Known *Anti Sars-Cov Protease Inhibitors* On Sars-Cov-2 Related Intracellular Reception Sites: *Docking Interaction* And Computer Aided Optimization Of Molecular Structure For *Chemioselective Interactions*

Sidhartha Parida (Corresponding Author)[1] , Satyabrata Jena[2] , Saswati Behera[3] , Swarnalata Mohapatra[4] , Anamika Gupta[5] , Sonali Tarei[6]

[1]Assistant Professor, Department of Pharmaceutics, School of Pharmacy Centurion University of Technology and Management, Odisha, India-756044 Email id- sidhartha1996parida@gmail.com
[2]Associate Professor, Department of Pharmaceutics, Bhaskar Pharmacy College Yenkapally, Moinabad, Hyderabad, India-500075
[3]Assistant Professor, Department of Pharmacology Gayatri Institute of Science and Technology (GIST), Gunupur, Rayagada, Odisha, India-765022
[4]Assistant Professor, Department of Pharmacology Hi-Tech College of Pharmacy, Pandra, Bhubaneswar, Odisha, India-751007
[5]Assistant Professor, Department of Pharmaceutical Chemistry Vijay Pharmacy College, Jaunpur, India-222139
[6]Assistant Professor, Department of Pharmaceutics Seemanta Institute of Pharmaceutical Science, Jharpokhria Bambay Chak, Mayurbhanj, Odisha, India-757086
DOI: 10.47750/pnr.2023.14.02.249

Abstract

Molecular docking and molecular dynamics aided virtual search of OliveNet™ directory identified potential secoiridoids that combat SARS-CoV-2 entry, replication, and associated hyperinflammatory responses. OliveNet™ is an active directory of phytochemicals obtained from different parts of the olive tree, Olea europaea (Oleaceae). Olive oil, olive fruits containing phenolics, known for their health benefits, are indispensable in the Mediterranean and Arabian diets. Secoiridoids is the largest group of olive phenols and is exclusive to the olive fruits. Functional food like olive fruits could help prevent and alleviate viral disease at an affordable cost. A systematized virtual search of 932 conformers of 78 secoiridoids utilizing Autodock Vina, followed by precision docking using Idock and Smina indicated that Nüzhenide oleoside (NZO), Oleuropein dimer (OED), and Dihydro oleuropein (DHO) blocked the SARS-CoV-2 spike (S) protein-ACE-2 interface; Demethyloleuropein (DMO), Neo-nüzhenide (NNZ), and Nüzhenide (NZE) blocked the SARS-CoV-2 main protease (M^{pro}). Molecular dynamics (MD) simulation of the NZO-S-protein-ACE-2 complex by Desmond revealed stability during 50 ns. RMSD of the NZO-S-protein-ACE-2 complex converged at 2.1 Å after 20 ns. During MD, the interaction fractions confirmed multiple interactions of NZO with Lys417, a crucial residue for inhibition of S protein. MD of DMO-M^{pro} complex proved its stability as the RMSD converged at 1.6 Å. Analysis of interactions during MD confirmed the interaction of Cys145 of M^{pro} with DMO and, thus, its inhibition. The docking predicted IC_{50} of NZO and DMO was 11.58 and 6.44 µM, respectively. Molecular docking and dynamics of inhibition of the S protein and M^{pro} by NZO and DMO correlated well. Docking of the six-hit secoiridoids to IL1R, IL6R, and TNFR1, the receptors of inflammatory cytokines IL1β, IL6, and TNFα, revealed the anti-inflammatory potential except for DHO. Due to intricate structures, the secoiridoids violated Lipinski's rule of five. However, the drug scores of secoiridoids supported their use as drugs. The ADMET predictions implied that the secoiridoids are non-toxic and pose low oral absorption. Secoiridoids need further optimization and are a suitable lead for the discovery of anti-SARS-CoV-2 therapeutics. For the moment, olive secoiridoids presents an accessible mode of prevention and therapy of SARS-CoV-2 infection.

Keywords- Anti SARS-COV, Protease Inhibitors,SARS-Cov-2,Intracellular Reception Sites, Docking Interaction, Computer Aided Optimization, Molecular Structure, Chemioselective Interactions.

INTRODUCTION

The current pandemic of coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has worsened persons' quality of life and socioeconomic status globally. Rigorous research for the prevention and therapy of SARS-CoV-2 infection using vaccines and small molecules are underway. Natural

