# Análisis *in silico* de la inmunogenicidad e interacción molecular de péptidos de plantas aromáticas con SARS-CoV-2

## *In silico* analysis of immunogenicity and molecular interaction of aromatic plant peptides with SARS-CoV-2

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Recibido: Septiembre 15 de 2022
Aceptado: Noviembre 20 de 2022
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**https://doi.org/10.47499/revistaaccb.v1i34.264**

**Resumen**

SARS-CoV-2 es un coronavirus de ARN que causa infecciones respiratorias como la actual pandemia de COVID-19. Los sistemas de salud combaten esta infección con cuidados paliativos; sin embargo, existen pocos tratamientos específicos para este patógeno. Este contexto representa la posibilidad de buscar tratamientos alternativos, como el uso de moléculas naturales. El objetivo de este estudio fue determinar *in silico* la interacción de péptidos de plantas aromáticas con proteínas específicas de SARS-CoV-2 que no comprometan la respuesta inmune. Se procesaron quinientos ochenta y tres péptidos con menos de 30 aminoácidos de *Thymus vulgaris L*., *Cymbopogon citratus*, *Salvia officinalis*, *Ocimum basilicum L* y *Zingiber officinale*. La metodología aplicó filtros de acuerdo a los más altos puntajes de docking molecular para encontrar 20 péptidos por cada planta. Los péptidos registraron interacción molecular fuerte de los sitios activos de las proteínas Spike RBD, S2 y Nsp4, empleando una energía de menos de –150 kcal/mol. La proteína Nsp4 mostró la mayor interacción con todas las especies. El 35% y el 65% de estos péptidos se registraron con baja activación de la respuesta inmune a través de la antigenicidad, puntuación inferior a 0,5 y ausencia de alergenicidad. Estos resultados indican el uso de moléculas de origen vegetal que pueden implementarse en el consumo para combatir la replicación viral del SARS-CoV-2.

**Palabras clave:** plantas aromáticas, péptidos, SARS-CoV-2, interacción molecular.

**Abstract**

SARS-CoV-2 is an RNA coronavirus that causes respiratory infections as the current COVID-19 pandemic. The health systems combat this infection with palliative care; however, there are few specific treatments for this pathogen. This context represents the possibility of searching for alternative treatments, such as using molecules from natural products. Our main objective was the *in silico* study of aromatic plant peptides and their interaction with specific proteins of SARS-CoV-2 that do not compromise the immune response. Five hundred eighty-three peptides with less than 30 amino acids from *Thymus vulgaris L., Cymbopogon citratus, Salvia officinalis, Ocimum basilicum L*, and *Zingiber officinale* were processed. The methodology applied filters according to the highest molecular docking scores to find 20 peptides for each plant species. The peptides show solid molecular interaction of the Spike RBD, S2, and Nsp4 proteins' active sites, using less than –150 kcal/mol energy. Nsp4 protein exposes the most interaction with all species. 35 and 65% of these peptides were recorded with low activation of the immune response through antigenicity, score below 0.5, and absence of allergenicity. These results indicate the use of plant-derived molecules that can be implemented in consumption to combat the viral replication of SARS-CoV-2.

**Keywords:** Aromatic plants, peptides, SARS-CoV-2, molecular docking.

## Introduction.

SARS-CoV-2 is an RNA coronavirus that deteriorates respiratory conditions observed during the current COVID-19 pandemic, which has claimed nearly 4.55 million deaths globally between 2020 and 2021 (1,2). The course of the infection causes inflammation response deregulation derived in chronic conditions (1), showing a sanitary emergency that requires rapid intervention. In some cases, the conventional drugs for intervention in this pathology are associated with side effects such as immune system activation and the presence of refractory patients; at the same time, SARS-CoV-2 generates pharmacological resistance (3). On the other hand, using the vaccine strategy is a preventive approach with side-effects reports and variation in availability due to government investment (4,5). This scenery makes it necessary to search for new alternative treatments that affect the viral life cycle without generating an effect on the host's immune response and that it is easily acquired.

Phytomedicines represent an alternative that uses plants and their extracts to combat pathogens (6). Among these, aromatic plants attract attention due to the bioactivity reports and their conventional use for several diseases (7,8). Plants such as ginger (*Zingiber officinale*), basil (*Ocimum basilicum* L), sage (*Salvia officinalis*), lemongrass (*Cymbopogon citratus*), and thyme (*Thymus vulgaris* L.) have been reported with antimicrobial and antiviral activity (9,10). In addition, some natural molecules, such as peptides of aromatic plants, have shown activity against other viruses such as HIV, Herpes virus, and dengue virus, among others (11). The peptide-based drug, typically of 2-50 amino acid residues, has several underlying biological characteristics such as better bioavailability, exuberant biological affinity or specificity to a particular target, and low toxicity represented by low immunogenic responses (12,13), highlighting them above a conventional drug. For the case of ginger, the approach *in silico* assays has shown compounds that interfere with the viability of SARS-CoV-2 (14,15). However, the interaction between natural peptides and components of the host's immune system remained to be determined.

*In silico* analysis of molecules allows for characterizing their structures with robustness systems and studying their interactions with proteins of interest of pathogens (16). Although there is a low percentage of characterized peptides from aromatic plants in this scenery, the main of this research was to evaluate with advanced bioinformatics tools the anti-SARS-CoV-2 activity and immune reactivity of small peptides from Ginger, basil, sage, lemongrass, and thyme. Furthermore, this study

allows us to define new ways to analyze combat alternatives for COVID-19.

