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Chemical constituents from coconut waste and their in silico evaluation as potential antiviral agents against SARS-CoV-2

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ABSTRACT

Eleven compounds were isolated from the ethyl acetate extract of Cocos nucifera L endocarp, jezonofol 1, scirpusin A 2, cassigal G 3, maackin A 4, threouguaiac rotic acid 8-vanillic acid ether 5, erythroguaiacyl rotic acid 8-vanillic acid ether 6, apigenin-7-O-β-D-glucoside 7, piceatannol 8, p-hydroxy-benzoic acid 9, protocatechuic acid 10 and vanillic acid 11. Compounds 1-7 were isolated for the first time from the plant. The isolated compounds were virtually screened against four critical components of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the main protease (M\textsuperscript{Pro}), papain-like protease (PL\textsuperscript{Pro}), nonstructural protein 13 (nsp13) and RNA dependent RNA polymerase (RdRp). Stilbene dimer 1-4 showed remarkable binding affinity towards the investigated targets (binding energy < -7.6 kcal/mol). Compounds 1, 3 and 4 interacted with the catalytic dyad (Cys145-His41) at the active pocket of M\textsuperscript{Pro} which is essential for achieving good inhibitory activity. Compounds 1-3 showed molecular interaction with the conserved ubiquitin-specific propeptide residues of PL\textsuperscript{Pro}, responsible for binding ability at different active sites of nsp13, which are crucial for decreasing the resistance caused by viral immune evasion. Compounds 2 and 3 showed the ability to bind at different active sites of nsp13, which is a key binding site for reducing antiviral resistance. Finally, compounds 1-3 showed the ability to bind with RdRp before and after RNA binding. Our findings suggested that the dimeric stilbene skeleton is a promising candidate for developing anti-COVID-19 drugs. Particularly, 1, 2 and 3, showed a promiscuity pattern binding to multiple targets of SARS-CoV-2 replication. Herein, 20 ns molecular dynamics (MD) simulations combined with molecular mechanics-generalized Born surface area (MM-GBSA) binding energy calculations were performed to estimate the binding affinity of the most potent three compounds against the viral SARS-CoV-2 targets. MM-GBSA calculations unveiled the outshine potency of compound 1 towards PL\textsuperscript{Pro} with a binding energy of ~60.7 kcal/mol. Structural and energetic analyses over 20 ns MD simulation displayed the high stability of compound 1 in complex with PL\textsuperscript{Pro}. The list of the compounds was considered herein forms a primer for clinical investigation in COVID-19 patients and directing for further antiviral examinations. Drug likeness properties of compounds 1-4 were evaluated.

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1. Introduction

The COVID-19 outbreak is an emerging health and economic crisis. The disease is caused by a novel severe acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Due to the lack of effective antiviral drugs, COVID-19 has become pandemic. Researchers have identified several molecular targets that are critical in the viral life cycle of SARS-CoV-2. In this manuscript we have focused on four therapeutic targets of SARS-CoV-2, the main protease (M\textsuperscript{Pro}), papain-like protease (PL\textsuperscript{Pro}), nonstructural protein 13 (nsp13), and RNA-dependent RNA polymerase (RdRp) (Kong et al., 2020). Upon entry into the host cell, the virus initiates the synthesis of two large replicative polyproteins, the viral proteases PL\textsuperscript{Pro} and M\textsuperscript{Pro}, and cleaves these polyproteins to release 16 nonstructural proteins (nsp5), including nsp13 and RdRp. These proteins are key components of replication-transcription complexes crucial for the life cycle of SARS-CoV-2 (Gil et al., 2020). Discovery of compounds that can block any of these key targets will feed the drug pipeline with potential antiviral candidates against COVID-19.
Natural products have been the main source for drug discovery for centuries. However, a large number of isolated compounds might pose a challenge to determine the corresponding biological activity. In this context, virtual screening proved to be a promising tool for prediction and recognition of pharmacological activities of natural products (Elgazar et al., 2019; Rollinger et al., 2008).

Cocos nucifera L. possesses numerous nutritional, industrial, and medicinal values (DebMandal and Mandal, 2011; Lima et al., 2015; Prades et al., 2016); however, its chemistry has been poorly investigated. Besides, recycling coconut waste as a sustainable source for natural compounds brings environmental and economic merits to overcome the depleting natural resources.

