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In Silico Screening of Breadfruit (*Artocarpus altilis*) Prenylated Flavonoids Identify Potential SARS-CoV Inhibitors

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a global health threat. Traditional herbals and dietary plants with medicinal values have a long antiviral history and, thus, are extensively studied in COVID-19 therapeutics development. Breadfruit (*Artocarpus altilis*) is a food crop with rich nutrient composition. This study screened selected breadfruit prenylated flavonoids for their potential inhibitory activities against the SARS-CoV family receptors using molecular docking and molecular dynamics (MD) simulation. The *A. altilis* prenylated flavonoids were selected as target ligands (artocarpin, artoindonesianin V, artonin M, cudraflavone A and cycloartobiloxanthone) and molecular targets from the SARS-CoV family were designated as receptors. Molecular docking was applied with the Lamarckian Genetic algorithm to measure the receptor-ligand orientation using AutoDock Vina software. The structural interactions of the receptor-ligand complexes were visualised using the Biovia Discovery Studio 4.5. Under all possible receptor-ligand combinations, the complexes' minimum binding affinities (MBA) ranged from -5.5 to -9.1 kcal/mol and held by hydrophobic interactions, hydrogen bonds and electrostatic attractions. Receptor-ligand complexes with the least MBA (<-6.0 kcal/mol) along with strong structural interactions were validated by MD simulation using the GROMACS software. The 5RE4-artocarpin and 5RE4-artoindonesianin V showed the

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highest hydrophobic interactions at MBA=-6.6 kcal/mol and -6.4 kcal/mol, respectively. The trajectory analysis of 5RE4-artocarpin and 5RE4-artoindonesianin V complexes was fairly stable throughout a 50 ns MD simulation run. The findings conclude that artocarpin and artoindonesianin V are good potential SARS-CoV family receptor inhibitors.

Keywords: Artocarpus altilis, COVID-19, dietary plant, herbal medicine, molecular docking, prenylated flavonoids, SARS-CoV-2, traditional medicine

INTRODUCTION

Breadfruit *(Artocarpus altilis),* commonly known as 'sukun' in Malaysia and Indonesia, is a multi-purpose tree. The fruit tree is native to New Guinea, Moluccas (Indonesia) and the Philippines (Sikarwar et al., 2018) and was first cultivated in the western Pacific. In tropical regions, the different tree parts are utilised as food (fruit), medicine (fruit, leaves and bark), building material (trunk) and feed (leaves) (Ragone, 2018). The evergreen tree belongs to the Moraceae family and bears large starchy, carbohydrate-rich seeded or seedless fruits. The tree starts to fruit within 3 to 5 years of establishment, thriving well in adverse conditions (Sofoini et al., 2018). Fruits are either oval or oblong, with an average weight of 1.5-2.0 kg (Figure 1). The monoecious tree has been an important staple food in the South-Pacific region for decades (Jamil et al., 2018).

Malaysia is a tropical country with great species diversity. An extensive range of fruit plants, from exotic (minor) to common (major) ones, are included in dietary consumption. Apart from their unique and desirable mouthfeel, taste, and flavour, these fruits are rich in nutraceutical values (Daley et al., 2020; Baba et al., 2016). As such, breadfruit is a well-known edible food often consumed as chips or incorporated in curries among rural Malaysians. It is also used in traditional folk medicine to combat inflammation and