products are inspiring sources of drugs, including antivirals. It is imperative to explore functional foods as therapeutic combat against SARS-CoV-2, as they are accessible and affordable. Focuses on nutritional supplements as a mode of prevention and therapy for SARS-CoV-2 infection have increased (Zabetakis et al., 2020). Olive fruits (olives) and oil are indispensable in the Mediterranean (Martinez-Gonzalez and Martin-Calvo, 2016) and Arabian diet (Al-Ruqaie et al., 2016) and are known for their health benefits. The northern provinces of Saudi Arabia have suitable climatic conditions and cultivate olive tree, Olea europaea (Oleaceae) for olive oil and olives (Hemida et al., 2014; Fraihat et al., 2017). Olive oil obtained from olives has earned nutritional and therapeutic value. Especially olive oil polyphenols demonstrated antioxidant, antihyperglycemic, anticancer, antilipidemic, antiviral, anti-inflammatory, cardioprotective, and immunomodulation properties (Rigacci and Stefani, 2016; Gorzynik-Debicka et al., 2018). The phytochemicals of olive leaves have also exhibited beneficial effects on ailments like diabetes, cancer, obesity, bacterial, and viral infections (Medina Pradas et al., 2019; Acar-Tek and Agagündüz, 2020). The green, black forms of olives consumed as table olives are high in plant polyphenols implicated in several diseases (Rahmani et al., 2014; Fernández-Poyatos et al., 2019; Conte et al., 2020). Olives contain secoiridoids, the largest group of polyphenols, in their glycoside and aglycone forms. Olives are also composed of other polyphenols-like flavonoids, triterpenes, and lignans (Hashmi et al., 2015). Olives are the primary source of secoiridoids, whereas oil contains the metabolites of secoiridoids (Sivakumar et al., 2018). Secoiridoids are useful in cancer therapy, heart diseases, neurodegeneration, immunoinflammatory, diabetes, obesity, and aging-related ailments (Celano et al., 2019; Castejón et al., 2020). Few studies are available on the antiviral property of secoiridoids against AIDS and influenza viruses (Omar, 2010; Vilaplana-Pérez et al., 2014). Oleuropein, a secoiridoid, possesses potent antiviral activity against parainfluenza type 3 virus, respiratory syncytial virus, hepatitis, and herpes mononucleosis (Omar, 2010). Oleuropein targeted the surface glycoprotein HIV-1 gp41 and HIV integrase, which blocked HIV entry and replication (Lee-Huang et al., 2007). A recent molecular docking study identified the flavonoids Cyanidin-3-rutinoside and Paeonidin-3-rutinoside from Olea europaea as inhibitors of SARS-CoV-2 main protease (Shawky et al., 2020). The spike protein (S protein), a surface structural protein of SARS-CoV-2, binds with a high affinity to human angiotensin-converting enzyme 2 (ACE-2), which facilitates the entry of the virus into the human host (Wrapp et al., 2020). Therefore, inhibition of spike protein and its interaction with ACE-2 can block virus entry into the host. The main protease (M^{pro}) of SARS-CoV-2, also known as 3CLpro, is responsible for the cleavage of polyproteins 1a and 1b, which is crucial for virus replication (Prajapat et al., 2020). The inhibition of virus replication is possible through the inhibition of M^{pro}. The S protein and M^{pro} are thus attractive targets for designing and discovering drugs to combat SARS-CoV-2 infection. On the host's invasion by SARS-CoV-2, there is a hyperinflammatory response by the immune system leading to acute respiratory distress and multiple organ failure. Elevated levels of pro-inflammatory cytokines, interleukin-1 (IL1β), interleukin-6 (IL6), and tumor necrosis factor-α (TNFα) in moderate to severe COVID-19 cases contribute to the hyperinflammatory response (Maiti et al., 2020; Tang et al., 2020). It is of paramount importance to block the actions of inflammatory cytokines to improve the patient's well-being and reduce the fatality rate of SARS-COV-2 infected patients. The receptors IL1R (Maiti et al., 2020), IL6R (Chen et al., 2017), and TNFR1 (Maiti et al., 2020) of cytokines are useful targets to evaluate the anti-inflammatory potential of secoiridoids. One of the rapid and efficient methods to identify potential small molecule therapeutics is utilizing virtual search methods like molecular docking (da Silva Rocha et al., 2019). OliveNet™ is an active directory of phytochemicals reported from olive leaves, olives, and olive oil (Bonvino et al., 2018).

MATERIALS AND METHODS

(i)Virtual Search of OliveNet™ Directory-We accessed OliveNet™ at https://mccordresearch.com.au/. Autodock Vina was the software used to perform the virtual search of OliveNet™ secoiridoids (Trott and Olson, 2010). The virtual search utilized a target-based docking to the structural and non-structural proteins of the virus (Maia et al., 2020). In this study, the drug targets were mainly the SARS-CoV-2 S protein, a structural protein, and M^{pro}, a non-structural protein. The virtual search process comprised the steps described herein.

(ii) Sources of SARS-CoV-2 Spike Protein, Main Protease, and Secoiridoids- We obtained the three-dimensional X-ray crystallographic structures of SARS-CoV-2 S protein and M^{pro} from the protein data bank using PDB IDs, 6LZG, and 6LU7, respectively. The targeted viral proteins were in their pdb file formats. The secoiridoids reported in OliveNet™ possess molecular weights between 184 and 2,692 g/mol. We filtered and used those secoiridoids with molecular weight <1,100 g/mol. Based on the molecular weight factor, we excluded oleuropein trimer, oleuropein tetramer, and oleuropein pentamer. Instead, we included the parent compounds tyrosol hydroxytyrosol that was within the molecular weight range. Finally, 78 secoiridoids contributed to the virtual search. OliveNet™, PubChem, and ZINC were the databases used to retrieve the structures of the chosen secoiridoids. We applied the canonical smiles of secoiridoids to generate the three-dimensional structures.

(iii) Preparation of SARS-CoV-2 Spike Protein, Main Protease, and Secoiridoids- Chimera 1.13.2 was the program used to prepare the target proteins (Pettersen et al., 2004). The 6LZG is the receptor-binding domain of the SARS-CoV-2 S protein complexed with the human ACE-2 receptor. The S protein chain B and the ACE-2 chain A were retained, and the ligand atoms were removed. The aim was to dock secoiridoids to the interface of S protein and ACE-2. In the case of 6LU7, it is the SARS-CoV-2 M^{pro} monomer complexed with a peptide inhibitor. The monomer chain A was retained, and the non-standard residues, including the inhibitor, was deleted. Afterward, the prepared protein structures underwent energy minimization to overcome unfavorable backbone and sidechain interactions through the steepest descent of 100 steps under the Amber ff99SB force field. Stabilization of the protein structures involved merging non-polar hydrogens, adding polar hydrogens, and assigning Kollmann charges.

SARS-CoV-2 target/PDB ID	Secoiridoid	Autodock Vina	Smina	Idock
		Binding energy (kcal/mol)		
S protein-ACE-2/6LZG	Nüzhenide oleoside	−8.90	−9.20	−7.60
	Oleuropein dimer	−8.70	−8.70	−7.04
	Dihydro oleeuropein	−8.70	−7.90	−6.94
	Chloroquine (reference drug)	−5.70	−5.90	−5.69
M^{pro}/6LU7	Demethyloleuropein	−8.90	−10.20	−8.85
	Neo–nüzhenide	−8.70	−9.70	−8.46
	Nüzhenide	−8.60	−9.20	−8.11
	Lopinavir (reference drug)	−7.80	−7.30	−7.91

Table 1 - Binding energies during precise docking of the three top-ranked olive secoiridoids to SARS-CoV-2 targets.

(iv) Molecular Docking of Secoiridoids to SARS-CoV-2 Spike Protein and Main Protease-Virtual search by docking secoiridoids to S protein-ACE-2 and M^{pro} utilized the Autodock Vina program. All the 932 conformers were docked to the binding sites identified and presented inside the grid. Repeated precision docking of secoiridoids to the targets using the software Idock (Li et al., 2012) and Smina (Koes et al., 2013) avoided the false-positive identification. We generated the S protein grid, surrounding Asn33, His34, Glu37, Asp38, Lys353, Ala387, Gln388, Pro389, Phe390, Arg393, Lys403, Tyr453, Tyr495, Gly496, Phe497, Ser494, and Tyr505, the critical interface residues of S protein-ACE-2 (Liu et al., 2020a; Shang et al., 2020). For M^{pro}, we centered the grid on Cys145 (Gurung et al., 2020; Liu et al., 2020b; Zhang et al., 2020). The parameters for docking were: maximum binding modes and energy enabled; exhaustiveness of search of 50.