Polyphenols were previously reported from green husk (Akhter et al., 2010) and husk fibers (Rencoret et al., 2013). The endocarp, despite being rich regarding its phenolic content (Nidhi et al., 2015), its phenolic constituents were not adequately identified. However, Singla and Dubey (2019) predicted some probable phytoconstituents in the endocarp by GC-MS analysis. For this, the authors have focused on investigating the chemistry of the endocarp (Elsbaey and Abdel Bar, 2017; Elsbaey et al., 2019).

Preceding our investigations, we isolated unique piceatannol dimers from the ethyl acetate extract of C. nucifera endocarp. Since stilbene-based compounds have been reported as potential drug candidates for COVID-19, this encouraged us to investigate their virtual binding interactions with the potential target receptors of SARS-CoV-2.

According to the literature, piceatannol was reported to possess a good binding affinity to the spike protein of SARS-CoV-2 (Pandey et al., 2020; Wahedi et al., 2020). In addition, synthetic resveratrol analogs were previously evaluated as potential inhibitors of SARS-CoV-2 (Li et al., 2006).

Meanwhile, the antiviral activity of some stilbene monomers was adequately reported, little is known about the efficacy of the oligomeric derivatives. For instance, piceatannol was reported as a potential antiviral agent against human cytomegalovirus (Wang et al., 2020), whereas resveratrol and pterostilbene exhibited antiviral activity against a wide group of viruses, including the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Pandey et al., 2020). This triggered us to perform a virtual screening study to evaluate the ability of the isolated piceatannol dimers to disrupt the viral invasion and replication mechanism by binding to the different molecular targets found in the SARS-CoV-2. In exploring chemical inhibitors to block SARS-CoV-2 viral replication, binding affinities of the most potent compounds were inspected using molecular dynamics (MD) simulations followed by molecular mechanics/generalized Born surface area (MM/GBSA) binding energy calculations. Multi-targets of SARS-CoV-2 involving main protease (MPro), papain-like protease (PLpro), non-structural protein 13 (nsp13), and RNA-dependent RNA polymerase (RdRp), were investigated and analyzed. Therefore, the aim of this study is to evaluate the antiviral potential of piceatannol dimers isolated from C. nucifera as drug candidates against COVID-19.

2. Materials and methods

2.1. Plant material, extraction, and isolation

Cocos nucifera L. var. typica (Tall) was collected from North Sulawesi, North Minahasa, in July 2015. The part used in this study is the sanded endocarp. Authentication was managed by Indonesian Palmae Crops Research Institute and by Ibrahim Mashaly, Professor of Ecology and Botany, Faculty of Science, Mansoura University. A specimen has been deposited at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, under the identification code (07-15-CN-Mansoura). For detailed extraction and isolation procedures, see the supplemental material (Fig. S58).

2.2. General experimental procedures

One and two-dimensional NMR spectroscopy was performed in methanol-d4 on Jeol NMR Spectrometer (500 MHz for 1H and 125 MHz for 13C) and Varian INOVA (600 MHz for 1H and 150 MHz for 13C). High resolution LC-MS analysis was performed using a Bruker maxis HD UHRS-TOF mass spectrometer with an Apollo II ion funnel ESI electrospray source. Chromatographic separation was carried out using silica gel G 60-230 (Merck, Germany), sephadex LH-20 (Sigma-Aldrich, Missouri, USA) and reversed phase silica gel (RP-C18, Bakerbond octadecyl C18, 40 µm) (Phillipsburg, NJ, USA). Thin-layer chromatography was carried out using Merck pre-coated silica gel F254 plates and using vanillin–sulfuric acid spray reagent.

