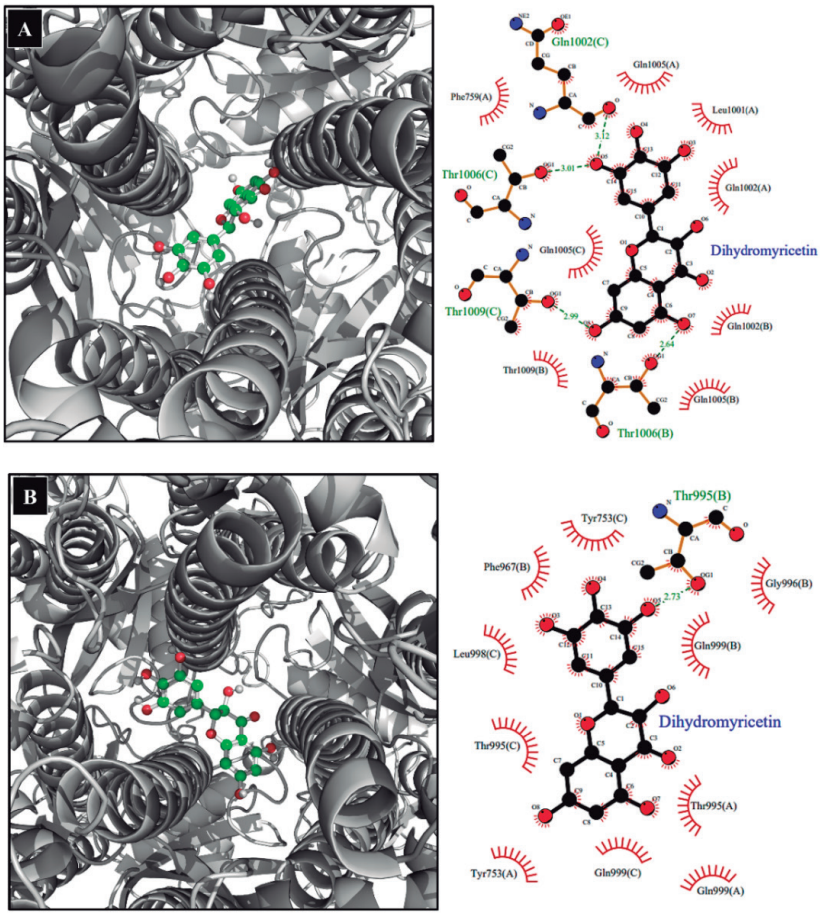


Figure 2 - SARS-CoV-2 Spike glycoprotein/DHM complexes:
(A) Delta variant. **(B)** Omicron variant. Left: 3D visualization. Right: 2D visualization.



CONCLUSIONS

The interaction of DHM against spike glycoprotein of pristine SARS-CoV-2 and its Delta and Omicron variants was investigated in this research. Such interactions were carried out and analyzed by molecular docking simulations. The molecular docking results were validated against a control structure, the galangin (a key component of standardized ethanolic extracts of propolis), which was able to inhibit *in vitro* proliferation of SARS-CoV-2 in previous studies in literature. In brief, DHM interacted with SARS-Cov-2 spike glycoproteins by forming both polar and non-polar contacts at the target site of such proteins. Moreover, DHM interacted in a similar way to the control against spike glycoproteins of Delta and Omicron variants, with slightly high ΔG values found for DHM. Based on docking simulation results and the way DHM interacted with the receptors (in comparison to the control), it is inferred that DHM could be evaluated as a potential antiviral agent in future experimental studies.

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