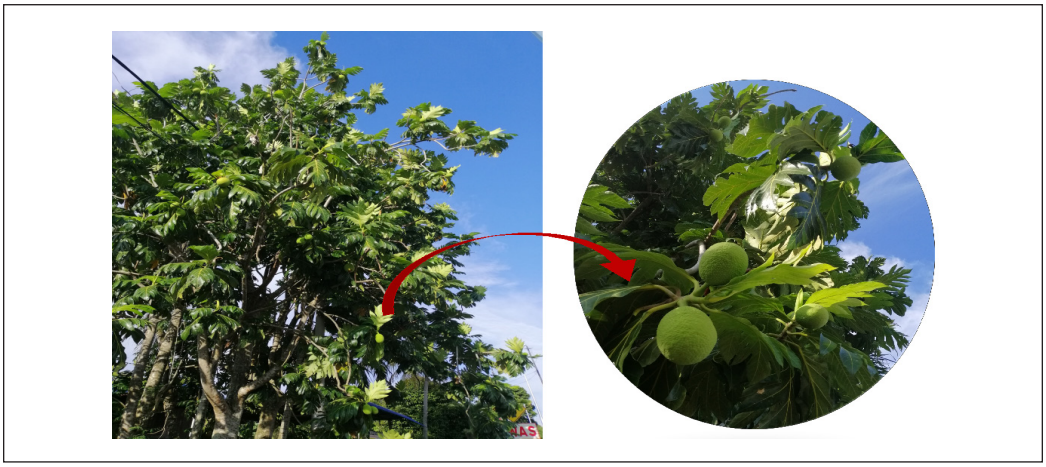

Figure 1. Breadfruit (*Artocarpus altilis*) tree along the roadside at Sungai Merab, Selangor. The image in circle is the zoom view of the unripe fruits

inflammation-associated diseases (Page, 2021; Lin et al., 2012; Fang et al., 2008; Wei et al., 2005). Breadfruit leaves hold pharmacological potential in treating liver cirrhosis, hypertension, renal function and diabetes (Jamil et al., 2018; Baba et al., 2016; Adewole & Oiewole, 2007). The leaves contain bioactive compounds with potent anti-allergenic, anti-inflammatory, anti-microbial and antioxidant activities (Leng et al., 2018). Latex and bark can be used to treat sprains, sciatica, and skin diseases, and the fruit extract has shown cytotoxic effects against human cancer cell lines (Jamil et al., 2018). In Indonesia, *Artocarpus* is used to treat inflammation and malaria fever (Hano et al., 1990).

Phytochemical analyses of various *Artocarpus* species have shown the occurrence of various bioactive compounds, particularly flavonoids, in different plant parts (Jalal et al., 2015): flavonoids, stilbenes, and 4-substituted resorcinols in *A. altilis* heartwood (Shimizu et al., 1998), cyclogeracommunin and artoflavone A in *A. communis* cortex (Lin et al., 2012), prenylflavonoids (Cidade et al., 2001), artomunoisoxanthone, artocommunol C, artochamin D, artochamin B, dihydroartomunoxanthonein in *A. communis* (Weng et al., 2006), cycloartelastoxanthone, artelastoheterol, cycloartobiloxanthone and arthonol A in *A. elasticus* (Ko et al., 2005), artocarpin, artoindonesianin, artonin M, cudraflavone and cycloartbiloxanthone in *A. altilis* heartwood and cortex (Hari et al., 2014; Lan et al., 2013; Amarasinghe et al., 2008; Hakim et al., 2006).

Flavonoids are polyphenols of naturally occurring antioxidants present in higher plants. They display free-radical scavenging, immunomodulating and antiviral properties implicated in pathological disorders such as carcinogenesis, ageing and inflammation (Shah et al., 2016; Lin et al., 2012). The general class of flavonoids have shown antiviral activities against influenza A virus (H1N1), hepatitis B and C virus (HBV/HVC), herpes simplex virus 1 (HSV-1), human immunodeficiency virus (HIV) and Epstein-Barr (Sofoini et al., 2018). No studies have reported on the potential inhibitory activities of breadfruit prenylated flavonoids against the coronavirus family.

Coronavirus disease-2019 (COVID-19) is an unprecedented health crisis of recent times. The disease sparks an inflammatory immune response with the burst of inflammatory cytokines leading to acute respiratory distress syndrome and multi-organ dysfunctionality (Tang et al., 2020). Herbal medicines are claimed to ease disease severity, improve clinical symptoms and reduce mortality. In previous studies, many plant extracts have demonstrated a broad range of immunomodulatory effects on the human immune system (Jantan et al., 2015). Further, plant-based medicines and supplements (traditional Chinese medicine, Ayurveda medicine) are reported to function effectively by minimising the burst of pro-inflammatory cytokine TNF, IL-6 and IL-8, among which are involved in the human immune response against SARS-CoV-2 infection (Rehman et al., 2021; Aucoin et al., 2021; Demeke et al., 2021; Paraiso et al., 2020; Liu et al., 2010).