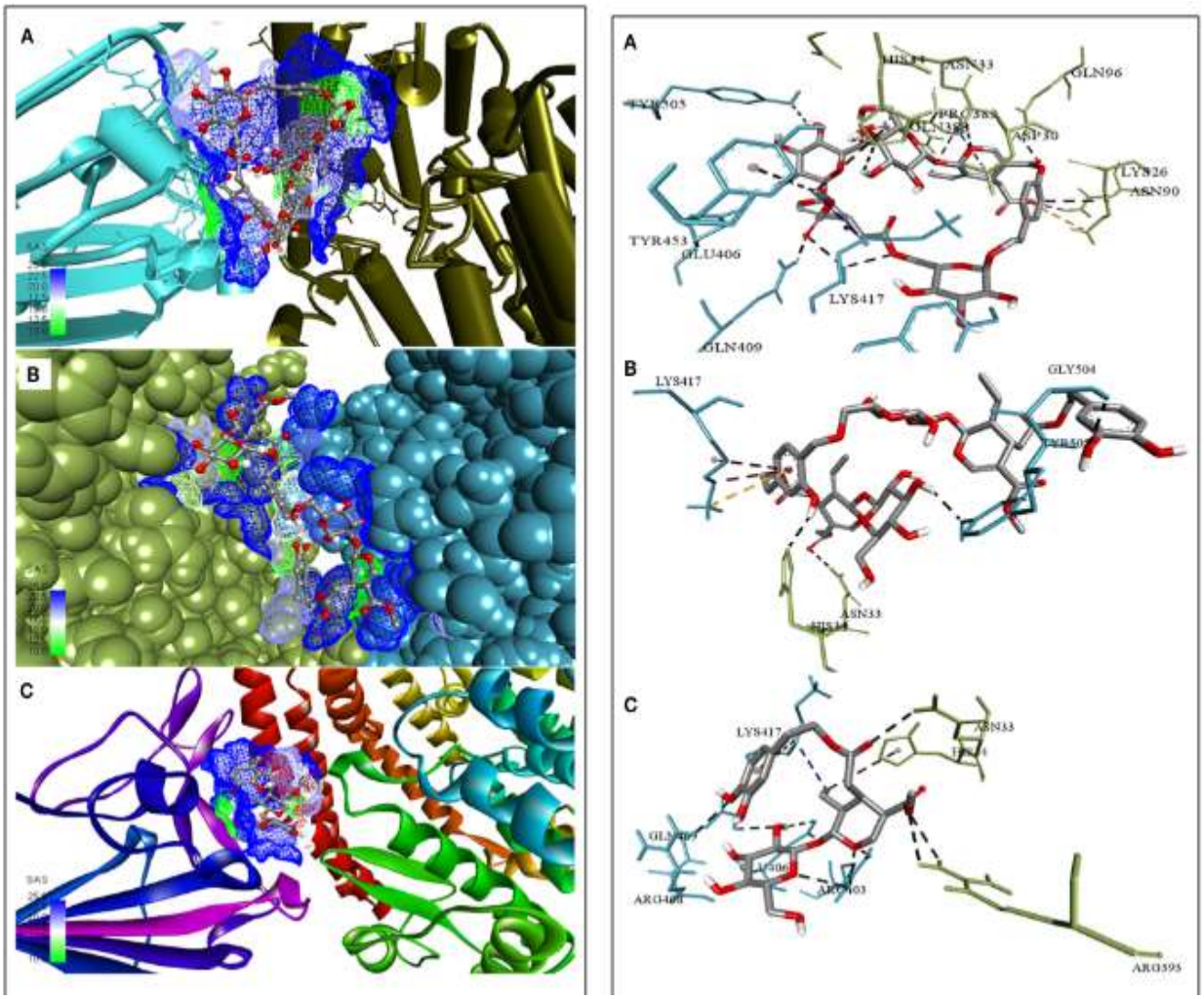

Figure 1- Most active secoiridoids docked to the SARS-CoV-2 spike protein-ACE-2 interface. (A) Nüzhenide oleoside (B) Oleuropein dimer (C) Dihydro oleuropein. Mesh around the binding site represents it's solvent accessible surface (SAS). All models show the spike protein in blue and ACE-2 in green. Panel (A) represents the SARS-CoV-2 S protein-ACE-2 in their secondary structure forms, (B) represents targets in the CPK model, and ribbons in C represent targets.

Figure 2 - Intermolecular interactions of (A) Nüzhenide oleoside (B) Oleuropein dimer (C) Dihydro oleuropein with SARS-CoV-2 spike protein-ACE-2 interface residues. Interaction with Lys417 of the spike protein is considered crucial for inhibition. Nature of bonds: black hydrogen bonds; yellow attractive charge; brown π-alkyl; blue alkyl-alkyl interactions.

(v) Prediction of Inhibition Constant-The binding affinity of the hit secoiridoids, in terms of the inhibition constant (IC_{50}) against S protein and M^{pro}, was predicted using Autodock tools (Morris et al., 2009). The search protocol for the best docking conformer consisted of a population of 150 individuals and a maximum of 25,000,000 energy evaluations in each run with other docking parameters at default. Overall, 100 conformations of each compound were generated with IC_{50} values. Finally, we carried out molecular dynamics studies to assess the stability of the complexes of NZO and DMO bound to respective targets.

(vi) Molecular Dynamics of Apo and Bound Forms of SARS-CoV-2 Spike Protein and Main Protease- Molecular dynamics (MD) simulation helps understand the dynamic motions of the atoms of protein targets and target-ligand complexes (Hospital et al., 2015). MD also unveils the conformational stability of target proteins and ligands before and after the interaction (De Vivo et al., 2016). MD of the apo (unbound) and the hit secoiridoids-bound forms of SARS-COV-2 S protein and M^{pro} were studied using the Desmond program (DE Shaw Research, 2020). After creating the topology, the apoproteins and the protein-ligand complexes were placed in the OPLS3e force field to study the number and stability of interactions. The complex was then immersed into a TIP3P water model at 300°K, maintaining 10 Å from the center of the box. The complex underwent energy minimization up to 5,000 steepest descent steps.