**Materials and methods.**

**Identification of peptides in plants.**

A descriptive and systematic review was carried out to identify peptides with antiviral activity reports, using a range of 2 years (2019-2021) as a selection filter. Additionally, the peptide sequences were verified with the Cybase platform (http://www.cybase.org.au/index.php, 17), which collects cyclic-type peptides with antiviral and antimicrobial activity. Small peptides (less than 30 amino acids) sequences were taken to be used as query sequences in the protein-protein BlastP (https://blast.ncbi.nlm.nih.gov/Blast.cgi) from the NCBI (National Center Biotechnology.). The alignments of these sequences with the organisms in the standard section: *Z. officinale* (Taxid:94328), *O. basilicum* (Taxid:39350), *S. officinalis* (Taxid:38868), *C. citratus* (Taxid:66014), and *T. vulgaris* (Taxid:49992) were executed. In BlastP, the peptides predicted and, with a report in proteins, were selected using the best percentage of identity.

**3D structure and anti-viral activity by docking molecular.**

The 3D structures to evaluate beta sheets or alpha chains were executed using the RPBS Web Portal PEP-FOLD 3.5 (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms:PEP-FOLD3, 18), using 100 simulations as the default value. The molecular docking allows for studying the Gibbs energy in the interaction. For this purpose, the nCOV Docking server (https://ncov.schanglab.org.cn/, 19) was used to select the Spike RBD domain and S2 subunit, and Nsp4 proteins as target proteins. We use the filter Gibbs energy >150 kcal/mol to select 20 peptides per plant, prioritizing the Spike RBD protein. Finally, molecular coupling was verified with the DINC-COVID Web Server platform (http://dinc-covid.kavrakilab.org/, 20). The ligand efficiency was calculated with docking molecular score / molecular mass.

**Physicochemical characterization and immune response of peptides.**

The physicochemical properties were identified using the bioinformatics tool ProtParam Tool (https://web.expasy.org/protparam/) from Expasy to determine the molecular weight, size, isoelectric potential, amino acid composition, atomic composition, half-life, instability index, allopathic index, and hydropathicity. Peptide solubility was determined using the SCRATCH Protein Predictor (http://scratch.proteomics.ics.uci.edu/) with the SOLpro function. Immune response was evaluated at two levels: Antigenicity with the ANTI GENpro function of SCRATCH Protein Predictor and allergenicity prediction using AllerTOP V.2.0 (https://ddg-pharmfac.net/AllergenFP/).

***Results.***

**Search and selection of peptides from plants.**

SARS-CoV-2 infection has developed with different epidemiological dynamics since the outbreak of the COVID-19 pandemic. The transmission of the infection has been affected by government control systems that incorporate vaccination status and self-care prevention measures in developing countries (4). In Colombia, four contagion peaks were detected between 2020 and 2022 (Fig. 1A). This scenario was the starting point for the search for pathological combat alternatives that deal with the biology of the pathogen. In this study, the molecular interaction between virus proteins with molecules of natural origin was sought, looking for the inactivation of the viral pathological synapse with the cells of the human system (21). We focus on the S2, Spike, and Nsp4 proteins found in the capsid's external structure (Fig. 1B) and facilitate interaction with angiotensin receptors. Initially, a search of peptide sequences in 5 aromatic plants, *Z. officinale*, *O. basilicum, S. officinalis, C. citratus* and *T. vulgaris* was carried out using 19 scientific articles. These sequences had antimicrobial activity reported in research and verified in Cybase. Using the NCBI online alignment tool (FIG. 1C), 583 peptides were found (22-38), with more than 80 sequences with a predicted structure for each plant (FIG. 1D) between 5 and 30 amino acids in length. The peptides were then characterized by their three-dimensional structure using homology modeling following the greedy algorithm (39) (Fig. 1C). This was to establish a molecular binding with viral proteins in the ncov web (40). The results show different configurations of the peptides found with beta sheets, alpha helices, and linear configurations, indicating folding possibilities that increase the molecular interaction capacity (41) (Fig. 1E).

