Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
ORIGINAL ARTICLE

Computational screening and biochemical analysis of *Pistacia integerrima* and *Pandanus odorifer* plants to find effective inhibitors against Receptor-Binding domain (RBD) of the spike protein of SARS-Cov-2

Gobindo Kumar Paul\textsuperscript{a,1}, Shafi Mahmud\textsuperscript{a,1}, Afaf A Aldahish\textsuperscript{b}, Mirola Afroze\textsuperscript{c}, Suvo Biswas\textsuperscript{a}, Swagota Briti Ray Gupta\textsuperscript{a}, Mahmudul Hasan Razu\textsuperscript{c}, Shahriar Zaman\textsuperscript{a}, Md. Salah Uddin\textsuperscript{a}, Mohammed H Nahari\textsuperscript{d}, Mohammed Merae Alshahrani\textsuperscript{d}, Mohammed Abdul Rahman Alshahrani\textsuperscript{d}, Mala Khan\textsuperscript{c,*,} , Md. Abu Saleh\textsuperscript{a,*}

\textsuperscript{a} Microbiology Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi 6205, Bangladesh
\textsuperscript{b} Department of Pharmacology and Toxicology, College of Pharmacy, King Khalid University, Abha 62529, Asir, Saudi Arabia
\textsuperscript{c} Bangladesh Reference Institute for Chemical Measurements, BRiCM, Bangladesh Council of Scientific and Industrial Research, Dhammondhi, Dhaka 1205, Bangladesh
\textsuperscript{d} Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Najran University, PO Box 1988, Najran 61441, Saudi Arabia

Received 29 August 2021; accepted 25 November 2021
Available online 1 December 2021

**KEYWORDS**

Plant extracts; SARS-CoV-2; Antioxidant;

**Abstract** Although World Health Organization-approved emergency vaccines are available in many countries, the mortality rate from COVID-19 remains high due to the fourth or fifth wave and the delta variant of the coronavirus. Thus, an effective mechanistic investigation in treating this disease is urgently needed. In this work, we extracted phytochemicals from two mangrove plants,
2.1. Sample collection

The leaves and fruits of *Pistacia integerrima* and *Pandanus odorifer*, assessing their potential actions against the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2. The antioxidant activities of *Pistacia integerrima* leaves and fruits were 142.10 and 97.13 µg/mL, respectively, whereas *Pandanus odorifer* leaves and fruits were 112.50 and 292.71 µg/mL, respectively. Furthermore, leaf extracts from both plants had lower cytotoxicity against *Artemia salina* than fruit extracts. Gas chromatography-mass spectrometry analysis revealed a total of 145 potential phytochemicals from these extracts. Three phytochemicals, 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol, displayed binding free energy values of –8.3, –7.5, and –8.1 Kcal/mol, respectively, in complexes with the spike protein of SARS-CoV-2. The root-mean-square deviation, solvent-accessible surface area, radius of gyration, root-mean-square fluctuations, and hydrogen bonds were used to ensure the binding stability of the docked complexes in the atomistic simulation. Thus, wet-lab validations are necessary to support these findings.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
was used to grind each sample individually (Jaipan, Family mate, Mumbai, India). Methanolic extracts were made using the procedure reported by Liu et al. (2008), in which 25 g of ground extracts were soaked in 100 mL methanol solvent and shaken continuously for 7 d at 25 °C in a shaker. The extracts were then filtered using Whatman No. 1 filter paper and allowed to evaporate at room temperature (Wavare et al., 2017). After evaporation, the yield of each extract was 1.95 g, 1.87 g, for Pistacia integerrima leaves and fruits and 1.07 g, 1.17 g for Pandanus odorifer leaves and fruits. The extracts were then kept at 4 °C in an airtight container until needed.

2.3. Antioxidant activity test

The antioxidant activity was estimated using DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) free radical scavenging assay established by Ilahi et al. (2013) with BHT (Butylated Hydroxy Toluene) as a standard (Sharma and Bhat, 2009). The BHT concentration was adjusted to 50, 100, 150, 200, and 250 µg/mL. The entire amount was then increased to 1 mL by adding methanol, followed by 2.5 mL of DPPH solution. After 30 min of conditioning at room temperature, the optical density was measured at 517 nm using a UV–visible spectrophotometer (Analytik Jena, Germany). The percentage scavenging capacity of DPPH was estimated using the formula Mahmud et al. (2021a), and the IC50 value was computed using a linear scatter graph in Microsoft Excel 2010.

2.4. Cytotoxicity test

An in vitro cytotoxicity test was conducted according to the method described by Achakzai et al. (2019) using Artemia salina (brine shrimp nauplii). Briefly, Artemia salina were hatched at room temperature in a particular tank, and the extract concentration of 25, 50, 100, 150, 200, and 250 µg/mL were placed in tubes. Next, 15 larvae were put in each test tube with 10 mL of artificially prepared seawater. Live and dead shrimps were counted after 24 h, and a lethal concentration (LC50) was obtained by plotting the results on an Excel regression scatter graph.