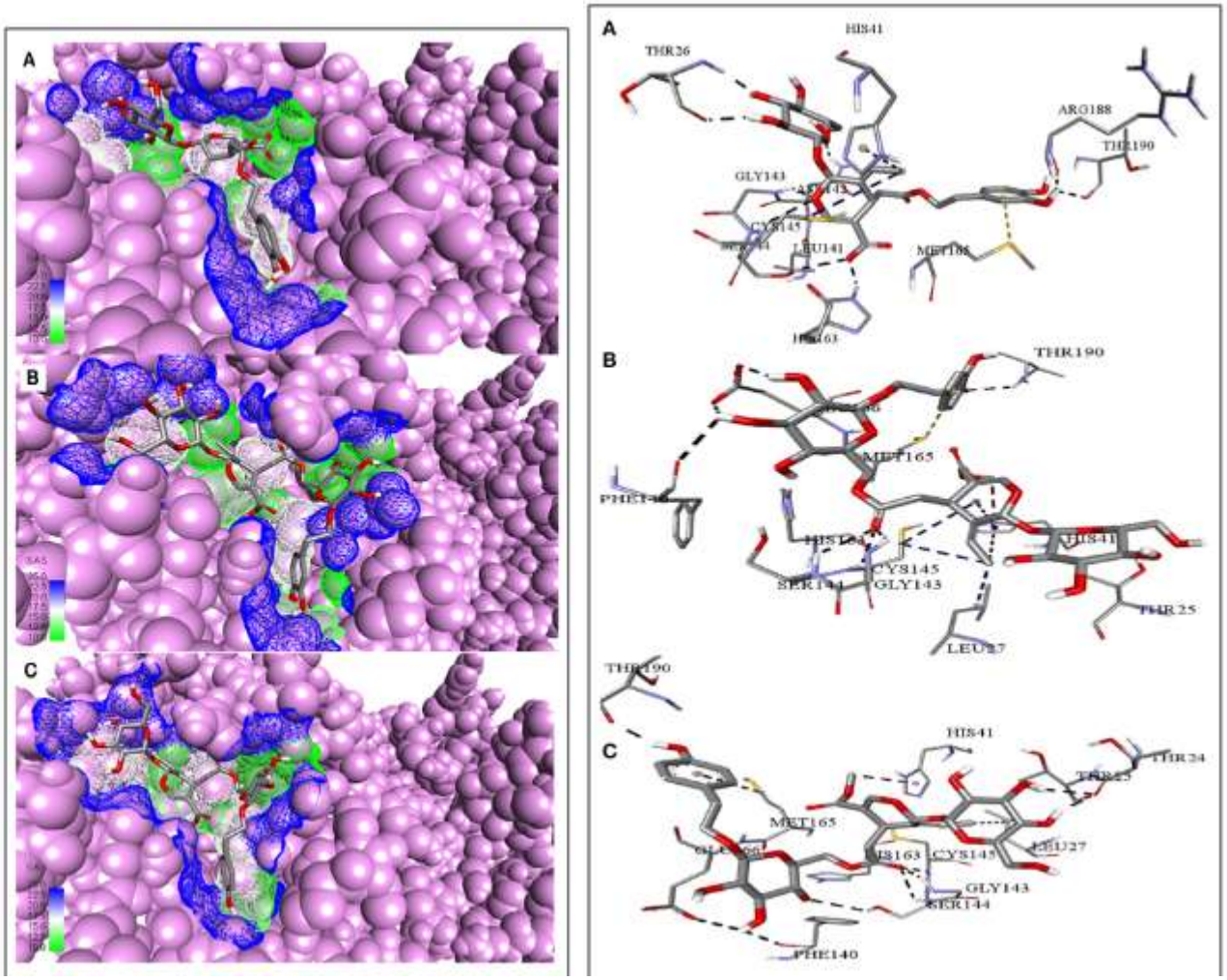

Figure 3- Most active secoiridoids docked to SARS-CoV-2 main protease. (A) Demethyloleuropein (B) Neo-nüzhenide (C) Nüzhenide. The viral protease is in the CPK model. Mesh around the binding site represents it's solvent accessible surface (SAS).

Figure 4- Intermolecular interactions of (A) Demethyloleuropein (B) Neo-nüzhenide (C) Nüzhenide with SARS-CoV-2 main protease. Interaction with Cys145 of the Mpro is considered crucial for inhibition. Nature of interactions: black—hydrogen bonds; yellow π sulfur; brown π-alkyl; blue alkyl-alkyl interactions.

(vii) Molecular Docking of Top-Ranked Secoiridoids to Inflammatory Protein Receptors- The three top-ranked secoiridoids obtained from docking to S protein and M^{pro} went through additional docking to cytokine receptors to assess the ability to inhibit the binding, thereby pro-inflammatory cytokine actions. Autodock Vina, Idock, and Smina tools were used for docking. The PDB IDs 1ITB, 1N26, and 1NCF correspond to the 3D structures of IL1R, IL6R, and TNFR1 receptors. We applied the previously prepared energy minimized structures of the secoiridoids for docking. The 3D structures of the cytokine receptors were stabilized using the Chimera tool. Methotrexate served as the standard drug for comparing the secoiridoids' inhibitory effect on IL1R. Methotrexate can directly inhibit the binding of IL1β to IL1R, resulting in inhibition of IL1β mediated cellular responses (Brody et al., 1993). A small-molecule inhibitor of IL6R, Chemiome CID5329098 (Chen et al., 2017), was used as the reference molecule for IL6R inhibition. Physcion-8-glucoside is an inhibitor of TNFR1 (Saddala and Huang, 2019), used as the reference molecule for TNFR1 inhibition. We applied the blind docking method that involves docking ligands to the whole surface of human cytokine receptors.