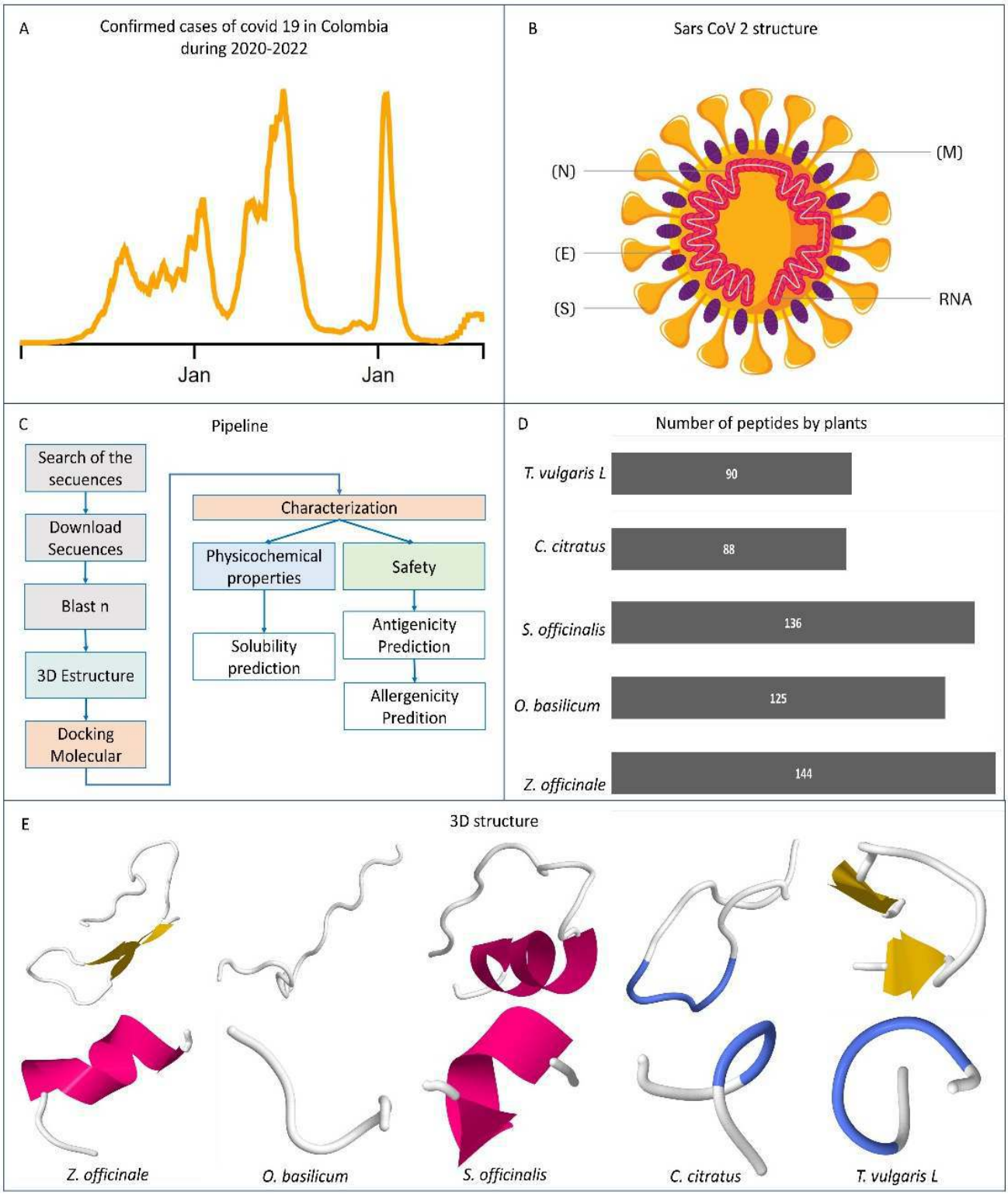


**Figure 1. Infection and case of SARS-CoV-2 and selection of peptides**. A. Number of cases of COVID-19 infection in Colombia reported by endcoronavirus.org/. B. Viral structure of SARS-CoV-2 that shows the antigenic proteins of interest. C. Workflow of this study to search and characterization of bioactivity. D. Number of peptides by the plant. E. Representative structure of peptides by the plant.

The molecular binding test revealed Gibbs energy values in a range between 0 and -350 (Supplementary 1). The RBD domain extra-capsid of Spike protein SARS-CoV-2 report six aa (L455, F486, Q493, S494, N501, and Y505) of solid interaction with ACE receptor in mammal cells. Using the Shan Chang lab software algorithm, our results show a firm binding with these amino acids; for example, peptide # 3 of *Z. officinale*, supports the possible interference of viral entry to host cells (42). Spike protein also presents other subunits to interact with ACE2 receptor, such as S2 subunit or fusion fragment, which after proteolytic cleavage S1-S2 induces the viral and host cell plasma membrane fusion. Our results show interaction with the connector domain (CD, residues 1037–1068) of S2, revealing alteration of protein fusion (42).

On the other hand, Nsp4 protein's role in viral replication report four predicted transmembrane domains. The peptides show strong interaction with cleavage sites to produce Nsp4-Nsp16, which could affect viral replication (43). Sequences with a binding score greater than -150 were discarded because their interaction was classified as weak. Twenty peptides per plant were selected for presenting more significant interaction. As shown by the heat maps in figure 2A, the Nsp4 protein is the one that interacts the most with most of the peptides of all plant species. Likewise, the peptides of the *Z. officinale* species are the ones that generate the most molecular union with all the proteins. For its part, the S2 protein reports less interaction with most peptides. The three-dimensional structure of the molecular docking indicates that the peptides have an affinity for the active sites of the target proteins (Fig. 2B, C, and D). These data were confirmed with the DINC-COVID Web server, where the correspondence between values was detected. This result suggests that viral replication processes could interfere. Physicochemical characterization of these molecules showed a solubility score between 0.5 to 0.9, a molecular weight of 570 to 2000 kDa, and as we expected, the half-life shows a range of 1 to 100 h in the mammal systems (Supplementary 2), according to high bioavailability reported to this molecules type (12,13). The highest half-life values that indicate the degradation time in the mammalian system have low interaction with the Nsp4 protein. However, some small peptides, such as #4 from *O. basilicum* have a half-life of more than 20 hours in mammalian cells, indicating an adequate period of bioactivity that could combat the virus. Next, the antigenicity and allergenicity of the peptides were evaluated using the database comparison algorithm by Antigenicity with ANTI GENpro function in SCRATCH Protein Predictor and AllerTOP V.2.0 (44,45). For the plant species *Z. officinale*, *O. basilicum, S. officinalis, C. citratus*, and *T. vulgaris*, 14, 14, 13, 12, and 11 peptides with antigenicity less than 0.5 were found, respectively; of these sequences 10, 7, 5, 5, and 5 peptides are non-allergenic, respectively. These results show no predicted side effects for the peptides found.