2.3. Molecular docking study

In order to investigate the ability of the isolated compounds to bind to the different targets involved in the replication of SARS-CoV-2, 3D structures of the main protease (MPro), papain-like protease (PLpro), and RNA-dependent RNA polymerase (RdRp), were obtained from PDB using the codes: 6LU7, 6WWU, and 7BV2 respectively, while the 3D structure of helicase (nsp13) was constructed based on the helicase structure of SARS-CoV using the pdb code: 6JYT, which shares similarity with SARS-CoV-2 up to 98.5%. The retrieved 3D structures were prepared using quick prep module in MOE, where water molecules were removed, bond orders were assigned, hydrogens were added, hydrogen bonds were optimized, charges were corrected, and the protein complex was minimized. The prepared PDB files of the protein were used in protein preparation module integrated in PyRx software for virtual screening (Dallakyan and Olson, 2015), where they were converted to pdbqt files and the active sites were defined according to Kong, et al. The grid box size was 30 × 30 × 30 and the coordinates were X: 34.9297, Y: 16.5271, and Z: 16.2044 for MPro; X: -10.85, Y: 12.58, and Z: 68.72 for PLpro; X: 21.83, Y: 69.71, and Z:3.32 for Remdesivir triphosphate (RTP) binding site of RdRp; X: 91.312, Y: 93.155, and Z: 102.826 for the RNA binding site (RNA site); X: 143.95, Y: 145.33, and Z: 156.87 for ADP binding site of nsp13; and X: 143.95, Y: 145.33, and Z: 156.87 for the nucleic acid binding site (nsp13; NCB site) (Kong et al., 2020). Compounds (1-11) were downloaded as mol2 file form zinc database (Sterling and Irwin, 2015), loaded to the ligand preparation module integrated in PyRx and converted to pdbqt. The molecular docking was proceeded using Autodesk vina as the docking engine, where exhaustiveness was set as 12 and the number of poses was 3. The software ranked the poses according to their binding free energy (ΔG), and the docked poses were subjected to analysis to determine how they interacted with amino acid residues in the active site using LigPlot+, which generates 2D presentation of complexes of the docked poses and proteins, where direct bonds are shown as dashed lines while hydrophobic interactions are shown as spline sections (Laskowski and Swindells, 2011). Predicted inhibition constant (K) was calculated based on the following equation:

ΔG = -RTln(k)

2.4. Molecular dynamics simulations

AMBER16 software (Case et al., 2016) was utilized to execute molecular dynamics (MD) simulations for the top potent identified compounds in complex with MPro, PLpro, nsp13 and RdRp. In the MD simulations, the identified compounds and multi-targets of SARS-CoV-2 were described
using general AMBER force field (GAFF2) and AMBER force field 14SB, respectively (Wang et al., 2004; Maier et al., 2015). The docked structures of the identified compounds complexed with the viral targets were neutralized by adding convenient counterions and were solvated in a truncated octahedron box of TIP3P water molecules with an average distance of 12 Å. The specifics of the employed MD simulations are described in Ref. (Ibrahim et al., 2020a, 2020b). In synopsis, the solvated inhibitor-target systems were primarily subjected to energy minimization for 5000 steps using a combination of steepest descent and conjugate gradient algorithms. After that, the inspected systems were progressively heated from 0 K to 300 K over 50 ps. The systems were then equilibrated for 1 ns MD. The production stage was subsequently carried out over 20 ns MD. GPU of pmemd (pmemd.cuda) in AMBER16 was applied to perform all MD simulations on the CompChem hybrid GPU/cluster (hpc.compchem.net).

2.5. MM-GBSA binding energy

The binding free energies ($\Delta G_{\text{binding}}$) of the most potent compounds in complex with the viral targets were estimated with the assistance of molecular mechanics-generalized Born surface area (MM-GBSA) approach (Massova, I. and P.A. Kollman., 2000). Based on the employed MD protocol, two thousand uncorrelated snapshots were obtained from the 20 ns MD simulations and subjected to MM-GBSA binding energy calculations. The MM-GBSA binding energy was estimated as the difference between the energy of the complex and the sum of energies of inhibitor and target.

2.6. Drug-likeness evaluation

The physicochemical parameters of the most promising compounds as SARS-CoV-2 inhibitors were expected with the online Molinspiration cheminformatics software (http://www.molinspiration.com). The anticipated parameters involved topological polar surface area (TPSA), number of hydrogen bond acceptors (nON), the number of rotatable bonds (nrotb), number of hydrogen bond donors (nOHNH), n-octanol/water partition coefficient (milogP), molecular weight (MWt), percent absorption (% ABS), and molecular volume (MVol). %ABS was evaluated as follows (Zhao et al., 2002): %ABS = 109 – [0.345 × TPSA]