2.5. Gas chromatography-mass spectroscopy analysis

The bioactive compounds from Pistacia integerrima and Pandanus odorifer plants leaves and fruits were analyzed using GC–MS (Shimadzu, Japan; Model GC–MS TQ 8040) analysis with an electronic ionization detector. A fused silica capillary column at a temperature of 50 °C was used (Rxi-5Sil MS, 30 m, 0.25 mm ID, and 0.25 µm). The samples were then taken in a fix split mode at 250 °C. The oven was preheated at 500 °C for 1 min, then 200 °C for 2 min, and finally 300 °C for 7 min. The ionization voltage was adjusted to 70 eV and the electron multiplier was set to 900 V. The unknown spectra of GC–MS were then compared to the spectra of existing chemicals housed in the Wiley or NIST libraries (Profiles et al., 2021). The names of the compounds, molecular formulas, and weights were then determined (A. et al., 2017).

2.6. Ligand preparation

The chemical compounds were obtained from Pistacia integerrima and Pandanus odorifer plant extracts using GC–MS analysis, and their SDF format of 3D structures were retrieved from the PubChem database (http://www.pubchem.ncbi.nlm.nih.gov) (Kim et al., 2016). The mmff94 force field from Avogadro was used to construct and improve the structures of the ligands (Hanwell et al., 2012).

2.7. Protein preparation

The spike receptor-binding domain of the SARS-CoV-2 structure was retrieved from Protein Data Bank (https://www.rcsb.org/) (PDB ID: 6M0J) and cleaned using Discovery Studio (Gao and Huang, 2011). Moreover, the A chain—including angiotensin converting enzyme-2 (ACE2)—was eliminated in Discovery Studio software. Next, a clean protein structure (Spike Protein S1) was minimized using the YASARA Dynamics software employing the AMBER14 force field (Land and Humble, 2018).

2.8. Molecular docking

Molecular docking of all identified compounds in Pistacia integerrima and Pandanus odorifer leaves and fruits extracts was performed using the AutoDock Vina software tool. The ligands were then converted to PDBQT format, with the box size and grid box center set to (X:26.29 Å, Y:12.60 Å, and Z:58.94 Å) and (X:50.33 Å, Y:67.27 Å, and Z:59.25 Å), respectively (Jaghoori et al., 2016; Nguyen et al., 2020; Trott and Olson, 2010). The level of thoroughness was set to 8. The total number of steps, updates steps, and energy difference for the ligand molecules were adjusted to 200, 1, and 0.1, respectively, utilizing the universal force field with conjugate gradient algorithms. The docking calculations were done with the PyRx program (version 0.8), while the binding interactions analysis was done with the Discovery Studio (version 3.0) (Gao and Huang, 2011) and PyMOL software (version 2.3) (Delano, 2002). Furthermore, the DockThor (Santos et al., 2020) and SwissDock program (Grosdidier et al., 2011) were also used to explore the binding energy of the ligand and protein complexes. For the docking investigation, the lowest binding scores were used, and energy was estimated in Kcal/mol.

2.9. Molecular dynamics simulation

A molecular dynamics simulation study was conducted in the YASARA dynamics package in the AMBER14 force field (Land and Humble, 2018; Wang et al., 2004). The docked complexes were initially cleaned and optimized. A hydrogen bond networking system was then applied to the docked complexes. A cubic simulation cell was generated using the TIP3P solvation model in periodic boundary conditions (Harrach and Drossel, 2014). The simulation conditions were set to pH 7.4, 310 K temperature, and 0.9% NaCl. The energy minimization
of the simulation system was achieved using the steepest gradient algorithms (5000 cycles) and annealing method. The simulation system’s time step was set to 2.0 fs. The long-range electrostatic interactions were calculated through the particle mesh Ewald (PME) by a cutoff radius of 8.0 Å (Essmann et al., 1995). The simulation trajectories were saved at every 100 ps interval. The final simulation was ran for 100 ns (Krieger and Vriend, 2015) while adhering to constant pressure and Berendsen thermostat. The root mean square deviation, solvent accessible surface area, radius of gyration, and hydrogen bond were studied using simulation trajectories (Afrose et al., 2021; Chowdhury et al., 2021; Mahmud et al., 2021d,e,a,f,g; Pramanik et al., 2021; Rakib et al., 2021; Uddin et al., 2021).

2.10. ADMET analysis

The Swiss ADME (http://www.swissadme.ch/) (Daina et al., 2017) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/) (Pires et al., 2015) tools were used for the evaluation of the pharmacological properties of ligands where canonical SMILES of the compound were used as the entry system for absorption, distribution, metabolism, and toxicity (ADMET) calculations.

2.11. Statistical analysis

Data were analyzed by GraphPad Prism (version 8.4), and all the values are reported as the mean ± standard error of the mean (SEM). Values were regarded as significantly different at ***p less than 0.001, **p less than 0.01, and *p less than 0.05, with one-way analysis of variance (ANOVA), followed by Dunnett’s test, while two-way ANOVA with repeated measures was used. The in vitro study was performed using triplicate measurements.