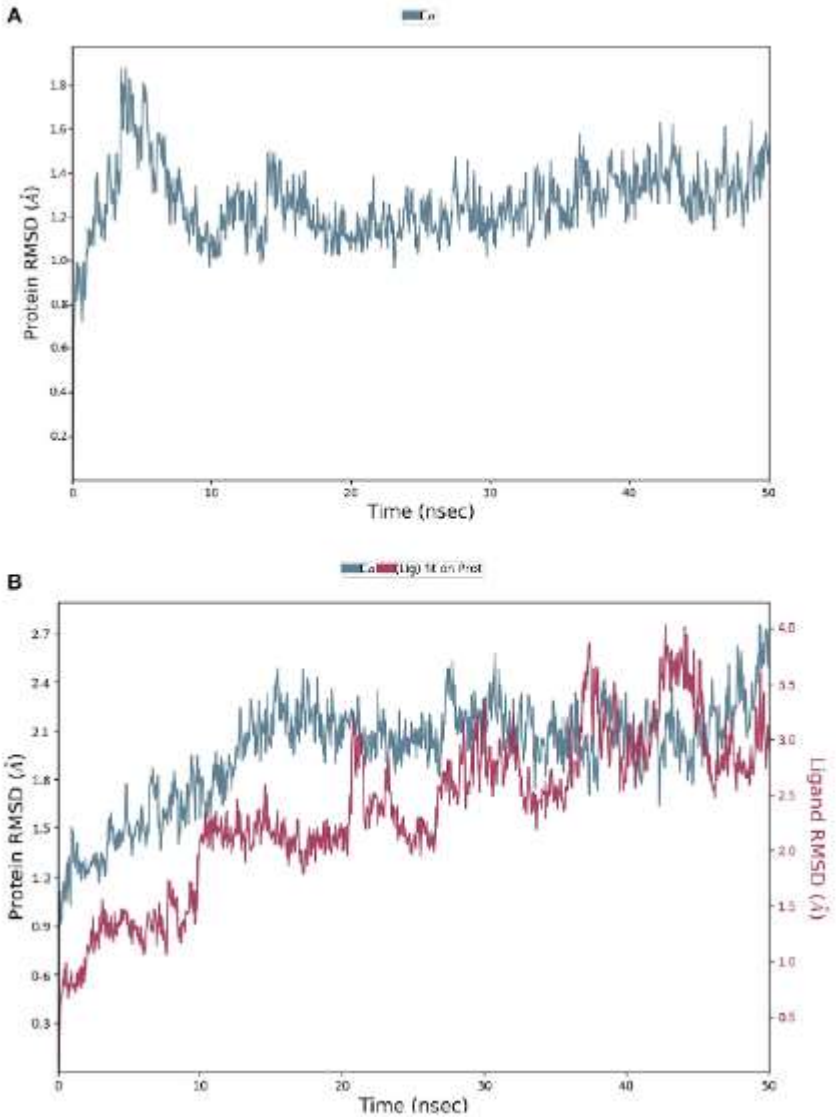

Figure 5- Results of MD of NZO-SARS-CoV-2 S-protein-ACE-2 complex. (A) RMSD of the apo form of SARS-CoV-2 S-protein-ACE-2. (B) RMSD of the NZO-SARS-CoV-2 S-protein-ACE-2 complex. The RMSDs were stable after 20 ns and converged at 2.1 Å.

(viii) Virtual Physicochemical, Pharmacokinetics, and Drug Score Screening- Secoiridoids have novel chemical structures that need an investigation of molecular, pharmacokinetic, and toxicity properties. Examining the secoiridoids' molecular properties like molecular weight, the number of hydrogen bond donor/acceptor groups, and the topological polar surface area, for Lipinski's violation provides insight into the oral bioavailability. The SwissADME server was used to predict the molecular properties and the bioavailability score (Daina et al., 2017). The admetSAR 2.0 online tool helped predict the distribution, toxicity, and LD_{50} (Yang et al., 2019). The OSIRIS property explorer (Osiris, 2020) was used to predict the drug score, which is a combination of drug-likeness score, lipophilicity, hydrophilicity, molecular weight, and the risk of toxicity of secoiridoids. The drug score helps to verify the overall quality of the secoiridoids to be potential drugs.

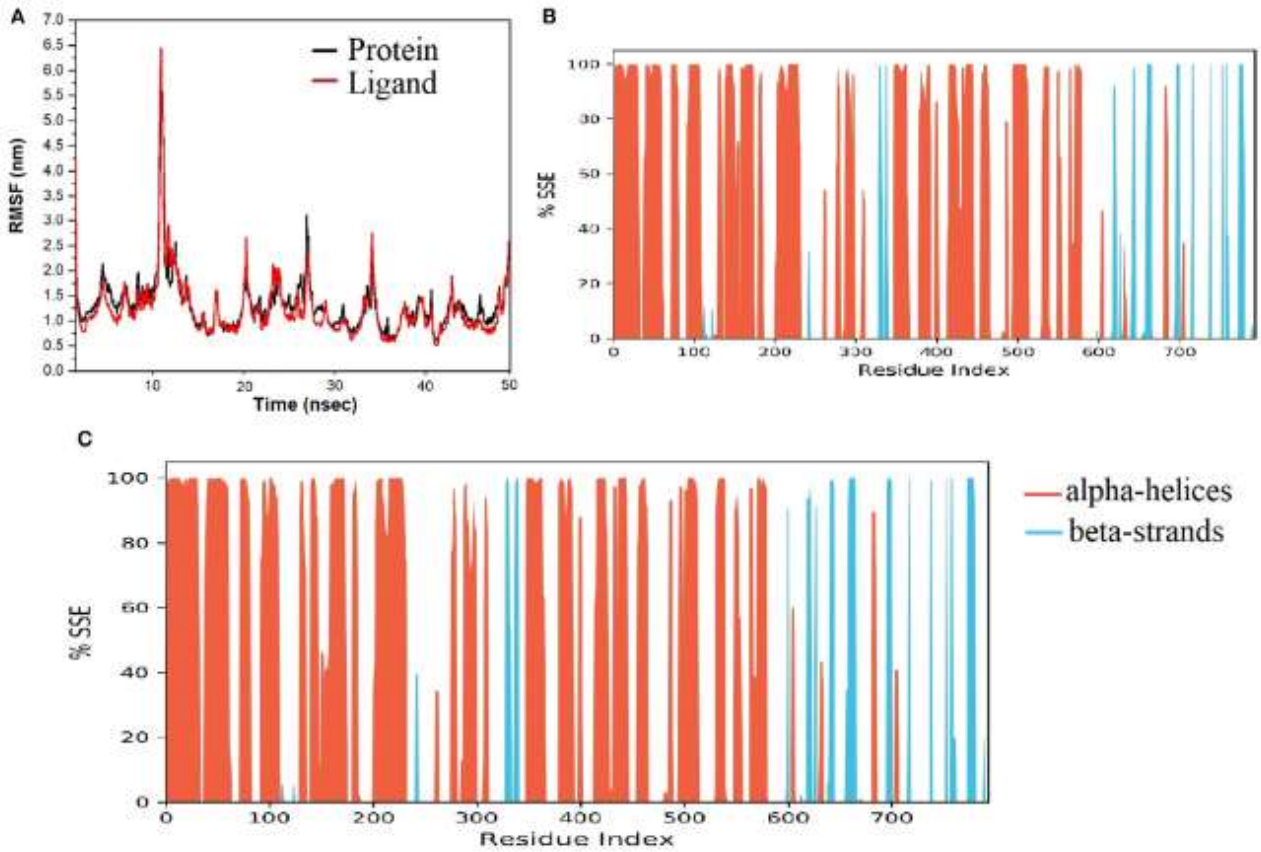

Figure 6 - Results of MD of NZO-SARS-CoV-2 S-protein-ACE-2 complex. (A) RMSF of the NZO-SARS-CoV-2 S-protein-ACE-2 complex. RMSF of protein and ligand were < 2 Å for most of the simulation time. (B) Secondary structure elements (SSE) of the apo form of SARS CoV-2 S-protein-ACE-2. (C) SSE of the NZO-SARS-CoV-2 S-protein-ACE-2 complex. No significant change in the percentage of SSE between the apo and bound proteins indicates conformational stability.