3. Results and discussion

3.1. Characterization of the isolated compounds

Apart from our previous investigations (Elsbaey and Abdel Bar, 2017; Elsbaey et al., 2019), the phytoconstituents of coconut endocarp were never identified. Eleven compounds (1-11) were isolated and identified from the ethyl acetate extract of C. nucifera (Fig. 1). They were identified as jezonofol (1) (Wada et al., 2009), scirpusin A (2) (Nakajima et al., 1978), cassigarol G (3) (Baba, et al., 1994), maackin A (4) (Kulesh et al., 1999), threouaguicar-gly–8′-vanillic acid ether (5), erythroguicar-gly–8′-vanillic acid ether (6) (Sakushima et al., 2003), apigenin-7-0-β-D-glucoside (7) (Moussaou et al., 2010), in addition to piceatannol (8), p-hydroxybenzoic acid (9), protocatechuic acid (10), and vanillic acid (11) (Elsbaey and Abdel Bar, 2017). Compounds 1-7, are reported for the first time from C. nucifera in this study. The NMR spectral data of compounds 1-6 are recorded in Tables 1-2, Tables S1-S2 and Fig. S1-S5.

Investigation of $^1$H and $^{13}$C NMR spectral data of compounds 5-6 (Table 2) and comparison with the published literature (Sakushima et al., 1995; Sakushima et al., 1997; Sakushima et al., 2003) revealed that Sakushima et al. (1997) miss-assigned the $\delta$-value of C-6" in the three isomers (Sakushima et al., 1997). Later, Sakushima et al. (2003) republished the NMR data of this isomer with the correct $\delta$-value of C-6". However, they had mistaken again when they gave these structures different numbering patterns for the benzoic acid moiety which is not matching with that for their recorded NMR data (Sakushima et al., 2003). Also, we recorded a different $\delta$-value for C-4" in 5 and 6 (145.6 and 145.8 ppm in methanol-d4, respectively) compared to $\delta$-151.0 ppm in acetone-d6, with a shift +5.4 and 5.2 ppm, respectively. Also, they have assigned H-5 to a signal at 7H 7.89, which is markedly different from our finding (6.98 ppm). These variations could not be due to the difference in the deuterated solvents used in each case. Therefore, this conflict in published NMR data of isomers 5 and 6 motivated us to republish it in the current report relied on confirmed positions using 1D and 2D NMR spectra.

3.2. Molecular docking study

To contribute to the efforts aiming at identifying potential antiviral compounds against SARS-CoV-2, the isolated compounds were virtually screened by docking against four molecular targets associated with the viral replication. The molecular docking studies showed the ability of the isolated compound to bind to multiple targets critical for this virus. The calculated binding free energies (E) and the predicted inhibition constants ($k_i$) were recorded in Table 3. The top four compounds in binding affinity are shown in Table 4 and Fig. 2-8, which depict their interactions with the different molecular targets under investigation.

In the case of Mpro, the oligomeric stibelines 1-4 showed better binding affinity than monomeric form 8 and other isolated compounds. This could be explained by their ability to interact with several key residues found in the active site of Mpro, including H-bonding interactions with Glu166, Cys145, His41, Thr25, Thr26, Arg188, Gln192, and Met49, and hydrophobic interactions, such as His164, Met165, and Gly143 (Fig. 2). Despite the structural similarity between compounds 4 and 2, they showed relatively different binding modes. However, compound 4 showed a slightly better binding affinity than 2 due to the different attachment position of the styryl moiety to the benzofuran ring that allowed H-bonding interactions of the resorcinol ring with Gln192. Similarly, 3,5-dihydroxy substituted benzene in compounds 3 and 8 might contributes to their better binding affinity. The predicted sub-micromolar $k_i$ might be explained in the light of other studies showed that Mpro inhibitors are known to interact with the catalytic dyad (Cys145 and His41), which is essential to achieve good inhibitory activity with this type of proteases (ul Qamar et al., 2020).