Due to their potency and safety, natural products are at a better edge than cytotoxic drugs (Ali-Reza et al., 2021). Under this context, screening the breadfruit phytochemicals

against SARS-CoV family receptors to shed information on their antiviral potentials is important to leverage the exploration of plant medicinal properties against infectious disease. In this study, the physical interaction of selected breadfruit prenylated flavonoid-bound SARS-CoV receptor complexes was evaluated via molecular docking and the most stable receptor-ligand complexes was validated by molecular dynamics simulation.

MATERIALS AND METHODS

Protein Files and Pre-Processing

The 3-dimensional structures of SARS-CoV family receptors were retrieved from the Protein Data Bank (PDB) (www.rcsb.org): membrane protein (PDB ID: 3I6G), main protease (PDB ID: 5RE4) and spike glycoproteins (PDB ID: 6VXX and 6VYB). A detailed structural view of each receptor is presented in Figure 2. The receptor files were pre-processed using AutoDock Tools 1.5.6 (Trott & Olson, 2010): remove water molecules, polar hydrogen atoms, and Kollman charges. The pre-processed receptor files were saved in PDBQT format.

Ligand Files and Pre-Processing

A sub-structure search was performed for the following breadfruit flavonoids using PubChem database (https://pubchem.ncbi.nlm.nih.gov): artocarpin (CID: 24850643), artoindonesianin V (CID: 10053761), artonin M (CID: 44258661), cudraflavone A (CID: 5316261) and cycloartobiloxanthone (CID: 10342859) (Figure 2). The ligand files obtained

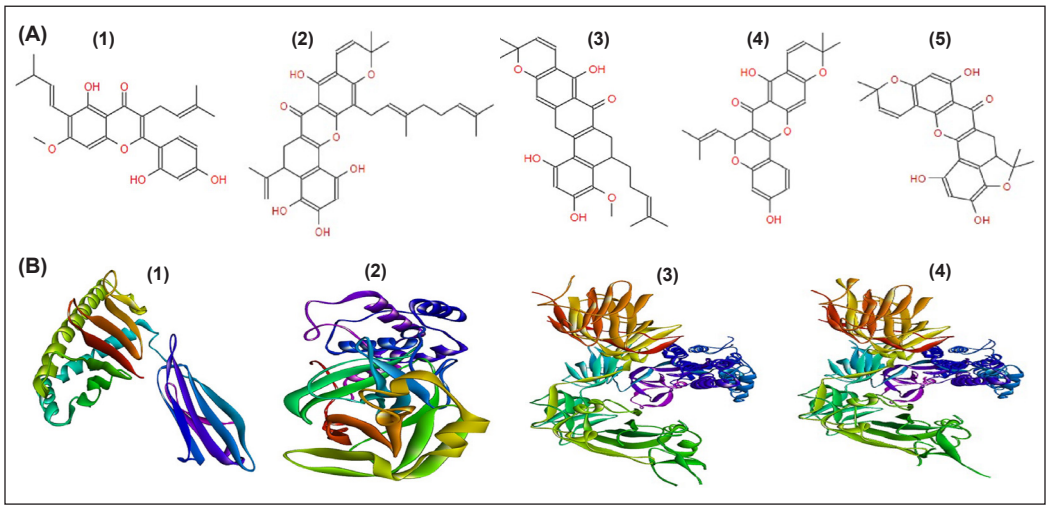

Figure 2. Ligand and receptor structure view. (A) *Artocarpus altilis* prenylated flavonoid 2D structures obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov): (1) artocarpin (CID: 24850643), (2) artoindonesianin V (CID: 10053761), (3) artonin M (CID: 44258661), (4) cudraflavone A (CID: 5316261) and (5) cycloartobiloxanthone (CID: 10342859). (B) The 3D structures of SARS-CoV family receptors retrieved from Protein Data Bank (www.rcsb.org): (1) membrane protein: 3I6G, (2) main protease: 5RE4, (3) spike glycoprotein:6VXX; and (4) spike glycoprotein: 6VYB.

in SDF format were converted into PDB format using Open Babel (www.cheminfo.org), a chemical toolbox for chemical structure inter-conversions (O'Boyle et al., 2011). These structures were then pre-processed using AutoDock Tools 1.5.6 (Trott & Olson, 2010), removing heteroatom, assigning torsion and adding Gasteiger charges. All ligand files were saved in PDBQT format.