Overall these results, and in contrast to those reported in the literature, show more potent binding energies with the viral target proteins studied in this study; for example, Wong et al. found the peptide EDKGMMHQQRMMEKAMNIPRMCGTMQRKCRMS binds to the RBD domain of the Spike protein with an energy -207.146 kcal/mol, which is relatively lower than our finding of the peptide IPCEDYVLACVFI that binds to the RBD domain of the Spike protein with the energy of -286.64 kcal/mol (46). Concerning the antigenicity and allergenicity scores, the possible secondary effects reported for some extracts of the before-mentioned plants are explained (47). In line with this finding, for future *in vitro* preclinical research experiments, purification of peptide extracts are necessary as has been carried out by (8) or through molecular techniques such as western block to isolate specific molecules.

***Discussion.***

The outbreak of COVID-19 was declared a pandemic of international concern from 2019 to date (48). Current research has focused its efforts on combating SARS-CoV-2 in two ways: 1) prevention of contact with biosecurity standards or vaccines application in the community (5, 49), and 2) in the case of patients, the use of palliative care and antiviral treatments (50, 51). Some antiviral drugs, such as hydroxychloroquine and chloroquine have been examined potentially to suppress SARS-CoV-2 replication (52). However, this fact represents difficulty in accessing the distribution and application of the active principle of drug cause due to the little government investment to include in the anti-SARS-CoV-2 public health plan, a context more recurrent in LATAM countries such as Colombia (53). According to this, new strategies more cost-effectively have been explored (54). Natural products of plant origin, as secondary metabolites and their derivatives, reported great chemical diversity and many therapeutic applications as antiviral potential (55). Therefore, these molecules as peptides from plants have been a trending topic in bioprospecting research. This point is confirmed by the anti-SARS-CoV-2 publications increasing

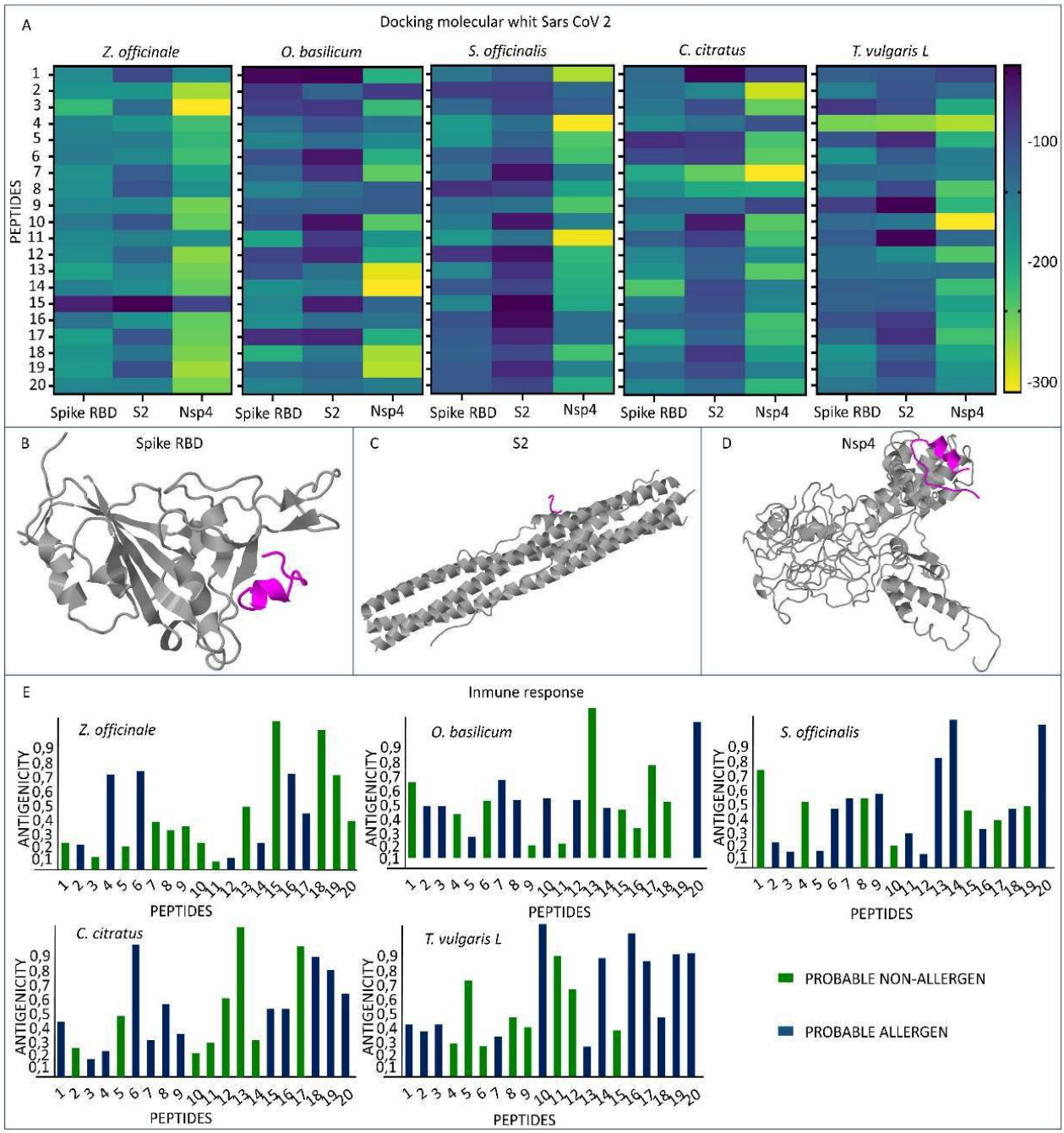


**Figure 2**. **Molecular interaction level and immune response**. A. Heat maps that show the Gibbs energy score result of docking molecular between 20 peptides by plant and Spike RBD domain and S2 subunit, and Nsp4 protein of SARS-CoV-2. Representative structure of docking molecular with Spike RBD (B), S2 (C), and Nsp4 (D). E. Score of antigenicity and allergenicity of peptides by the plant.

during 2020 and 2021 (49).