Regarding the results of molecular docking with PI3K, compounds 1-4 were the top-ranked inhibitors, respectively. These compounds showed the ability to form H-bonds with the key amino acid residues, including Arg166, Tyr264, Leu162, Met208, and Asp164 (Fig. 3). These residues are conserved ubiquitin–specific protease residues that are responsible for the ability of the virus to escape immune response in host cells (Ratia et al., 2008). In addition, analysis of the binding mode of these compounds showed that they interact with the amino acids found in two hydrophobic subsites S3 and S4, such as Pro248, Tyr264, and Lys157 (Fig. 3). This implies that their inhibitory action might be achieved by allosteric inhibition. Such interactions were absent in the case of compounds 5, 6, and 8 due to their inaccessibility to these pockets. Compound 7 showed remarkable binding affinity, but it does not interact with amino acid residues in the hydrophobic pockets implying that its inhibition mode would be different from compounds 1-4.
Concerning nsp13, compounds 1-4 showed the best binding affinity when the docking was performed using NCB active site coordinates (Fig. 4), while compounds 2-4 and 7 were the top-ranked when the ADP binding site was used (Fig. 5). This implies that compounds 2 and 3 can bind to different active sites in the same enzyme, which would decrease the possibility of resistance that was reported previously in different type of corona virus (Fang et al., 2007). For ADP active site, compounds 2-4 interacted extensively through hydrogen-bonding with residues Gly285, Arg443, Lys320, Ala316, Lys288, and hydrophobic interaction with amino acids Arg442, Gly287, His290, Gly538, Lys320, and Ser289 (Fig. 5). It is worthy to note that all of the docked poses of the compounds were aligned to the known natural inhibitor scutellarein (Yu et al., 2012), yet they achieved better binding affinity.

Compound 7 (Fig. 7D) also achieved good binding affinity which is consistent with previous reports indicating that flavonoids are known to bind effectively to ATP binding site (Miron et al., 2017). On the other hand, compounds 2 & 3 showed different binding modes from compound 1 and scutellarein.

Finally, the molecular docking of the isolated compounds in different conformations of RdRp, before and after RNA binding to the active site, was investigated. Compounds 1-4 achieved better binding affinity over other compounds and the known inhibitor Remdesivir. They also showed the ability to bind to RdRp before and after RNA binding (Fig. 6-7).

It was noticed that compound 1, 2, and 4 tends to bind to RNA bases more frequently than compound 3 which has binding mode similar to Remdesivir, yet its aromatic ring was protruding toward the RNA binding site allowing to form more hydrogen bonds and hydrophobic interaction with purine and pyrimidine base in the RNA such as A19, A11, U9, U18, U20, U12, U10, A13, and G16.

As discussed, the analysis of molecular docking results indicates that the dimeric stilbene skeleton is a promising candidate for developing the next generation of SARS-CoV-2 drugs. Compounds 1-3, in

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**Fig. 1.** Chemical structures of compounds isolated from the ethyl acetate fraction of *Cocos nucifera* L. endocarp.
particular, showed a promiscuity pattern binding to four key targets of viral replication, endowing synergistic antiviral activity, and low possibility of the emergence of future resistance. On the other hand, piceatannol, or the monomeric stilbene form, showed much less binding affinity than the dimeric form. Therefore, dimeric stilbenes might be used as building blocks for designing more potent antiviral agents against SARS-CoV-2, which is consistent with several reports addressing the antiviral activity of this class of compounds (Mattio et al., 2020).

3.3. Molecular dynamics simulation and binding energy calculations

Molecular dynamics (MD) simulations were applied to boost the trustworthiness of predicted the binding energies of the most potent compounds in complex with multi-targets of SARS-CoV-2. Compounds 1–3 complexed with PLpro, NCB site of nsp13, and RdRp, and compounds 2–4 complexed with the ADP site of nsp13, as well as compounds 1, 3, and 4 with Mpro, were further investigated by MD over 20 ns simulation time. Based on the gathered compound-target snapshots over the production stage of 20 ns, the binding energies (ΔGbinding) were calculated using the MM-GBSA approach and presented in Table 5. What is striking about the figures in Table 5 that most compounds displayed promising binding affinities against PLpro with binding affinities (ΔGbinding) with values of −60.7, −42.2, and −44.6 kcal/mol for compound 1, 2, and 3, respectively. On the other hand, the most of compounds demonstrated intermediate binding affinities towards Mpro, RdRp, helicase/NBC site, and helicase/ADP site with MM-GBSA binding energies ranged from −37.4 to −43.1 kcal/mol, from −24.0 to −30.7 kcal/mol, from −37.5 to −39.9 kcal/mol and −37.5 to −41.9 kcal/mol, respectively.