Molecular Docking and Visualisation

Four different SARS-CoV family receptors were docked with breadfruit prenylated flavonoids (ligands) under all receptor-ligand, pair-wise combinations. All pre-processed structure files (PDBQT format) were docked using AutoDock Vina 1.1.2 (Trott & Olson, 2010). A grid box of 40x40x40 encompassing the active residues of the receptor was set based on the x, y and z coordinates of the receptor binding pocket region. The Lamarckian Genetic algorithm was applied using its default settings. For each receptor-ligand complex, the docking procedure was repeated thrice. The best conformation for each complex was determined by the minimum binding affinity (MBA) expressed in kcal/mol, root-mean-square-deviation (RMSD) and the extent of favourable interactions between the receptor residues (RR) and ligand atoms. All receptor-ligand complexes were visualised using the Biovia Discovery Studio 4.5 (Dhurga et al., 2016).

Molecular Dynamics Simulation

Based on the molecular docking output, the best receptor-ligand complex with the least minimum binding affinity and fairly high number of interactions was selected and validated by molecular dynamics (MD) simulation using GROMACS version 5.1.4 (http://gromacs.org) and CHARMM General Force Field (cGENFF) program (Vanommeslaeghe et al., 2010). The CHARMM36 all-atom force field (Feb 2021) was retrieved from the MacKerell lab website (http://mackerell.umaryland.edu). The ligand-receptor complex was solvated in a dodecahedron box (edge box set at 10 A°) using the TIP3P water model. The system was neutralised with Na^+/Cl^- ion addition. The energy minimisation steps were set as follows: the maximum number of steps set= 50 000, and the energy step size=0.01. The 'nsteps' set for NVT and NPT equilibration was fixed at 50 000 ns. Molecular dynamic simulation of the ligand-receptor complex was performed under a 50 ns run (Lemkul, 2018; Pronk et al., 2013). The following parameters evaluated the trajectory analysis of the MD run: (1) root-mean-square-deviation (RMSD), (2) root-mean-square-fluctuation (RMSF), (3) radius of gyration (Rog), and (4) the number of hydrogen bonds.

RESULTS AND DISCUSSION

Although COVID-19 has shifted from pandemic to endemic status, ongoing control measures are cautiously deliberated as the viral-specific medication is yet to be discovered.

The most common control measures exercised routinely include regular hand washing, social distancing at crowded places, masking, isolation of infected persons and self-care or immune system enhancement via dietary consumption of nutritious food (Das et al., 2021). The likelihood of being infected with COVID-19 is directly correlated with personal health practices and compliance with general measures (WHO, 2020). Numerous studies on identifying COVID-19 inhibitors have indicated that phytochemicals are an excellent source of therapeutic ingredients.

In this regard, plant-based food (fruits and vegetables) enriched with potent phytochemicals could be exploited for immune system protection and preparation against invading viral infections. No previous research had reported on the breadfruit phytochemicals' inhibitory potential against SARS-CoV family receptors of COVID-19. Scientific information enables prediction, informs, and prepares effective, innovative solutions and holistic management strategies against COVID-19 (Skariyachan et al., 2020). In this study, the breadfruit phytochemicals from the prenylated flavonoid class were screened and evaluated via computational structure-based design (CSD) to understand and shed meaningful insights on breadfruit therapeutics' role against COVID-19 molecular targets.

In recent times, especially considering the COVID-19 pandemic, computational approaches have been rapidly deployed for phytochemical screening, antiviral agent identification, and drug discovery and development (Jorgensen, 2004). With the advent of high-throughput computational architectures coupled with algorithms dedicated to high-level computations, the implementation of CSD, which includes molecular docking and MD simulation, has been accelerated significantly. In a protein-ligand (pharmacophores) docking, the analysis calculates and evaluates the free natural affinity of the ligand to the protein active site. It infers the potential occurrence of interactions between pharmacophores. The protein-ligand conformations are ranked using a scoring function. On the other hand, the MD simulation evaluates the protein-ligand conformations' strength of interactions according to Newton's law of motion (De Vivo et al. 2016).