The speciation of aromatic plants against predators such as insects has generated a highly active secondary metabolism with many peptides (57). This study found highly favorable results for some peptides from the five selected aromatic plants. The domains of SARS-CoV-2 proteins were selected due to their positive antecedents with the peptide-based inhibitor as a first-in-class treatment for COVID-19. We generally perceive higher binding with the Nsp4 protein, medium binding with the RBD protein, and low binding with the S2 protein. This similar trend in the results binding between plants selected with the three SARS-CoV-2 domains can be explained by an association between aromatic plant synthesis pathways that have been evolutionarily conserved (57). The peptides that yielded an excellent blast match in NCBI, mainly denoted a report by predictive algorithm, and a few matched with reported proteins, which made it impossible to use control sequences in molecular docking and antigenicity experiments. Considering the different molecular masses of peptides in the range of 525.69 and 2978.4 kDa (Supplementary 2), we evaluated the ligand efficiency of all peptides with the first model of docking molecular that show higher binding, Nsp4. The heat map in table 1 shows that small peptides have more ligand efficiency; for example, peptide # 1 of *C. citratus with* five amino acids results in -0.56 score of ligand efficiency; this is probably due to more possibility of chemical interaction with exposed radical groups. Mostly, this analysis exhibit *S. officinalis* with more ligand efficiency than other plants, according to the reports of its antiviral activity (58).

**Table 1**. Ligand efficiency between peptides and Nsp4.

| # Peptide | *Z. officinale* | *O. basilicum* | *C. citratus* | *S. officinalis* | *T. vulgaris* |
|---|---|---|---|---|---|
| 1 | -0,18 | -0,17 | -0,56 | -0,53 | -0,38 |
| 2 | -0,21 | -0,47 | -0,13 | -0,34 | -0,42 |
| 3 | -0,25 | -0,17 | -0,26 | -0,16 | -0,22 |
| 4 | -0,13 | -0,40 | -0,31 | -0,45 | -0,31 |
| 5 | -0,19 | -0,44 | -0,29 | -0,28 | -0,17 |
| 6 | -0,20 | -0,25 | -0,15 | -0,22 | -0,36 |
| 7 | -0,14 | -0,15 | -0,22 | -0,22 | -0,22 |
| 8 | -0,16 | -0,34 | -0,14 | -0,15 | -0,27 |
| 9 | -0,11 | -0,39 | -0,23 | -0,47 | -0,28 |
| 10 | -0,22 | -0,18 | -0,13 | -0,28 | -0,15 |
| 11 | -0,25 | -0,28 | -0,22 | -0,26 | -0,29 |
| 12 | -0,18 | -0,33 | -0,16 | -0,36 | -0,27 |
| 13 | -0,10 | -0,22 | -0,15 | -0,36 | -0,44 |
| 14 | -0,13 | -0,26 | -0,35 | -0,36 | -0,25 |
| 15 | -0,25 | -0,32 | -0,35 | -0,38 | -0,32 |
| 16 | -0,25 | -0,42 | -0,22 | -0,25 | -0,20 |
| 17 | -0,16 | -0,24 | -0,21 | -0,31 | -0,21 |
| 18 | -0,19 | -0,17 | -0,18 | -0,28 | -0,27 |
| 19 | -0,17 | -0,20 | -0,15 | -0,38 | -0,28 |
| 20 | -0,19 | -0,27 | -0,12 | -0,15 | -0,33 |

Computer-aided virtual screening approaches play an essential role as cost-effective and take less time than experimental studies to reach the drug to the market (59). Molecular docking used to identify peptides with binding affinity to three target receptors of SARS-CoV-2 was similar to other studies using *in silico* tools (59, 60). For example, peptides with the stage of development theoretical such as Inhibitors 1-4 peptide, have shown excellent results in targeting SARS-CoV-2 RDB (60); at the same time, this receptor also has been targeted by SBP1 (61) and Spikeplug (62) peptides in a preclinical advance. Our results show an average free energy similar to DRAMP00877 and DRAMP02333 peptides with −245.612 and −243.441 KJ/mol, respectively (63). We expect the inclusion of the peptides found in this study as modulators phytochemicals in future research causes interference in a downstream signaling process viral, focusing on preventing the SARS-CoV-2

entry into the host cell through protein inhibition (64). Out of 583 screened ligands, some compounds have low binding affinity, probably due to low noncovalent binding according to their structure.

On the other hand, most of the reports of binding to SARS-CoV-2 proteins focus on molecular docking (65); however, few extend to determining immunogenicity. This study's selection of non-allergenic peptides with low antigenicity demonstrates bioactive potential, probably with low side effects. The safety of these peptides should be evaluated in future in vitro studies. Likewise, other *in silico* tests could be included to determine the antigenic presentation of the peptides found. The study of the immunogenicity of the peptides with the highest molecular docking score allows the positioning of an innovative methodology with the inclusion of checkpoints to select reactivity and safety.