RESULTS

(i)Virtual Search-The virtual search of the OliveNet™ directory using Autodock Vina, Idock, and Smina was successful. The grids generated around the binding site of the 3D crystalline structures of the S protein-ACE-2 receptor interface (6LZG) and the catalytic Cys145 of M^{pro} (6LU7) provided reliable results. The virtual search using Autodock Vina generated 932 energy minimized conformers of the screened secoiridoids, which were then docked to the SARS-CoV-2 targets and graded based on the binding energy. All the docked conformers of secoiridoids had the potential to bind to the SARS-CoV-2 S protein's active site and its interface with ACE-2 with binding energies in the range of −8.9 to −4.1 kcal/mol. Similarly, the olive secoiridoids have shown binding affinities between −8.9 and −4.3 kcal/mol toward the binding site of SARS-CoV-2 M^{pro}. Nüzhenide oleoside (NZO), Oleuropein dimer (OED), and Dihydro oleuropein (DHO) secured the top three ranks, respectively, for binding to SARS-COV-2 S protein-ACE-2 receptor complex. Demethyloleuropein (DMO), Neo-nüzhenide (NNZ), and Nüzhenide (NZE) were the three top-ranked secoiridoids exhibiting efficient binding to SARS-COV-2 M^{pro}. Hence, the top-ranked secoiridoids passed through the next step of precise docking using the Autodock Vina, Idock, and Smina tools.

(ii) Molecular Docking of Secoiridoids to SARS-CoV-2 Spike Protein and Main Protease- The three top-ranked secoiridoids on precise docking in Autodock Vina, Idock, and Smina displayed better binding efficiency at the S protein-ACE-2 interface and M^{pro} binding sites than the reference drugs shown in Table 1. The secoiridoid-SARS-CoV-2 target complexes obtained from precise docking in Autodock Vina, when probed in Discovery Studio Visualizer, unraveled the in-silico binding inhibitory mechanisms. Figures 1A–C show the 3D best binding poses of NZO, OED, and DHO docked to the S protein-ACE-2 target interface. The intermolecular bonds of NZO, OED, and DHO with the S protein-ACE-2 interface are shown in Figures 2A–C. NZO interacted with the S protein-ACE-2 interface through 10 hydrogen bonds and 1 attractive charge interaction (Figure 2A). Four hydrogen bonds existed with S protein, and six hydrogen bonds were with the ACE-2. Lys417 of the S protein was involved in the formation of two hydrogen bonds with NZO. The attractive charge interaction was between the cationic Lys26 of the ACE-2. Six hydrophobic interactions of π-alkyl and

alkyl-alkyl type also existed in the NZO-S protein-ACE-2 complex. Hydrogen bonds were the main forces stabilizing the bound form of NZO as they were shorter than the hydrophobic bonds. OED and DHO interacted through four and nine hydrogen bonds, respectively. OED also established an electrostatic charge interaction with cationic residue Lys417 of the S protein (Figure 2B). Two hydrophobic interactions between Lys417 of S protein and DHO is also significant (Figure 2C). Chloroquine interacted with S protein through a hydrogen bond with Glu406, a π-alkyl interaction with Lys417, besides a hydrogen bond, a π-cation interaction with His34, and an attractive charge interaction with Glu37 of ACE-2. Table 1 shows the results of precise docking of the top-ranked secoiridoids to Mpro. The binding energies of olive compounds were less than the reference drug Lopinavir. Figure 3 shows the active conformations of the DMO (Figure 3A), NNZ (Figure 3B), and NZE (Figure 3C) bound to the Mpro. DMO established nine hydrogen bonds with Mpro (Figure 4A). Besides other significant interactions, one hydrogen bond with catalytic Cys145 and one hydrophobic π-alkyl interaction with catalytic His41 predicted for DMO indicated its inhibitory property. NNZ established seven hydrogen bonds with Mpro, of which one bond was with the catalytic Cys145 (Figure 4B). NNZ also interacted with the catalytic duo Cys145-His41 through two hydrophobic alkyl-alkyl and three π-alkyl interactions, espectively. The predicted hydrogen bonds were stable than the hydrophobic interactions due to their shorter bond lengths.NZE also made nine hydrogen bonds with the Mpro. The active Cys145 residue was engaged in hydrogen bonding with NZE (Figure 4C),

(iii) Prediction of Inhibition Constant-AutoDock predicted IC_{50} against the screened drug targets helped confirm the inhibitory property of the top-ranked secoiridoids NZO and DMO. The IC_{50} of NZO against SARS-CoV-2 S protein was 11.58 μM, and the IC_{50} of DMO against Mpro was 6.44 μM.

(iv) Molecular Dynamics of Apo and Bound Forms of SARS-CoV-2 Spike Protein and Main Protease-The spike-ACE-2 receptor complex's apoprotein, when subjected to MD simulation studies, had a zero-net charge, 91,253 total atoms, 67.355 mM of Na+ ions, 50.690 mM of Cl− ions surrounded by 26,184 water molecules. The final simulation box for the S-protein-ACE-2-NZO complex consisted of 26,110 water molecules, 68.243 mM of Na+ ions, and 50.834 mM of Cl− ions, possessing a zero-net charge, and 91,167 total atoms maintained at 300◦K. For an MD run of 50 ns, RMSD and RMSF were predicted for the apo and bound forms. A ligand's interaction can ward off unfolding and stabilize the protein (Mazal et al., 2018). Hence, we analyzed the protein's secondary structures before and after docking to understand the conformational changes due to ligand binding. Figure 5 shows the results of RMSD analysis of the spike protein-ACE-2 interface before and after docking NZO. The spike protein- ACE-2 apo form attained an equilibrium after 10 ns (Figure 5A). A stable conformation was achieved by the target protein at RMSD 1.2 Å, an acceptable value for protein structures. RMSD of the NZO complex with S-protein was stable after 20 ns and got fixed and converged at 2.1 Å that disclosed the stability of the complex (Figure 5B).