The molecular docking analysis predicted the interaction between the receptor residues (RRs) and ligand atoms under the lowest energy conformation at root-mean-square deviation (RMSD) =0. The prenylated breadfruit ligands (artocarpin, artoindonesianin V, artonin M, cudraflavone A and cycloartobiloxanthone) were docked with SARS-CoV family receptors, and the minimum binding affinity (MBA) for all the pair-wise receptor-ligand complexes ranged at -5.5 to -9.1 kcal/mol (Table 1). These values were comparable to numerous previous studies reported on SARS-CoV-2 molecular targets and phytochemical computational docking analyses (Kaspi et al., 2022; Khaerunnisa et al., 2020). The SARS-CoV-2 spike protein (PDB ID: 6LU7) bound with gingerol (ginger), allicin (garlic), curcumin, demethoxycurcumin (turmeric), catechin, epicatechin-gallate (tea), nelfinavir,

lopinavir, kaempferol, quercetin, luteolin-7-glucoside and naringenin (phytochemicals) complexes showed MBA= -4.03 to -7.6 kcal/mol (Khaerunnisa et al., 2020). In another study, a total of 18 different compounds isolated from honey and propolis (Dawood, 2020) and java tea (Mohd Kaspi et al., 2022) showed MBA= -5.6 to -7.8 kcal/mol through an *in silico* docking against SARS-CoV-2 molecular targets.

Table 1
The ligand-receptor minimum binding affinity is expressed as energy in kcal/mol

Ligand	Receptors (Protein Data Bank ID)			
	3I6G	5RE4	6VXX	6VYB
artocarpin	-7.2	-6.6	-5.5	-5.5
artoindonesianin V	-8.4	-6.4	-6.1	-5.4
artonin M	-8.5	-7.1	-6.3	-6.5
cudraflavone A	-8.0	-6.8	-5.8	-6.1
cycloartobiloxanthone	-9.1	-7.7	-6.3	-6.0

Note. The first row represents the SARS-CoV receptors, while the first column represents the breadfruit prenylated flavonoids. All numerical values denote the minimum binding affinities expressed in kcal/mol

The receptors selected in this study represented the structural components of SARS-CoV: i) spike protein (6VXX and 6VYB), ii) main protease (5RE4) and iii) membrane protein (3I6G). The first represents the viral envelope type 1 transmembrane S glycoprotein, largely distributed protruding on the surface of mature SARS-CoV-2. The spike protein is the cognate receptor facilitating the viral entry into host cells (ACE2) and then initiates infection. Initial infection is mediated through the fusion of the viral spike protein into the host cell membrane. The main protease (M^{pro}) controls the proteolysis of large polyproteins and papain-like protease (PL^{pro}). Upon SARS-CoV-2, the positive-stranded genomic RNA attaches to the host ribosome for translation into polyproteins. With proteolysis, these polyproteins are orderly packaged into new virions. The membrane and envelope proteins modulate the maturation and retention processes for successful virion assembly (Boson et al., 2021).

The MBA for all the prenylated breadfruit ligand-bound complexes ranged from -6.3 to-5.5 kcal/mol for spike protein receptors. Slightly in a much smaller range, the MBA of main protease-bound ligand complexes ranged between -7.7 to -6.4 kcal/mol. The membrane protein-bound ligand complexes showed the least MBA range at -7.2 to -9.1 kcal/mol. The cycloartobiloxanthone-bound complexes showed the least MBA range at -9.1 to -6.0 kcal/mol, followed by artonin M-bound complexes at -8.5 to -6.5 kcal/mol (Table 1). All the receptor-ligand complexes were held by hydrogen bond, hydrophobic interaction and/or electrostatic interaction except 6VXX-artocarpin and 6VYB-cudraflavone A complexes. In most receptor-ligand complexes, at least two interactions (hydrogen bond and hydrophobic interactions) were present except 6VXX-cycloartobiloxanthone (Table 2).

Table 2
Interactions between the receptor residues and ligand atoms of the ligand-receptor complexes