3. Results

3.1. DPPH scavenging activity

The free radical scavenging activities of the leaves and fruits extracts of Pistacia integerrima and Pandanus odorifer were demonstrated in Figures 1 and S1. The scavenging percentage of both plants’ leaves and fruits extracts increased gradually as the concentration of the extracts increased. The IC_{50} value of Pistacia integerrima leaves and fruits were 142.10 and 173.58 µg/mL, respectively, while Pandanus odorifer leaves and fruits were 112.50 and 292.71 µg/mL, respectively, while the BHT standard was 145.96 µg/mL. Thus, these results specified that IC_{50} value for fruits is higher (90.37 µg/ml) than for BHT. Thus, these data signify that Pandanus odorifer plant leaves have the superior antioxidant activity than other extracts (Fig. 1), because it has 50% scavenging at 112.50 µg/mL, which is lower than other concentrations.

3.2. Cytotoxic activity

The cytotoxicity of these methanolic plant extracts are shown in Fig. 2 and Figure S2. The LC_{50} value of Pistacia integerrima plant leaves and fruits was 95.54 and 82.27 µg/mL, respectively, while Pandanus odorifer plant leaves and fruits was 117.00 and 67.51 µg/mL. Therefore, these findings clearly demonstrated that Pandanus odorifer plant leaves have less toxic activity than other investigated extracts (Fig. 2) since it revealed 50% mortality at 117.00 µg/mL, which was higher than other concentrations.

3.3. GC–MS analysis

Both Pistacia integerrima and Pandanus odorifer leaves had 40 compounds in GC–MS analysis, whereas fruits had 20 and 45

![Fig. 1](image-url)  
Fig. 1  Antioxidant activity of Pistacia integerrima and Pandanus odorifer plant leaves and fruits extracts. A indicates DPPH scavenging activity of all extracts from both plants and B indicates IC_{50} value of all extracts from both plants. Different significant letters indicate significant differences between mean ± SD of replications (n = 3) at a P ≤ 0.05 significant level.
compounds, respectively (Fig. 3 and S3-S6). The International Union of Pure and Applied Chemistry name, molecular formula, canonical SMILES, and retention time of the compounds are presented in Table S3-S6. The major compounds of *Pistacia integerrima* leaf extracts are Lup-20(29)-en-3-ol, acetate, (3.beta.) (C32H52O2) has the highest concentration (15.24%), followed by beta-sitosterol (C29H50O) with 13.29%, 24-Noroleana-3,12-diene (C29H46) with 12.32%, 9-octadecenamide, (Z) (C18H35NO) with 10.75%. The most prominent compounds of *Pistacia integerrima* fruit extracts include 4-O-Methylmannose (C7H14O6) with the highest proportion (79.26%), followed by gamma-sitosterol (C29H50O) with 38.17%, and stigmasterol (C29H48O) with 37.00%. Moreover, the most significant components of *Pandanus odorifer* leaf extracts are trans-geranylgeraniol (C20H34O) with 30.27%, 3-cyclohexene-carboxylic acid, 6,6-dimethyl-4-(4-morpholyl)-2-oxo-methyl ester (C14H21NO4) with 27.64%, and 5-methyl-Z-5-docosene (C23H46) with 27.15%.

3.4. Docking analysis

Molecular docking analysis was carried out to explore the binding interaction and identify lead molecules with a higher affinity for SARS-Cov-2 spike receptor-binding domain. The three compounds (Fig. 4); 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol had a binding energy...
of − 8.3, −7.5, −8.1 Kcal/mol, respectively (Table 1), which is higher than the other compounds of *Pistacia integerrima* and *Pandanus odorifer* plants. Moreover, the DockThor and SwissDock program was also employed to further validate the docking energy where 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol had −5.985, −6.002, −5.712 Kcal/mol energy in DockThor and −7.38, −7.48, −7.25Kcal/mol, respectively, in SwissDock. Regarding the compounds, 28-demethyl-beta-amyrone had two Pi-alkyl interactions at Phe374, Phe342, and an alkyl bond at Leu368, as well as a Pi-sulfur bond at Trp436 residues (Fig. 5). The 24-Noroleana-3,12-diene and spike protein domain-receptor complexes were stabilized by two alkyl bonds at Val367, Leu368, three pi-alkyl bonds at Phe374, Phe342, and one Pi-sulfur bond at Phe342 (Fig. 6). Moreover, the stigmasterol had two pi-alkyl interactions points at Leu335 and Leu368 residues, whereas three pi-alkyl bonds at Phe374, Phe342, and Phe338 residues (Fig. 7).