(v) Molecular Docking of Top-Ranked Secoiridoids to Inflammatory Protein Receptors-We used Autodock Vina, Idock, and Smina for molecular docking to avoid false-positive identification of potential cytokine receptor inhibitors. The docking tools evaluated the binding energies and scored the secoiridoids. The six secoiridoids, when docked to the inflammatory cytokine receptors, exhibited good binding affinity except for DHO. IL1R interactions with the reference drug Methotrexate was similar to the interactions of secoiridoids. The binding site for the inhibitors of IL1R comprised of Asn216, Leu237, Asp239, Ala241, Lys244, Ile250, Glu252, Glu259, Tyr261, Thr277, Thr294, Ile303, Ala305, and Tyr307 shows a high binding affinity with Methotrexate and NZO due to less binding energy of −7.8 kcal/mol. NZO and DMO, the identified SARS-CoV-2 inhibitors, can inhibit IL1R because of stronger hydrogen bond interactions with Ile250 and Tyr261 compared to Methotrexate.

(vi) Virtual Physicochemical, Pharmacokinetics, and Drug Score Screening-Olive secoiridoids are natural products possessing new, intricate structures. Their physicochemical and ADMETox properties influence the biological activity; hence we proceeded to predict them. Table 2 presents the results of virtual physicochemical, pharmacokinetics, and drug score screening of the six-hit secoiridoids. The predicted human intestinal absorption of secoiridoids was lesser than Lopinavir. All the secoiridoids

violated Lipinski's rule of five due to their molecular weight higher than 500, the number of hydrogen bond acceptors like oxygen atoms greater than ten, and the number of
hydrogen bond donors like -OH greater than five. Secoiridoids possess a topological polar surface area >150 Å2, which might pose problems in oral absorption, but the predicted
bioavailability was favorable.

DISCUSSION

The Mediterranean and Arabian diet consist of consuming olive fruits (olives) regularly. OliveNetTM is the exclusive, unique database of phytochemicals of the olive tree, Olea europaea. The largest group of polyphenols called secoiridoids reported in OliveNetTM were mainly from olives (MacCord Research OLIVEAMINE R©, 2017). The other groups of phenols like
Catechol, Gallic acid, flavonoids like Hesperidin, Luteolin, Quercetin, Rutin reported in the OliveNetTM have undergone rigorous virtual screening for anti-SARS-CoV-2 activity as they
are commonly present in most of the plants. The research question was whether the consumption of olives could prevent SARS-CoV-2 entry and replication. Therefore, the study aimed
to explore the secoiridoids of olives reported in OliveNetTM directory for their potential to combat SARS-COV-2 entry, replication by inhibition of the interaction of SARS-CoV-2 spike protein with human ACE-2 receptor, and inhibition of SARS- CoV-2 Mpro. The methodology was to search OliveNetTM by virtual means applying molecular docking using the Autodock
Vina technique. The three-dimensional structure minimization using LigPrep yielded 932 conformers of secoiridoids. Virtual search involved molecular docking of all the 932 conformers to S protein and Mpro in Autodock Vina. The Autodock Vina technique scores the binding poses based on the binding energy in kcal/mol. The secoiridoids NZO, OED, and DHO topped the score list for the best inhibitory potential against the SARS-CoV- 2 S protein-ACE-2 receptor. NNZ, NZE, and DMO were the top scorers for their lowest binding energies to interact and inhibit SARS-CoV-2 Mpro. We repeated the molecular docking of the above-mentioned top-ranked conformers of secoiridoids using Autodock Vina, Idock, and Smina techniques for precision. Virtual search predictions were precise because we obtained similar results with all three techniques applied for docking. On probing the 3D structure of the complexes obtained from precise docking, the secoiridoids' inhibitory binding to SARS-CoV-2 targets was explicit. The residues Glu406, Arg408, Gln409, Lys417, Tyr453, Gly504, and Tyr505 of the S protein and Lys26, Asp30, Asn33, His34, Asn90, Gln96, Gln388, Pro389, and Arg393 residues of the ACE-2 receptor marked the binding site for olive secoiridoids. Asp30, Asn33, His34, Pro389, Arg393, Lys417, Tyr453, and Gly504 amino acids are crucial for the binding of the SARS- CoV-2 S protein with human ACE-2 receptor, hence for its entry (Wahedi et al., 2020). The potential of olive secoiridoids to bind to the critical residues at the interface suggests that
they can prevent the viruses' S protein binding to ACE-2 and block the entry of SARS-CoV-2 into the host (Khan et al., 2020). Olive secoiridoids exhibited lesser binding energy and higher binding efficiency than Chloroquine. Though the binding site was the same, Chloroquine made lesser hydrogen bonds than secoiridoids. Exploring the 3D structures of secoiridoids bound to M^{pro}
provided insight into the mechanism of binding and inhibition. The binding site of M^{pro} was composed of Thr24, Thr26, Leu27, His41, Met49, Phe140, Leu141, Asn142, Gly143, Ser144,
Cys145, His163, Met165, Glu166, Arg188, Gln189, and Thr190. The SARS-CoV-2 M^{pro} is a cysteine protease, and targeting the catalytic Cys145-His41 residues is indispensable for achieving
efficient inhibition of virus replication in-vivo (Liu et al., 2020b, Zhang et al., 2020). The secoiridoids were able to bind to the active residues Cys145 and His41 of M^{pro}, indicating potential
virus replication inhibition. Olive secoiridoids and Lopinavir interacted similarly with Cys145 and His41, but Lopinavir's binding energy was more than that of secoiridoids. The IC50
values of the most active secoiridoids confirmed their inhibitory potential against SARS-CoV-2 S protein and M^{pro}. Therefore, the secoiridoids NZO and DMO were chosen against S protein and M^{pro}, respectively, and carried over to molecular dynamics simulation studies. Molecular dynamics of apoproteins and protein-ligand complexes constitute a significant paradigm in assessing the
conformational stability of protein and its complex with anyligand in a simulated biological environment (Hospital et al., 2015).