Receptor-ligand complex	Hydrogen bond	Hydrophobic interaction	Electrostatic interaction	**Total number of interactions**
3I6G-artocarpin	ARG97, TRP147, GLN155	TYR99, LEU156, VAL152, HIS114, TRP147, TYR159, VAL152	-	**10**
3I6G-artoindonesianin V	THR73	TYR99, TYR159, ALA69, LYS66,	ARG97	**6**
3I6G-artonin M	ARG97, LYS146,	TYR159, TRP147, VAL76, HIS70, TYR99, ALA150, VAL152	ARG97	**10**
3I6G-cudraflavone A	TYR7, ARG97, GLU63, GLU63, LYS66	LEU156, LYS66	LYS66	**8**
3I6G-cycloartobiloxanthone	ARG97, LYS146, TRP147	TRP147, ALA150, VAL152	-	**6**
5RE4-artocarpin	VAL77	GLN74, VAL73, ARG76, ARG76, VAL77	ARG76	**7**
5RE4-artoindonesianin V	GLN74, PHE66	ARG76, ILE78, VAL77	ARG76	**6**
5RE4-artonin M	ASP92	VAL73, ARG76	ARG76	**4**
5RE4-cudraflavone A	ARG76	ARG76, VAL73	ARG76	**4**
5RE4-cycloartobiloxanthone	GLN74, LEU75, LEU67	VAL77	ARG76	**5**
6VXX-artocarpin	-	-	-	**-**
6VXX-artoindonesianin V	ASN87	THR236	-	**2**
6VXX-artonin M	PHE86, ASN87	THR236, PRO85	-	**4**
6VXX-cudraflavone A	ASN196	THR236	-	**2**
6VXX-cycloartobiloxanthone	GLN115, ASN234	-	-	**2**
6VYB-artocarpin	ASN87, ASN196	THR236, LEU54, ILE197	-	**5**
6VYB-artoindonesianin V	ASN87, ASN196, ASP88	THR236	-	**4**
6VYB-artonin M	ASN87, THR236	THR236, PRO85	-	**4**
6VYB-cudraflavone A	-	-	-	**-**
6VYB-cycloartobiloxanthone	ASN234, ASN196, ASN87	THR236	-	**4**

Based on the MBA values and the extent of structural interactions characterisation, the 5RE4-artocarpin and 5RE4-artoindonesianin V complexes were selected for further validation by MD simulation. The root-mean-square-fluctuation (RMSF) of 5RE4-artocarpin and 5RE4-artoindonesianin V ranged from 0.08-0.4 and 0.1-0.5, respectively (Figure 3A). A high RMSF suggests the occurrence of the flexible region within the structure complex, whereas a much lower value may implicate the presence of a secondary structure

(O'Boyle et al., 2011). The results may also suggest the stabilising effect of artocarpin and artoindonesianin V on the structure complex. The root-mean-square-deviation (RMSD), which corresponds to the conformational stability and dynamics of the complexes, was calculated for all the backbone residues. In general, the receptor-ligand complex structure remained stable for 50 ns at the following range: 5RE4-artocarpin; 0.18-0.32 ns and 5RE4-artoindonesianin V; 0.2-0.5 ns (Figure 3B). The RMSD values indicate fairly strong complex structural stability at adhering the ligand molecule at the binding site throughout the simulation run. The radius of gyration of the 5RE4-artocarpin complex was slightly higher than the 5RE4-artoindonesianin V complex (Figure 3C). Likewise, the number of hydrogen bonds formed within the 5RE4-artocarpin complex was greater than 5RE4-artoindonesianin V. At least three hydrogen bonds were consistently present throughout the 50 ns MD simulation in both the 5RE4-artocarpin and 5RE4-artoindonesianin V complexes (Figure 3D).

Prenylated flavonoids have wide pharmacological properties, highly beneficial to human health; they are anti-inflammation, anti-Alzheimer, antioxidant, anti-diabetes, vasorelaxant and cytotoxic (Shi et al., 2021). Artocarpin is abundantly distributed in the genus of Artocarpus (*Artocarpus communis, Artocarpus integrifolia, A. lakoocha*).