**Conclusion**

The computational analysis of this research defined new bioactivity for peptides predicted found in alignment with the plants of study. The interaction between SARS-CoV-2 proteins Spike RBD domain and S2 subunit, and Nsp4 with plant peptides indicated a higher binding potential that could interfere with the viral replication. Our findings show that some peptides are associated with scores of antigenicity and allergenicity; in this line to future directions for in vitro experiments, the molecule's purification is necessary. This pre-clinical study represents an alternative that could be included to combat the COVID-19 pandemic.

**Declarations.**

**Acknowledgements.**

The authors thank the support in the design of the research to Pablo Fernández and Fedra Ortíz.

**Author Contributions.**

Conceptualization and Methodology, all authors; Data Curation and Validation: JPG; Formal Analysis, all authors; Writing-Original Draft Preparation, JPG; Writing-Review and Approval, all authors. All authors have read and agreed to the published version of the manuscript.

**Funding.**

The authors gratefully acknowledge funding by the Departamento de Biología de la Universidad de Nariño and Semillero de Investigación Biológica Aplicada a la Biodiversidad y a la Biotecnología en la Región (SIBIO).

**Competing interests.**

The authors declare that they have no conflict of interest with funders or personal relationship that could have had a bearing on the work presented in this paper.

**Consent to Participate.** Not applicable.

**Availability of data and material**. Not applicable.

**Code availability**. Not applicable.

## Referencias


1. Joshi A, Sunil Krishnan G, Kaushik V. Molecular docking and simulation investigation: effect of beta-sesquiphellandrene with ionic integration on SARS-CoV2 and SFTS viruses. J Genet Eng Biotechnol. 2020; 18(1):1–8.
2. Mohamadian M, Chiti H, Shoghli A, Biglari S, Parsamanesh N, Esmaeilzadeh A. COVID-19: Virology, biology and novel laboratory diagnosis. J Gene Med. 2021; 23(2):e3303.
3. Vilas Boas LCP, Campos ML, Berlanda RLA, de Carvalho Neves N, Franco OL. Antiviral peptides as promising therapeutic drugs. Cell Mol Life Sci. 2019; 76(18):3525–42.
4. Amponsa-Achiano K, Frimpong JA, Barradas D, Bandoh DA, Kenu E. Leveraging Lessons Learned from Yellow Fever and Polio Immunization Campaigns during COVID-19 Pandemic, Ghana, 2021. Emerg Infect Dis. 2022; 28(13):S232-S237.
5. Calina D, Docea AO, Petrakis D, Egorov AM, Ishmukhametov AA, Gabibov AG, et al. Towards effective COVID-19 vaccines: Updates, perspectives and challenges (Review). Int J Mol Med. 2020; 46(1):3-16.
6. Sharma A, Khanna S, Kaur G, Singh I. Medicinal plants and their components for wound healing applications. Futur J Pharm Sci 2021 71. 2021; 7(1):1–13.
7. Chen K, Wu W, Hou X, Yang Q, Li Z. A review: Antimicrobial properties of several medicinal plants widely used in Traditional Chinese Medicine. Food Qual Saf. 2021; 5:1–22.
8. Tang SS, Prodhan ZH, Biswas SK, Le CF, Sekaran SD. Antimicrobial peptides from different plant sources: Isolation, characterisation, and purification. Phytochemistry. 2018; 154:94–105.
9. Patil SM, Ramu R, Shirahatti PS, Shivamallu C, Amachawadi RG. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. Heliyon. 2021; 7(5):e07054.
10. Abou Baker DH, Amarowicz R, Kandeil A, Ali MA, Ibrahim EA. Antiviral activity of Lavandula angustifolia L. and Salvia officinalis L. essential oils against avian influenza H5N1 virus. J Agric Food Res. 2021; 4:100135.
11. Agarwal G, Gabrani R. Antiviral Peptides: Identification and Validation. Int J Pept Res Ther 2020 271. 2020; 27(1):149–68.
12. Bruckdorfer T, Marder O, Albericio F. From production of peptides in milligram amounts for research to multi-tons quantities for drugs of the future. Curr Pharm Biotechnol. 2004; 5(1):29-43.
13. Lien S, Lowman HB. Therapeutic peptides. Trends Biotechnol. 2003; 21(12):556-62.
14. Mehmood A, Khan S, Khan S, Ahmed S, Ali A, xue M, et al. In silico analysis of quranic and prophetic medicinals plants for the treatment of infectious viral diseases including corona virus. Saudi J Biol Sci. 2021; 28(5):3137–51.
15. Shah B, Modi P, Sagar SR. In silico studies on therapeutic agents for COVID-19: Drug repurposing approach. Life Sci. 2020; 252:117652.
16. Tamay-Cach F, Villa-Tanaca ML, Trujillo-Ferrara JG, Alemán-González-Duhart D, Quintana-Pérez JC, González-Ramírez IA, Correa-Basurto J. In Silico Studies Most Employed in the Discovery of New Antimicrobial Agents. Curr Med Chem. 2016; 23(29):3360-3373.
17. Mulvenna J, Wang C, Craik C. CyBase: a database of cyclic protein sequence and structure, Nucleic Acids Research, 2006; 34,(1):D192–D194.
18. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, Tufféry P. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex, Nucleic Acids Research. 2016; 44,(w1):W449–W454.
19. Kong R, Yang G, Xue R, Liu M, Wang F, Hu J, et al. COVID-19 Docking Server: a meta server for docking small molecules, peptides and antibodies against potential targets of COVID-19, Bioinformatics. 2020; 36,(20):5109–5111.