CONCLUSION

In summary, the research identified olive secoiridoids as inhibitors of SARS-CoV-2 entry and replication. The virtual search generated 932 conformers of 78 secoiridoids in the OliveNetTM, unraveling Nüzhenide oleoside (NZO) and Demethyloleuropein (DMO) as the most active SARS-CoV- 2 spike protein and Mpro inhibitors. The potential of olive secoiridoids to combat the hyperinflammatory responses in COVID-19 is an additional benefit. Molecular dynamics of the virus targets bound to the secoiridoids confirmed virus protein stability and secoiridoids interactions. Due to intricate molecular structures, secoiridoids may present problems in oral absorption. Given the longstanding use of olive fruits in Mediterranean and Arabian diets, the bioavailability of secoiridoids can be explicated but needs investigation. Also, the dose of secoiridoids that can combat the virus entry and replication, the amount of secoiridoids in different forms of olives, and recommended daily intake require further research. Besides, in-vitro and in-vivo
studies to substantiate the virtual anti-SARS-CoV-2 activity of olive secoiridoids are imminent. The explored secoiridoids are novel leads for the design, discovery, and development of anti- SARS CoV-2 therapeutics. For now, the known safety of olives as a functional food and the explored anti-SARS-COV-2 activity of olive secoiridoids afford a plausible intervention of SARS-CoV-2
infection and associated hyperinflammatory responses.

REFERENCES

[1] Acar-Tek, N., and Agagündüz, D. (2020). Olive leaf (Olea europaea L. folium): potential effects on Glycemia and Lipidemia. Ann. Nutr. Metab. 76, 63–68. doi: 10.1159/000505508

[2] Alagna, F., Mariotti, R., Panara, F., Caporali, S., Urbani, S., Veneziani, G., et al. (2012). Olive phenolic compounds: metabolic and transcriptional profiling during fruit development. BMC Plant Biol. 12:162. doi: 10.1186/1471-2229-12-162

[3] Ali, M. U., Zulqarnain, M. S. J., ur Rehman, M. A., Rehman, M. A., Alsamadany, H., and Shah, Z. H. (2019). Metabolic and biochemical profiling of phenolic compound and their biosynthesis in oil crops. BJSTR 3, 13652–13661. doi: 10.26717/BJSTR.2019.18.003166

[4] Al-Ruqaie, I., Al-Khalifah, N., and Shanavaskhan, A. (2016). Morphological cladistic analysis of eight popular Olive (Olea europaea L.) cultivars grown in Saudi Arabia using Numerical Taxonomic System for personal computer to detect phyletic relationship and their proximate fruit composition. Saudi J. Biol. Sci. 23, 115–121. doi: 10.1016/j.sjbs.2015.05.008.

[5] BIOVIA (2015). Dassault Systèmes, Discovery Studio Visualizer, v16.1.0.15350. San Diego: Dassault Systèmes. https://discover.3ds.com/discovery-studio- visualizer-download (accessed June 20, 2016).

[6] Bonvino, N. P., Karagiannis, T. C., Boskou, D.-C., Zafiris, E., Hung, A., Ray, N. B., et al. (2018). OliveNetTM. Database (Oxford). doi: 10.1093/database/bay016

[7] Brody, M., Böhm, I., and Bauer, R. (1993). Mechanism of action of methotrexate: experimental evidence that methotrexate blocks the binding of interleukin 1β to the interleukin 1 receptor on target cells. Eur. J. Clin. Chem. Clin. Biochem. 10:667. doi: 10.1515/cclm.1993.31.10.667

[8] Castejón, M. L., Montoya, T., Alarcón-de-la-Lastra, C., and Sánchez- Hidalgo, M. (2020). Potential protective role exerted by Secoiridoids from Olea europaea L. in cancer, cardiovascular, neurodegenerative, aging-related, and immunoinflammatory diseases. Antioxidants 9:149. doi: 10.3390/antiox9020149

[9] Celano, M., Maggisano, V., Lepore, S. M., Russo, D., and Bulotta, S. (2019). Secoiridoids of olive and derivatives as potential coadjuvant drugs in cancer: a critical analysis of experimental studies. Pharmacol. Res. 142, 77–86. doi: 10.1016/j.phrs.2019.01.045

[10] Chen, Q., Niu, X., and Li, N. (2017). Exploring the natural chemiome to target interleukin-6 receptor (IL-6R) cytokines: an atomic scale investigation for novel rheumatoid arthritis drug discovery. Braz. J. Pharm. Sci. 53:e17256. doi: 10.1590/s2175-97902017000317256

[11] Conte, P., Fadda, C., Del Caro, A., Urgeghe, P. P., and Piga, A. (2020). Table olives: an overview on effects of processing on nutritional and sensory quality. Foods 9:514. doi: 10.3390/foods9040514

[12] da Silva Rocha, S. F., Olanda, C. G., Fokoue, H. H., and Sant'Anna, C. M. (2019). Virtual screening techniques in drug discovery: review and recent applications. Curr. Top. Med. Chem. 19, 1751–1767. doi: 10.2174/1568026619666190816101948

[13] Daina, A., Michielin, O., and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7:42717. doi: 10.1038/srep.42717

[14] DE Shaw Research (2020). Download Desmond. https://www.deshawresearch.com/ downloads/download_desmond.cgi/ (accessed July 10, 2020).

[15] De Vivo, M., Masetti, M., Bottegoni, G., and Cavalli, A. (2016). Role of molecular dynamics and related methods in drug discovery. J. Med. Chem. 59, 4035–4061. doi: 10.1021/acs.jmedchem.5b01684

[16] Fernández-Poyatos, M., Ruiz-Medina, A., and Llorent-Martínez, E. (2019). Phytochemical profile, mineral content, and antioxidant activity of Olea europaea L. cv. Cornezuelo table olives. Influence of in vitro simulated gastrointestinal digestion. Food Chem. 297:124933. doi: 10.1016/j.foodchem.2019.05.207

[17] Fraihat, S., Gilbert-López, B., Molina-Díaz, A., and Sabouni, I. (2017). Physicochemical characterization of olive oil from Aljouf area of Saudi Arabia. Int. J. Chemtech. Res. 10, 1004–1010.

[18] Gao, Y. M., Xu, G., Wang, B., and Liu, B. C. (2020). Cytokine storm syndrome in coronavirus disease 2019: a narrative review. J. Int. Med. doi: 10.1111/joim.13144

[19] Ghanbari, R., Anwar, F., Alkharfy, K. M., Gilani, A.-H., and Saari, N. (2012). Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)—a review. Int. J. Mol. Sci. 13, 3291–3340. doi: 10.3390/ijms13033291

[20] Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Marino Gammazza, A., Knap, N., et al. (2018). Potential health benefits of olive oil and plant polyphenols. Int. J. Mol. Sci. 19:686. doi: 10.3390/ijms19030686

[21] Gurung, A. B., Ali, M. A., Lee, J., Farah, M. A., and Al-Anazi, K. M. (2020). Unravelling lead antiviral phytochemicals for the inhibition of SARS- CoV-2 Mpro enzyme through in silico approach. Life Sci. 255:117831. doi: 10.1016/j.lfs.2020.117831.