To recognize the nature of the dominant interactions, decomposition of the MM/GBSA binding energy was performed. The calculated energy components for compounds 1–2 and 3–PLpro complexes are summarized in Table 6. As well, the estimated energy components for the most potent compounds in complex with the other SARS-CoV-2 targets are listed in Table S3-S6. To obtain a better view on which energy terms have more in energy components, the estimated energy components were compared. As shown in Table 6 and Table S3-S6, the electrostatic (Eele) and van der Waals

### Table 1

| H/C no. | δCppm (f in Hz) | δHppm (f in Hz) | 1 | 2 | 3 | 4 |
|--------|----------------|----------------|---|---|---|---|
| 1  | 132.0 | 134.7 | 125.0 | 133.6 |
| 2  | 115.6 | 7.12 (d, 2.1) | 113.7 | 6.76 (d, 1.9) | 111.7 | 7.23 (d, 1.0) | 114.2 | 6.82 (d, 2) |
| 3  | 146.8 | 146.4 | 145.4 | 146.5 |
| 4  | 147.7 | 146.4 | 144.6 | 146.4 |
| 5  | 116.4 | 6.86 (d, 8.1) | 116.2 | 6.74 (d, 8.1) | 116.5 | 8.00 (s) | 116.2 | 6.75 (d, 8.4) |
| 6  | 120.6 | 7.01 (dd, 8.2, 2.1) | 118.4 | 6.65 (d, 8.5, 1.9) | 124.4 | 119.0 | 6.70* |
| 7  | 93.7 | 5.35 (d, 10.8) | 94.8 | 5.31 (d, 6.4) | 75.5 | 4.78 (dd, 10.2, 1) | 95.2 | 5.35 (d, 8.4) |
| 8  | 49.1 | 4.45 (dd, 10.7) | 58.3 | 4.34 (d, 6.4) | 76.3 | 4.73 (dd, 10.0, 1, 0.5) | 59.4 | 4.38 (d, 8.5) |
| 9  | 137.2 | 147.5 | 136.5 | 142.4 |
| 10  | 122.4 | 107.4 | 10.17 (d, 1.5) | 103.8 | 6.83 (d, 2.5) | 107.7 | 6.14* |
| 11  | 160.0 | 160.2 | 156.1 | 159.8 |
| 12  | 96.8 | 6.11 (d, 1.8) | 102.1 | 6.18* | 104.9 | 6.38 (d, 2.5) | 102.4 | 6.18 (r, 2) |
| 13  | 159.6 | 160.2 | 158.1 | 159.8 |
| 14  | 104.2 | 6.16 (d, 1.7) | 107.4 | 6.17 (d, 1.5) | 112.5 | 112.7 | 107.7 | 6.14* |
| 1  | 132.0 | 139.0 |
| 2  | 115.6 | 7.12 (d, 2.1) | 120.0 |
| 3  | 146.8 | 162.7 |
| 4  | 147.7 | 96.8 | 6.25 (2) |
| 5  | 116.4 | 6.86 (d, 8.1) | 159.7 |
| 6  | 120.6 | 7.01 (dd, 8.2, 2.1) | 104.3 | 6.63 (d, 2) |
| 7  | 93.7 | 5.35 (d, 10.8) | 123.6 | 6.59 (d, 12.5) |
| 8  | 49.1 | 4.45 (dd, 10.7) | 131.4 | 6.82 (d, 16) |
| 9  | 137.3 | 130.3 | 140.9 | 141.1 |
| 10  | 122.4 | 128.7 | 6.65 (d, 8.5) | 105.9 | 6.49 (d, 2.1) | 105.7 | 6.41 (d, 2.1) |
| 11  | 160.0 | 116.3 | 7.05 (d, 8.5) | 159.7 | 159.8 |
| 12  | 96.8 | 6.11 (d, 1.8) | 158.3 |
| 13  | 159.6 | 116.3 | 7.05 (d, 8.5) | 159.7 | 159.8 |
| 14  | 104.2 | 6.16 (d, 1.7) | 128.7 | 6.65 (d, 8.5) | 150.9 | 150.9 |