20. Hall-Swan S, Devaurs D, Rigo M, Antunes D, Kavraki L, Zanatta G. DINC-COVID: A webserver for ensemble docking with flexible SARS-CoV-2 proteins. Computers in Biology and Medicine. 2021; (139):104943
21. Wani AR, Yadav K, Khursheed A, Rather MA. An updated and comprehensive review of the antiviral potential of essential oils and their chemical constituents with special focus on their mechanism of action against various influenza and coronaviruses. Microb Pathog. 2021; 152:104620.
22. Bokesch HR, Pannell LK, Cochran PK, Ii RCS, Mckee TC, Boyd MR. A Novel Anti-HIV Macrocyclic Peptide from Palicourea condensata. 2001; 249–50.
23. Hiu KOUEIC. An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides. 1999; 96:8913–8.
24. Panya A, Yongpitakwattana P, Budchart P, Sawasdee N, Krobthong S, Paemanee A, et al. Novel bioactive peptides demonstrating anti-dengue virus activity isolated from the Asian medicinal plant Acacia Catechu. Chem Biol Drug Des. 2019; 93(2):100–9.
25. Hu E, Wang D, Chen J, Tao X. Novel cyclotides from hedyotis diffusa induce apoptosis and inhibit proliferation and migration of prostate cancer cells. Int J Clin Exp Med. 2015; 8(3):4059–65.
26. Kalkhoran S, Benowitz NL, Rigotti NA. Prevention and Treatment of Tobacco Use: JACC Health Promotion Series. J Am Coll Cardiol. 2018; 28;72(9):1030-1045.
27. Auvin-Guette C, Baraguey C, Blond A, Xavier HS, Pousset JL, Bodo B. Pohlianins A, B and C, cyclic peptides from the latex of Jatropha pohliana ssp. molissima. Tetrahedron. 1999; 55(38):11495–510.
28. Pinto MEF, Batista JM, Koehbach J, Gaur P, Sharma A, Nakabashi M, et al. Ribifolin, an orbitide from jatropha ribifolia, and its potential antimalarial activity. J Nat Prod. 2015; 78(3):374–80.
29. Jack HW, Tzi BN. Sesquin, a potent defensin-like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides. 2005; 26(7):1120–6.
30. Pränting M, Lööv C, Burman R, Göransson U, Andersson DI. The cyclotide cycloviolacin O2 from Viola odorata has potent bactericidal activity against Gram-negative bacteria. J Antimicrob Chemother. 2010; 65(9):1964–71.
31. Ireland DC, Wang CKL, Wilson JA, Gustafson KR, Craik DJ. Cyclotides as natural anti-HIV agents. Biopolym - Pept Sci Sect. 2008; 90(1):51–60.
32. Camargo Filho I, Cortez DAG, Ueda-Nakamura T, Nakamura C V., Dias Filho BP. Antiviral activity and mode of action of a peptide isolated from Sorghum bicolor. Phytomedicine. 2008; 15(3):202–8.
33. Gustafson KR, Walton LK, Sowder RC, Johnson DG, Pannell LK, Cardellina JH, et al. New circulin macrocyclic polypeptides from Chassalia parvifolia. J Nat Prod. 2000; 63(2):176–8.
34. Hallock YF, Sowder RC, Pannell LK, Hughes CB, Johnson DG, Gulakowski R, et al. Cycloviolins A-D, anti-HIV macrocyclic peptides from Leonia cymosa. J Org Chem. 2000; 65(1):124–8.
35. Zou XG, Hu JN, Li J, Yang JY, Du YX, Yu YF, et al. iCellular uptake of [1–9-NαC]-linusorb B2 and [1–9-NαC]-linusorb B3 isolated from flaxseed, and their antitumor activities in human gastric SGC-7901 cells. J Funct Foods. 2018; 48:692–703.
36. Chen B, Colgrave ML, Daly NL, Rosengren KJ, Gustafson KR, Craik DJ. Isolation and characterization of novel cyclotides from Viola hederaceae: Solution structure and anti-HIV activity of vhl-1, a leaf-specific expressed cyclotide. J Biol Chem. 2005; 280(23):22395–405.
37. Baraguey C, Auvin-Guette C, Blond A, Cavelier F, Lezenven F, Pousset JL, et al. Isolation, structure and synthesis of chevalierins A, B and C, cyclic peptides from the latex of Jatropha chevalieri. J Chem Soc - Perkin Trans 1. 1998; (18):3033.
38. Daly NL, Clark RJ, Plan MR, Craik DJ. Kalata B8, a novel antiviral circular protein, exhibits conformational flexibility in the cystine knot motif. Biochem J. 2006; 393(3):619–26.
39. Maupetit J, Derreumaux P, Tufféry P. A fast method for large-scale de novo peptide and miniprotein structure prediction. J Comput Chem. 2010; 31(4):726-38.
40. Kong R, Yang G, Xue R, Liu M, Wang F, Hu J, et al. COVID-19 Docking Server: a meta server for docking small molecules, peptides and antibodies against potential targets of COVID-19. Bioinforma-