### 3.5. ADMET analysis

The ADMET calculations were used to adjudicate the drug-likeness properties of the selected three compounds (Table 2 and Tables S7.1–S7.4). According to the Lipinski rule of five, any molecule with a molecular weight less than 500 g/mol (MW ≤ 500 g/mol) is a feasible drug candidate (Chen et al., 2020). The molecular weights of the three potential compounds were 410.686, 394.687, and 412.702 g/mol for 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol, respectively. To be a plausible drug candidate, a compound’s H-bond acceptors and H-bond donors should not be more than ten and five, respectively, according to the Lipinski rule of five (Alodeani et al., 2015). The numbers of H-bond acceptors were 1, 0, and 1, and the H-bond donors were 0, 0, and 1 for 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol, respectively. The topological polar surface area (TPSA) score must be within the range of 0 Å² to 140 Å² for being a potential drug candidate (Jagannathan, 2019). The TPSA scores were 17.07, 0.00, and 20.23 Å², whereas BBB permeability was 0.705, 0.848, and 0.771 for 28-demethyl-beta-amyrone, 24-noroleana-3,12-diene, and stigmasterol, respectively. The Human Intestinal Absorption were 96.643%, 96.674%, and 94.97%, respectively, for 28-demethyl-beta-amyrone, 24-noroleana-3,12-diene, and stigmasterol confirming the high absorption rate of the selected three compounds in the intestine. Moreover, the three compounds were recognized as P-gp non-substrate in addition to appearing as non-toxic in conformity with AMES toxicity and hepatotoxicity.
profiling. In addition, three potential molecules were thought to fulfill the Lipinski rule of five, with one violation in each compound; however, one violation is always acceptable for being a viable therapeutic candidate (Daina et al., 2017).

3.6. Molecular dynamics simulation

The molecular dynamics simulation of the docked complexes was conducted to analyze the structural stability and the equilibration of the ensemble across the atomistic simulations. Fig. 8(a) demonstrates the root mean square deviations of the C-alpha atoms from the docked complexes. Fig. 8(a) indicates that at the start of simulations, all three complexes, 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol exhibited an initially higher trend. This trend might have occurred as a result of the complexes' increased flexibility. The complexes attained a steady state after 10 ns of the simulation times. The stigmasterol had a somewhat

| Complex                      | Amino Acid Residues | Bond Type | Distance (Å) | Docking Energy (Kcal/mol) | Docking Energy (Kcal/mol) | Docking Energy (Kcal/mol) |
|------------------------------|---------------------|-----------|--------------|----------------------------|---------------------------|---------------------------|
|                              |                     |           |              | AutoDock Vina              | Dockthor                  | SwissDock                 |
| 28-demethyl-beta-amyrone     | Phe342              | H         | 2.02         |                            |                           |                           |
|                             | Leu368              | H         | 2.46         |                            |                           |                           |
|                             | Phe342              | PA        | 5.20         |                            |                           |                           |
|                             | Ser371              | H         | 2.47         |                            |                           |                           |
|                             | Trp436              | H         | 2.07         |                            |                           |                           |
|                             | Arg509              | H         | 2.01         |                            |                           |                           |
|                             | Trp508              | H         | 2.44         |                            |                           |                           |
|                             | Trp436              | APS       | 3.99         |                            |                           |                           |
|                             | Phe374              | PA        | 4.82         |                            |                           |                           |
|                             | Trp436              | PA        | 4.51         |                            |                           |                           |
|                             | Phe338              | H         | 2.01         |                            |                           |                           |
|                             | Tyr365              | H         | 2.46         |                            |                           |                           |
|                             | Ser375              | H         | 2.55         |                            |                           |                           |
|                             | Asn437              | H         | 2.20         |                            |                           |                           |
|                             | Ile368              | PA        | 5.20         |                            |                           |                           |
|                             | Val511              | PA        | 3.93         |                            |                           |                           |
|                             | Ile434              | PA        | 5.17         |                            |                           |                           |
|                             | Phe342              | H         | 2.18         |                            |                           |                           |
|                             | Val367              | H         | 2.54         |                            |                           |                           |
|                             | Phe338              | H         | 2.03         |                            |                           |                           |
|                             | Leu368              | H         | 2.46         |                            |                           |                           |
|                             | Ser371              | H         | 2.80         |                            |                           |                           |
| 24-Noroleana-3,12-diene      | Phe342              | PA        | 5.20         |                            |                           |                           |
|                             | Phe374              | PA        | 5.17         |                            |                           |                           |
|                             | Asp364              | H         | 2.54         |                            |                           |                           |
|                             | Tyr365              | H         | 2.46         |                            |                           |                           |
|                             | Ile434              | PA        | 5.37         |                            |                           |                           |
|                             | Val511              | PA        | 3.93         |                            |                           |                           |
|                             | Leu335              | H         | 2.61         |                            |                           |                           |
|                             | Phe338              | H         | 2.03         |                            |                           |                           |
|                             | Val341              | H         | 2.14         |                            |                           |                           |
|                             | Phe342              | H         | 2.02         |                            |                           |                           |
|                             | Leu368              | H         | 2.46         |                            |                           |                           |
|                             | Ser371              | H         | 2.47         |                            |                           |                           |
|                             | Leu368              | A         | 4.87         |                            |                           |                           |
|                             | Phe338              | PA        | 4.69         |                            |                           |                           |
|                             | Phe342              | PA        | 4.96         |                            |                           |                           |
|                             | Phe374              | PA        | 5.28         |                            |                           |                           |
|                             | Asn334              | H         | 2.63         |                            |                           |                           |
|                             | Cys335              | H         | 2.03         |                            |                           |                           |
|                             | Tyr365              | H         | 2.46         |                            |                           |                           |
|                             | Val362              | H         | 2.51         |                            |                           |                           |
|                             | Cys336              | PA        | 4.69         |                            |                           |                           |
|                             | Ile358              | PA        | 4.91         |                            |                           |                           |
|                             | Ala363              | PA        | 5.02         |                            |                           |                           |
|                             | Ile434              | PA        | 5.37         |                            |                           |                           |
|                             | Val511              | PA        | 3.93         |                            |                           |                           |
greater RMSD than the other two complexes at 40–60 ns, which could explain the higher flexibility of the complexes. The RMSD of all three complexes was less than 2.5, indicating that they have a stable and rigid overall profile. Furthermore, the stable and steady nature of the protein systems is defined by the hydrogen bond patterning of the simulating systems. The hydrogen bond for all three complexes, 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol, was stable and did not fluctuate much [Fig. 8 (b)].