*Signal obscured

### Table 2

| H/C no. | δH ppm (f in Hz) | δC ppm (f in Hz) | 1 | 2 | 3 | 4 |
|---------|-----------------|-----------------|---|---|---|---|
| 1  | 7.50, 1H, d (2.0) | 512.9* | 112.9* | 107.9* |
| 2  | 149.4 | 149.5 |
| 4  | 152.0 | 151.9 |
| 5  | 7.06, 1H, d (8.0) | 114.4 | 6.98, 1H, d (8.0) | 114.9 |
| 6  | 123.2 | 7.52, 1H, brd (8.0) | 123.1 |
| 7  | 167.6 | 167.4 |
| 1  | 120.0 | 118.4 | 7.04 (d, 8.3) |
| 2  | 114.4 | 6.98, 1H, d (8.0) |
| 3  | 123.2 | 7.52, 1H, brd (8.0) |
| 4  | 167.6 | 167.4 |
| 5  | 167.6 | 167.4 |
| 6  | 123.2 | 123.1 |
| 7  | 167.6 | 167.4 |
| 1  | 51.1 | 51.1 |
| 2  | 51.1 | 51.1 |
| 3  | 51.1 | 51.1 |
| 4  | 51.1 | 51.1 |

**Integration is abnormally less than expected value due to steric effect at C-7.**
3. Post-MD dynamics analysis

Molecular docking calculations, as well as MD simulations combined with MM-GBSA binding energy calculations, exposed the investigated compounds as prospective SARS-CoV-2 inhibitors. MD-based analyses would be demanded to exhibit structural and energetic stabilities for the investigated compounds in complex with Mpro, PLpro, RdRp, helicase/NCB site, and helicase/ADP site interactions. The structural and energetical analyses involved binding energy per-frame and root-mean-square deviation (RMSD).

MM-GBSA binding energy per-frame for the most three potent compounds with PLpro were estimated through 20 ns MD simulations (Fig. 9A). As well, the MM-GBSA binding energy per-frame for the three most potent compounds in complex with the other viral targets were illustrated in Fig. S5A-S5A-57A. As shown in Fig. 9A, it is apparent that the overall stabilities for compound 1-PLpro, compound 2-PLpro, and compound 3-PLpro complexes were remarked during the MD simulation with average binding energies ($\Delta G_{\text{binding}}$) of $-60.7$, $-42.2$, and $-44.6$ kcal/mol, respectively. In other respects, the investigated compounds in complex with the other viral targets also demonstrated satisfactory stability throughout the MD simulation (Fig. 9A).
Fig. 2. 3D presentation of top 4 ranked compound in the active site of Main protease (Mpro). PDB code: 6LU7; A) Jezonofol (1) (yellow), B) Cassigarol G (3) (red), C) Maackin A (4) (green), and D) Scirpusin A (2) (magenta) aligned to the co-crystallized ligand, peptide-like inhibitor (blue).

Fig. 3. 3D presentation of top 4 ranked compounds in the active site of papain like protease (PLpro). PDB code: 6WUU; A) Scirpusin A (2) (yellow), B) Cassigarol G (3) (red), C) Jezonofol (1) (green), and D) Maackin A (4) (magenta) aligned to the co-crystallized ligand, peptide-like inhibitor (blue).
Fig. 4. 3D presentation of top 4 ranked compounds in the NCB active site of nsp13, PDB code: edited 6JYT; A) Jezonofol (1) (yellow), aligned to the standard inhibitor, scutellarein (Blue), B) Cassigarol G (3) (red), C) Scirpusin A (2) (green), and D) Maackin A (4) (magenta).

Fig. 5. 3D presentation of top 4 ranked compounds in the ADP active site of nsp13, PDB code: edited 6JYT; A) Cassigarol G (3) (yellow), B) Scirpusin A (2) (red), C) Maackin A (4) (green), and D) Apigenin-7-O-β-D-glucoside (7) (magenta) aligned to the standard inhibitor, scutellarein (blue).
Fig. 6. 3D presentation of top 4 ranked compounds in the RTP binding site of RdRp, PDB code: 7BV2; A) Jezonofol (1) (yellow), B) Cassigarol G (3) (red), C) Scirpusin A (2) (green), and D) Maackin A (4) (magenta) aligned to the standard inhibitor, Remdesivir triphosphate, RTP (blue).