tics. 2020; 36(20):5109–11.
41. Santos-Silva CA dos, Zupin L, Oliveira-Lima M, Vilela LMB, Bezerra-Neto JP, Ferreira-Neto JR, et al. Plant Antimicrobial Peptides: State of the Art, In Silico Prediction and Perspectives in the Omics Era. Bioinform Biol Insights. 2020; 2;14:1177932220952739.
42. Hasan A, Paray BA, Hussain A, Qadir FA, Attar F, Aziz FM, et al. A review on the cleavage priming of the spike protein on coronavirus by angiotensin-converting enzyme-2 and furin. J Biomol Struct Dyn. 2021; 39(8):3025-3033.
43. Yan, W., Zheng, Y., Zeng, X. et al. Structural biology of SARS-CoV-2: open the door for novel therapies. Sig Transduct Target Ther. 2022; 7(26). DOI: 10.1038/s41392-022-00884-5
44. Cheng J, Randall AZ, Sweredoski MJ, Baldi P. SCRATCH: a protein structure and structural feature prediction server. Nucleic Acids Res. 2005; 33(Web Server issue).
45. Dimitrov I, Bangov I, Flower DR, Doytchinova I. AllerTOP v.2--a server for in silico prediction of allergens. J Mol Model. 2014; 20(6).
46. Wong FC, Ong JH, Kumar DT, Chai TT. In Silico Identification of Multi-target Anti-SARS-CoV-2 Peptides from Quinoa Seed Proteins. Int J Pept Res Ther 2021 273. 2021; 27(3):1837–47.
47. Türkmenoğlu A, Özmen D. Allergenic components, biocides, and analysis techniques of some essential oils used in food products. J Food Sci. 2021; 86(6):2225–41.
48. Mishra NP, Das SS, Yadav S, Khan W, Afzal M, Alarifi A, et al. Global impacts of pre- and post-COVID-19 pandemic: Focus on socio-economic consequences. Sens Int. 2020; 1:100042.
49. Ma H, Zhu J, Liu J, Zhang X, Liu Y, Yang Q. Hospital biosecurity capacitation: Analysis and recommendations for the prevention and control of COVID-19. J Biosaf Biosecur. 2020; 2(1):5-9.
50. Gilissen J, Pivodic L, Unroe KT, Van den Block L. International COVID-19 Palliative Care Guidance for Nursing Homes Leaves Key Themes Unaddressed. J Pain Symptom Manage. 2020; 60(2):e56-e69.
51. Yu WL, Toh HS, Liao CT, Chang WT. A Double-Edged Sword-Cardiovascular Concerns of Potential Anti-COVID-19 Drugs. Cardiovasc Drugs Ther. 2021; 35(2):205-214.
52. Juurlink DN. Safety considerations with chloroquine, hydroxychloroquine and azithromycin in the management of SARS-CoV-2 infection. CMAJ. 2020; 27;192(17):E450-E453.
53. Almeida-Espinosa A, Sarmiento-Ardila JA. COVID-19: implications of SARS-CoV-2 in Colombia. Gac Med Mex. 2020; 156(4):334-338.
54. Huang J, Tao G, Liu J, Cai J, Huang Z, Chen JX. Current Prevention of COVID-19: Natural Products and Herbal Medicine. Front Pharmacol. 2020; 16;11:588508.
55. Bhuiyan FR, Howlader S, Raihan T, Hasan M. Plants Metabolites: Possibility of Natural Therapeutics Against the COVID-19 Pandemic. Front Med (Lausanne). 2020; 7;7:444.
56. Ashaolu TJ, Nawaz A, Walayat N, Khalifa I. Potential "biopeptidal" therapeutics for severe respiratory syndrome coronaviruses: a review of antiviral peptides, viral mechanisms, and prospective needs. Appl Microbiol Biotechnol. 2021; 105(9):3457-3470.
57. Arimura G, Kost C, Boland W. Herbivore-induced, indirect plant defences. Biochim Biophys Acta. 2005; 15;1734(2):91-111.
58. Schnitzler P, Nolkemper S, Stintzing FC, Reichling J. Comparative in vitro study on the anti-herpetic effect of phytochemically characterized aqueous and ethanolic extracts of Salvia officinalis grown at two different locations. Phytomedicine. 2008; 15(1-2):62-70.
59. Terstappen GC, Reggiani A. In silico research in drug discovery. Trends Pharmacol Sci. 2001; 22(1):23-6.
60. Kim JH, Park YI, Hur M, Park WT, Moon YH, Koo SC, et al. The inhibitory activity of methoxyl flavonoids derived from Inula britannica flowers on SARS-CoV-2 3CLpro. Int J Biol Macromol. 2022; 1;222(Pt B):2098-2104.
61. Zhang G., Pomplun S., Loftis A.R., Tan X., Loas A., Pentelute B.L. Investigation of ACE2 N-terminal fragments binding to SARS-CoV-2 spike RBD. bioRxiv. 2020. . https://doi.org/10.1101/2020.03.19.999318

62. Romano M., Ruggiero A., Squeglia F., Berisio R. An engineered stable mini-protein to plug SARS-Cov-2 Spikes. bioRxiv. 2020. https://doi.org/10.1101/2020.04.29.067728
63. Mahmud S, Biswas S, Kumar Paul G, Mita MA, Afrose S, Robiul Hasan M, et al. Antiviral peptides against the main protease of SARS-CoV-2: A molecular docking and dynamics study. Arab J Chem. 2021; 14(9):103315.
64. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012; 4(6):1011-33.
65. Zahran EM, Albohy A, Khalil A, Ibrahim AH, Ahmed HA, El-Hossary EM, et al. Bioactivity Potential of Marine Natural Products from Scleractinia-Associated Microbes and In Silico Anti-SARS-COV-2 Evaluation. Mar Drugs. 2020; 16;18(12):645.

**Supplementary 1.**
https://drive.google.com/file/d/1hD2RpkL7aG0pcIo_6wPBlK_E7aw5n0zm/view?usp=sharing
**Supplementary 2.**
https://drive.google.com/file/d/1wB_5NJww_B1MmImsn_gtsADccJRIQKf7/view?usp=sharing