Moreover, the solvent accessible surface area of the complexes was investigated to better understand the changes in the protein’s surface area. The higher SASA, the more the surface area expands, and the lower the SASA, the more the protein is truncated. The 24-Noroleana-3,12-diene had a somewhat greater SASA at 40–45 ns times denoting the extension of the surface area of the complexes [Fig. 9 (a)]. However, the SASA of stigmasterol was lower than that of all three complexes, indicating that the complexes were more condensed with time. The radius of gyration of the complexes determines their liability and mobile nature. Higher Rg indicates that the complexes are more flexible. The 24-Noroleana-3,12-diene had lower Rg than the other two complexes, which indicate a comparatively stable profile of these complexes [Fig. 9 (b)]. The other two complexes had minor variances, but they did not differ much.

4. Discussion

Currently, battling COVID-19 has become a major issue for the entire world due to the virus’s global exposure. Moreover, the virus’s breakout is becoming more severe and causing an
increasing number of deaths. Although many vaccines have been recognized by the WHO, vaccine adoption rates vary by country (Sallam, 2021), and vaccines are not readily available in poor countries, particularly in low-income countries. As of July 20, 2021, only 26.3% of the global population has received at least one dose of vaccine. In contrast, only 1.1% of people in poor countries are now under a vaccination program to administer at least one dose (https://ourworldindata.org/covidvaccinations?country=OWID_WRL&fbclid=I

**Table 2** Pharmacological assessment of the screened hit ligand molecules.

| Parameters                      | 28-demethyl-beta-amyrone | 24-Noroleana-3,12-diene | Stigmasterol |
|---------------------------------|--------------------------|-------------------------|--------------|
| Molecular Weight                | 410.686 g/mol            | 394.687 g/mol           | 412.702 g/mol |
| Num. H-bond acceptors           | 1                        | 0                       | 1            |
| Num. H-bond donors              | 0                        | 0                       | 1            |
| TPSA (S)                        | 17.07 Å²                 | 0.00 Å²                 | 20.23 Å²     |
| BBB permeability                | 0.705                    | 0.848                   | 0.771        |
| Human Intestinal Absorption     | 96.643 %                 | 96.674 %                | 94.97 %      |
| P-glycoprotein substrate        | No                       | No                      | No           |
| AMES Toxicity                   | No                       | No                      | No           |
| Lipinski rule of five           | Yes; 1 violation         | Yes; 1 violation        | Yes; 1 violation |
| Hepatotoxicity                  | No                       | No                      | No           |

**Fig. 7** Docking simulation between spike receptor-binding domain of SARS-CoV-2 and stigmasterol, respectively, where (a) Cartoon view, (b) 3D view, and (c) Surface view.

**Fig. 8** The molecular dynamics simulation. (a) Root mean square deviation of the three docked complexes, (b) hydrogen bond of the docked complexes.
SARS-CoV-2 variants have been identified globally, and the U.S. Centers for Disease Control and Prevention (CDC) classifies them as variants of concern, variants of interest, and variants of significant consequence (Abdool Karim and de Oliveira, 2021). Variants are mostly created through virus mutations, and SARS-CoV-2 variants have recently been discovered in large numbers. Three of them, the B.1.1.7 variant, the 501Y.V2 variant, and the P.1 variant, have become important concerns since they have roughly 23, 23, and 35 mutations, respectively, and have spread rapidly throughout numerous nations. Those mutations are mostly found in the spike protein’s receptor-binding domain, which improves the RBD’s affinity for ACE-2. Several varieties can cause more serious infections, with some being able to spread faster than others and, more crucially, evade the host body’s immune response, which grows after infection and despite immunization (Abdool Karim and de Oliveira, 2021; Walensky et al., 2021).