Fig. 7. 3D presentation of top 4 ranked compounds in the RNA binding site of RdRp, PDB code: 7BV2; A) Jezonofol (1) (yellow), B) Cassigarol G (3) (red), C) Scirpusin A (2) (green), and D) Maackin A (4) (magenta).
These findings revealed good enough stabilities of the ligand-enzyme complexes.

To explore the dynamic stability of the investigated compound-target complexes, the root-mean-square deviations (RMSDs) for the whole complex backbone atoms were calculated (Fig. 9B and Fig. S54B-S57B). The RMSD plot demonstrated that all investigated systems realized equilibrium in a short time. The RMSD values of all systems fluctuated around 0.3 nm. The current results confirm that all investigated compounds are tightly bonded in the active site and do not influence the overall topology of the viral targets. Eventually, these post-MD energetic and structural analyses proved the high stability of the investigated compound-target complexes over 20 ns MD simulations.

Fig. 8. 2D representation of interactions of, A) Jezonofol (1) in the active site of main protease (Mpro), PDB code: 6LU7, B) Jezonofol (1) in the active site of papain-like protease (PLpro), PDB code: 6WUU, C) Cassigarol G (3) in the NCB active site of nsp13, PDB code: edited 6JYT, D) Cassigarol G (3) in the ADP active site of nsp13 aligned to the standard inhibitor, scutellarein (blue), PDB code: edited 6JYT, E) Jezonofol (1) in the RTP binding site of RdRp, PDB code: 7BV2, and F) Cassigarol G (3) in the RNA binding site of RdRp, PDB code: 7BV2.
3.5. Drug-likeness evaluation

To estimate the drug-likeness properties of compounds (1-4), their pharmacokinetic properties were evaluated considering Lipinski’s rule using Molinspiration cheminformatics software. The estimated parameters are tabulated in Table 7. What is striking about the values in Table 7 is the mlogP being approximately five for the four scrutinized compounds (calc. in range 5.0 to 5.5), proposing that the investigated compounds have satisfactory permeability through the cell membrane. Furthermore, their molecular weights were found to be less than 500 (calc. in range 468.5 to 484.5), should be facilely transported, diffused, and absorbed. Additionally, the number of hydrogen bond acceptors (nON) was less than ten on the basis of Lipinski’s rule, and the number of hydrogen bond donors (nOHNH) was in the range 6 to 7. It is worth noting that this little superfast in hydrogen bond donors will not have a considerable influence on molecule transportation as well as diffusion, where it has been documented that many FDA-approved drugs proceeded beyond the conventional hydrogen bond donors of 5 (Mullard, 2018). As well, TPSA of all auspicious molecules was noticed in range 134.5 to 154.7 Å², which was an ideal benchmark of the bioavailability of the scrutinized compounds. Besides, the estimated %ABS was in the range 55.6% to 62.6%, indicating that the scrutinized compounds may have good cell membrane permeability and oral bioavailability.

| Table 7 |
| Predicted physiochemical parameters of the most promising compounds as SARS-CoV-2 inhibitors |

| Compound     | mLogP | TPSA | nON | nOHNH | nviolation | Nrotb | MolVol | MWt | %ABS  |
|--------------|-------|------|-----|-------|------------|-------|--------|-----|-------|
| Jezonofol (1)| 5.0   | 147.7| 8   | 6     | 2          | 2     | 386.7  | 480.4 | 58.1% |
| Scirpusin A (2)| 5.5 | 147.7 | 8   | 6     | 2          | 2     | 397.5  | 482.4 | 58.1% |
| Cassigarol G (3)| 5.2 | 134.5| 7   | 6     | 2          | 4     | 399.4  | 468.5 | 62.6% |
| Maackin A (4)| 5.1   | 154.7| 8   | 7     | 2          | 4     | 407.4  | 484.5 | 55.6% |

4. Conclusion

This study pointed out the importance of coconut waste as a sustainable source for naturally occurring compounds with prospected medicinal value. Four piceatannol dimers were isolated from Cocos nucifera L. endocarp for the first time. The docking study revealed their ability to bind to multiple key targets that are critical for SARS-CoV-2 replication, endowing promiscuity, synergistic activity, and low incidence of resistance. Our findings reveal that piceatannol...
dimers are promising anti-COVID-19 drug candidates and open the door to future in vitro and in vivo studies of this important group against SARS-CoV-2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2021.05.018.

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