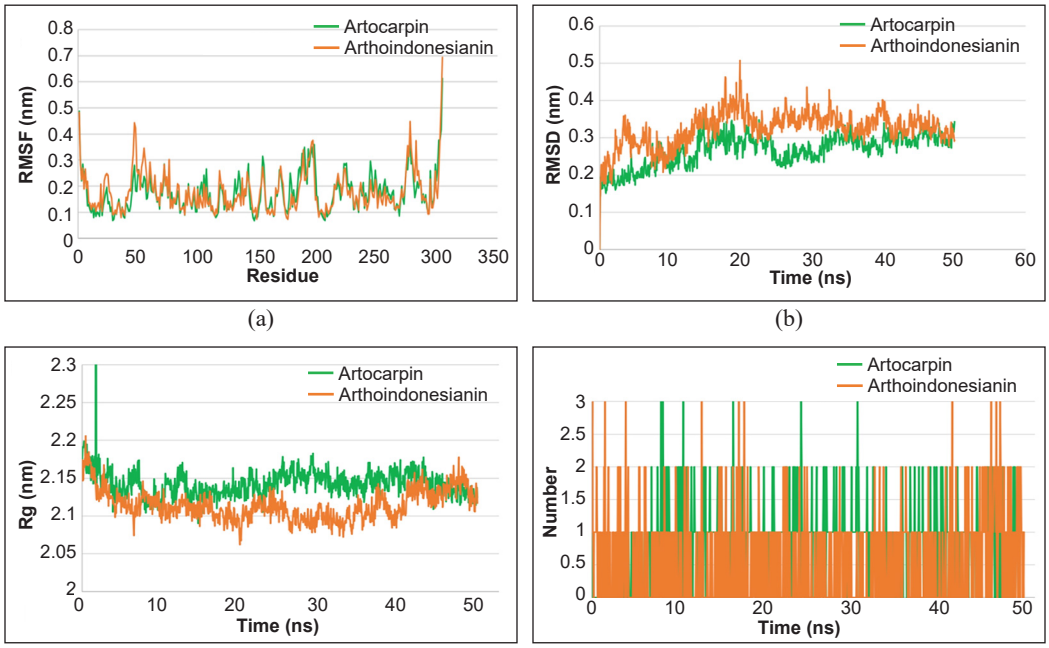

Figure 3. Trajectory analysis of 5RE4-artocarpin and 5RE4-artoindonesianin V, receptor-ligand complexes under a 50 ns molecular dynamics (MD) simulation run at 300 K (temperature) and 1 bar pressure. The green and orange lines represent 5RE4-artocarpin and 5RE4-artoindonesianin V, respectively. (a) Root-mean-square-deviation (RMSD), (b) Root-mean-square-fluctuation (RMSF), (c) Radius of gyration (Rg) and (d) The number of hydrogen bonds

Previous reports have demonstrated interesting biological activities of artocarpin, such as inhibitory effects on melanin biosynthesis, antibacterial activities, and cytotoxicity. On the other hand, the artoindonesianin V is not only found in stem bark extracts of *A. altilis* (Shamaun et al., 2010) but is also present in the heartwood of *A. champeden*, locally known as chempedak in Malaysia. Artoindonesianin V has shown cytotoxic effects against murine leukaemia P38 cells (Hakim et al., 2006). The artonin (art) M has been reported to be present in *A. altilis* (Hano et al., 1990) and *A. rotunda* (Suhartati et al., 2008). No studies have recorded specific medicinal effects/actions against human cell lines (Bailly, 2021). Similarly, cudraflavone A and cycloartobiloxanthone medicinal properties are least reported compared to other prenylated flavonoids identified in the genus Artocarpus. Cudraflavone A is reportedly present in *A. communis* root bark (Shieh & Lin, 1992) and *A. altilis*. In a study by Septama et al. 2018, artocarpanone and artocarpin compounds were shown to suppress the phagocytosis of phagocyte cells.

Considering the new norm of living with COVID-19, there is an ever-growing awareness among the public for better health management. The demand for dietary supplements, herbal products, and fortified diets is soaring. Phytochemicals are excellent sources for pharmacological applications often integrated as immune health boosters (Chang et al., 2021). Non-pharmaceutical interventions in infectious disease management are gaining momentum with the inclusion of dietary bioactive compounds, which are good nutraceuticals. Under this context, breadfruit prenylated flavonoids from a readily available fruit crop within the tropical region can be integrated into the human diet, enhancing the immune health system with better protection against the SARS-CoV-2 of COVID-19 (Bhat & Paliyath, 2016; Shi et al., 2021).

CONCLUSION

Breadfruit prenylated flavonoids hold good therapeutic potential in COVID-19 prevention and management. The current findings predicted a good minimum binding affinity between artocarpin, artoindonesianin V, artonin M, cudraflavone A and cycloartobiloxanthone and SARS-CoV family receptors. The MD simulation analysis of artocarpin and artoindonesianin V compounds provides valuable insights into developing new breadfruit-based therapeutic agents for COVID-19 treatment and management. However, complementary laboratory scale experiments are required prior to upstream application strategies.

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