As a result, producing a traditional medicine by screening a new phytochemical component could be beneficial in treating the virus in this case. We therefore selected two mangrove plants’ leaves and fruits and isolated their components to identify new lead molecules against SARS-CoV-2 using molecular docking and dynamics. Some studies have previously shown that natural plant chemicals can successfully combat pathogenic disease (Joshi et al., 2020; Khaerunnisa et al., 2020), and mangrove plants have also been used for therapeutic purposes since ancient times (Dahibhate et al., 2018).

The higher antioxidant activity of plants is a crucial factor in the creation of new drugs. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is a good way to assess plant antioxidant activity (Choi et al., 2000). Furthermore, certain phytochemical substances have anticancer properties (XiaoPing et al., 2009). In the current investigation, both *Pistacia integerrima* and *Pandanus odorifer* leaf extracts had higher antioxidant activity (142.10 g/mL and 112.50 g/mL, respectively) than their fruits extracts (173.58 g/mL and 292.71 g/mL, respectively) (see Fig. 1). In vitro brine shrimp fatality experiments are also useful for determining the toxicity of plants (Carballo et al., 2002). Both *Pistacia integerrima* and *Pandanus odorifer* leaf extracts showed less toxicity in toxicity tests (95.54 g/mL and 117.00 g/mL, respectively) than their fruits extracts (82.27 g/mL and 67.51 g/mL, respectively) (Figure-2). The GC-MS analysis of *Pistacia integerrima* and *Pandanus odorifer* plant showed 145 phytochemical compounds. Because the antioxidant content of both plant leaves was higher and the toxicity of their fruits extract was lower, these leaves can be considered biological agents with a lot of potential for molecular docking investigations. Finally, potent phytochemicals against the spike receptor-binding domain protein of SARS CoV-2 were identified through computational research.

Plant antioxidants can protect cells from damage by free radicals, and molecular docking studies are used to determine the interaction dynamics of a protein complex (Mahmud et al., 2021b). The results of these two investigations were combined to determine the pharmacological characteristics of the leaf extracts. Antioxidant activity can be checked via molecular docking under *in silico* conditions, but in this study, the antioxidant activity was assessed under lab conditions and molecular docking was used only to assess the properties of phytochemicals derived from *Pistacia integerrima* and *Pandanus odorifer* plants against target proteins.

A structure-based computer-aided virtual screening technique known as molecular docking effectuates a distinct amalgamation of positions, orientations, and conformations through the assessment of the interaction between a protein and a plausible drug molecule utilizing computer-inured three-dimensional structures with the purpose of structure-premised drug design (Chaudhary and Mishra, 2016; Roblin, 1953). In the computational drug discovery process, protein pliability is important for ligand binding. If biologists or researchers seek to figure out how a protein, ligand, or other molecule works, the motions of those unique biomolecules will be quite helpful and provide a wealth of information about their functions. The grandiose diversity of essential bio-molecular systems, such as conformational variation, protein flexion, ligand nexus, and eliciting atomic sites, can be captured using a simulation (Hollingsworth and Dror, 2018).

In docking studies, binding at the active regions of proteins causes the targeted protein to be inhibited (Mahmud et al., 2021c, 2021b). The complexes 24-Noroleana-3,12-diene and
stigmasterol had non-bonded contacts with the active points Phe374 in this investigation, as well as many interactions around the active points of the targeted protein, which could be responsible for the target protein’s inhibition. In addition, numerous descriptors derived from simulated trajectories for docked structures suggested tight conformations and a less flexible character of the complexes. The binding stability of the complexes is correlated with the root mean square deviations for the C-alpha atoms of the docked complexes, radius of gyrations, solvent accessible surface area, root mean square fluctuations and hydrogen bond of the systems. In addition, the top three potential candidates fulfilled the Lipinski rule of five with one violation in each compound, where each compound has a molecular weight less than 500 g/mol, and the H-bond acceptors and H-bond donors are not more than ten and five, respectively. Though each molecule has only one violation of the Lipinski rule of five, which is MlogP > 4.15, one violation is nevertheless always acceptable for being a feasible therapeutic candidate (Daina et al., 2017). As a result, chemicals from Pistacia integerrima and Pandanus odorifer plants may inhibit the receptor domain of the spike protein. These findings must be confirmed through wet-lab synthesis and additional in vivo investigations.

5. Conclusion

Plants, as opposed to synthetic substances, are becoming a more viable source for therapeutic application due to their comprehensive appearance, reduced side effects, and broad specificity. Due to the scarcity of effective vaccinations, the mortality rate from the COVID-19 pandemic is rising by the day. Consequently, we tested the efficiency of leaf and fruit extracts from two mangrove plants (Pistacia integerrima and Pandanus odorifer) as potential SARS-CoV-2 inhibitors. Both plants had significant antioxidant and cytotoxicity effects when methanolic extracts of leaves and fruits were tested. The molecular docking study identified three potential compounds, 28-demethyl-beta-amyrone, 24-Noroleana-3,12-diene, and stigmasterol, as having the maximum binding energy in complexes with the spike receptor-binding domain protein of SARS-CoV-2. This study may aid researchers in developing a viable therapy for SARS-CoV-2. As a result, additional in vivo and in vitro validation is required for the confirmation and safety profiles of the findings of this study.

Funding

This research was partially supported by the Ministry of Science and Technology, Government of the People’s Republic of Bangladesh. The authors are also grateful to the Deanship of Scientific Research at King Khalid University for funding this study through the Small Research Group Project, under grant number GRP/340 /42.

CRediT authorship contribution statement

Gobindo Kumar Paul: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. Shafi Mahmud: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. Afaf A Aldahish: Funding acquisition, Project administration. Mirola Afroze: Formal analysis, Investigation. Swagota Briti Ray Gupta: Data curation, Methodology. Mahmudul Hasan Razu: Formal analysis, Investigation. Shahriar Zaman: Resources, Writing – review & editing. Md. Salah Uddin: Resources, Writing – review & editing. Mohammed H Nahari: Data curation, Methodology. Mohammed Merae Alshahrani: Data curation, Methodology. Mohammed Abdul Rahman Alshahrani: Data curation, Methodology. Mala Khan: Project administration, Resources, Supervision, Writing – review & editing. Md. Abu Saleh: Conceptualization, Funding acquisition, Project administration, Resources, Software, Supervision, Writing – review & editing.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103600.

References

A. Hussein, R., A. El-Annsary, A., 2019. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. Herb. Med. 10.5772/intechopen.76139.
A., S., M., D., B., A., C., C., B., G., G., E., B., D., T., 2017. In vitro evaluation of the antioxidant, cytoprotective, and antimicrobial properties of essential oil from Pistacia vera L. Variety Bronte Hull. Int. J. Mol. Sci. 18.
Abdool Karim, S.S., de Oliveira, T., 2021. New SARS-CoV-2 Variants — Clinical, Public Health, and Vaccine Implications. N. Engl. J. Med. 384, 1866–1868. https://doi.org/10.1056/nejmc2100362.
Achakzai, J.K., Anwar Panzeazi, M., Kakar, M.A., Kakar, A.M., Kakar, S., Khan, J., Khan, N.Y., Khilji, I., Tareen, A.K., 2019. In Vitro Anticancer MCF-7, Anti-Inflammatory, and Brine Shrimp Lethality Assay (BSLA) and GC-MS Analysis of Whole Plant Butanol Fraction of Rheum rubi (WBFRR). Biomed Res. Int. 2019. https://doi.org/10.1155/2019/3264846.
Afrose, S., Hasan, R., Sharmin, M., Shimu, S., 2021. Antiviral peptides against the main protease of SARS-CoV-2: A molecular docking and dynamics study. Arab. J. Chem. 103315. https://doi.org/10.1016/j.arabjc.2021.103315.
Alodeani, E.A., Arshad, M., Izhari, M.A., 2015. Anti-uropathogenic activity, drug likeness, physicochemical and molecular docking assessment of (E-)-N0-(substituted-benzylidene)-2-(quinolin-8-yloxy) acetylhydrazide. Asian Pac. J. Trop. Biomed. 5, 676–683. https://doi.org/10.1016/j.ijpab.2015.04.010.
C., P., M.E., M., A.T., P., G., D.C., V., P., 2020. The “Three Italy” of the COVID-19 epidemic and the possible involvement of SARS-CoV-2 in triggering complications other than pneumonia. J. Neurovirol.
Carballo, J.L., Hernández-Inda, Z.L., Pérez, P., García-Grávalos, M. D., 2002: A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol. 2. https://doi.org/10.1186/1472-6875-2-17.
Chaudhary, K.K., Mishra, N., 2016. A Review on Molecular Docking: Novel Tool for Drug Discovery. JSM Chem 4, 1029.
Chen, X., Li, H., Tian, L., Li, Q., Luo, J., Zhang, Y., 2020. Analysis of the Physicochemical Properties of Acaricides Based on Lipinski’s Rule of Five. J. Comput. Biolog. 27, 1397–1406. https://doi.org/10.1089/cmb.2019.0323.

Choi, H.S., Sun Song, H., Ukeda, H., Sawamura, M., 2000. Radical-scavenging activities of citrus essential oils and their components: Detection using 1,1-diphenyl-2-picrylhydrazyl. J. Agric. Food Chem. https://doi.org/10.1021/jf990551q.

Chowdhury, K.H., Chowdhury, M.R., Mahmud, S., Tareq, A.M., Hanif, N.B., Banu, N., Ali Reza, A.S.M., Emran, T. Bin, Simal-Gandara, J., 2021. Drug repurposing approach against novel coronavirus disease (COVID-19) through virtual screening targeting SARS-CoV-2 main protease. Biology (Basel). 10, 1–14. https://doi.org/10.3390/biology10010002.

Corrêa Giron, C., Laaksonen, A., Barroso da Silva, F.L., 2020. On the interactions of the receptor-binding domain of SARS-CoV-1 and SARS-CoV-2 spike proteins with monoclonal antibodies and the receptor ACE2. Virus Res. 285. https://doi.org/10.1016/j.virusres.2020.198021.

Dahibhate, N.L., Saddhe, A.A., Kumar, K., 2018. Mangrove Plants as Potential Inhibitor of COVID-19 Main Protease (M pro) from Several Medicinal Plant Compounds by Molecular Docking Study. Preprints. 1–14.

Kim, C., Ryu, D.K., Lee, J., Kim, Y.I, Seo, J.M., Kim, Y.G., Jeong, J.H., Kim, M., Kim, J.I., Kim, P., Bae, J.S., Shim, E.Y., Lee, M.S., Kim, M.S., Noh, H., Park, G.S., Park, J.S., Son, D., An, Y., Lee, J. N., Kwon, K.S., Lee, J.Y., Lee, H., Yang, J.S., Kim, K.C., Kim, S., Woo, H.M., Kim, J.W., Park, M.S., Yu, K.M., Kim, S.M., Kim, E.H., Park, S.J., Jeong, S.T., Yu, C.H., Song, Y., Gu, S.H., Oh, H., Koo, B.S., Hong, J.J., Ryu, C.M., Park, W.B., Oh, M. don, Choi, Y.K., Lee, S.Y., 2021. A therapeutic neutralizing antibody targeting receptor binding domain of SARS-CoV-2 spike protein. Nat. Commun. 12, 1–10. 10.1038/s41467-020-20602-5.

Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A., Wang, J., Yu, B., Zhang, J., Bryant, S.H., 2016. PubChem substance and compound databases. Nucleic Acids Res. 44, D120–D1213. https://doi.org/10.1093/nar/gkv951.

Krieger, E., Vriend, G., 2015. New ways to boost molecular dynamics simulations. J. Comput. Chem. 36, 996–1007. https://doi.org/10.1002/jcc.24392.

Tian, L., Li, H., Chen, G., Zhang, Y., 2020. 1001 Ways to run AutoDock Vina for virtual screening. J. Comput. Aided. Mol. Des. 30, 237–249. https://doi.org/10.1007/s10822-016-9900-9.

Joshi, T., Joshi, T., Sharma, P., Mathpal, S., Pandit, H., 2020. In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking 4529–4536.

Khaerunnisa, S., Kurniawati, H., Awalluddin, R., Suhartati, S., 2020. Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool for chemoinformatics, chemical space visualization, and prediction of the rule of five. J. Cheminform. 4. https://doi.org/10.1186/1758-2946-4-17.

Nalakshmi, J., Hemanth, A., Chandrasekhar, R., Dinesh, S., Karthick, S., Wahi, N., 2021. A virtual screening study on the inhibition of main protease of SARS-CoV-2 using in silico techniques. J. Comput. Des. 30, 237–249. https://doi.org/10.1007/s10822-016-9900-9.

Pak. J. Pharm. Sci. 26, 949–952.
Computational screening and biochemical analysis of *Pistacia integerrima* and *Pandanus odorifer* plants

Mahmud, S., Paul, G.K., Biswas, S., Afrrose, S., Mita, M.A., Hasan, M.R., Shimu, M.S.S., Hossain, A., Promi, M.M., Ema, F.K., Chidambaram, K., Chandrasekaran, B., Alqahtani, A.M., Emran, T. Bin, Saleh, M.A., 2021c. Prospective Role of Peptide-Based Antiviral Therapy Against the Main Protease of SARS-CoV-2. Front. Mol. Biosci. 8. https://doi.org/10.3389/fmolb.2021.628585.

Mahmud, S., Raif, O., Paul, G.K., Promi, M.M., Sharmin, M., Shimu, S., Biswas, S., Emran, T. Bin, Dhma, K., 2021d. Designing a multi-epitope vaccine candidate to combat MERS-CoV by employing an immunoinformatics approach. Sci. Rep. 1–21. https://doi.org/10.1038/s41598-021-92176-1.

Sallam, M., 2021. COVID-19 vaccine hesitancy worldwide: A concise systematic review of vaccine acceptance rates. Vaccines 9, 1–15. https://doi.org/10.3390/vaccines9020160.

Santos, K.B., Guedes, I.A., Karl, A.L.M., Dardenne, L.E., 2020. Highly Flexible Ligand Docking: Benchmarking of the DockThor Program on the LEADS-PEP Protein-Peptide Data Set. J. Chem. Inf. Model. https://doi.org/10.1021/acs.jcim.9b00905.

Boblin, R.O., 1953. Medicinal chemistry. Chem. Eng. News 31, 48–49. https://doi.org/10.1021/cent-v031n001.p048.

Wang, J., Wolf, R.M., Caldwell, J.W., Kollman, P.A., Case, D.A., 2004. Development and testing of a general Amber force field. J. Comput. Chem. 25, 1157–1174. https://doi.org/10.1002/jcc.20035.

Wang, J., Wolf, R.M., Caldwell, J.W., Kollman, P.A., Case, D.A., 2004. Development and testing of a general Amber force field. J. Comput. Chem. 25, 1157–1174. https://doi.org/10.1002/jcc.20035.