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SARS-CoV-2 main protease inhibition by compounds isolated from *Luffa cylindrica* using molecular docking

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**Abstract**

In this study, chemical investigation of methanol extract of the air-dried fruits of *Luffa cylindrica* led to the identification of a new δ-valerolactone (1), along with sixteen known compounds (2-17). Their chemical structures including the absolute configuration were elucidated by extensive spectroscopic analysis and electronic circular dichroism analysis, as well as by comparison with those reported in the literature. For the first time in literature, we have examined the binding potential of the isolated compounds to highly conserved protein, M\(^{\text{pro}}\) of SARS-CoV-2 using the molecular docking technique. We found that the isolated saponins (14-17) bind to the substrate-binding pocket of SARS-CoV-2 M\(^{\text{pro}}\) with docking energy scores of −7.13, −7.29, −7.47, and −7.54 kcal.mol\(^{-1}\), respectively, along with binding abilities equivalent to an already claimed N3 protease inhibitor (−7.51 kcal.mol\(^{-1}\)).

**Introduction**

*Luffa cylindrica*, a subtropical vegetable, belonging to the Cucurbitaceae family, is also known as a vegetable sponge or sponge gourd. This plant is widely cultivated in Asia, India, Brazil, and the USA.\(^3\) The fruits and seeds of *Luffa* contain various bioactive compounds, such as phenolics,\(^1\) flavonoids,\(^1\) lufﬁns,\(^2,3\) sapogenins,\(^4\) and triterpenoids.\(^5\) *Luffa* possesses wide pharmaceutical activities such as anticancer,\(^6\) anti-inflammatory,\(^1\) anti-HIV-1 (human immunodeﬁciency virus 1),\(^6\) antiallergic,\(^6\) anti-infectious,\(^6\) antifungal,\(^7\) and antibacterial\(^7\) activities. For instance, oleaenic acid isolated from *L. cylindrica* demonstrated inhibition of NO production at 10 μM in an LPS/IFN-γ-induced cell model occurred.\(^8\) It has been reported that the peptides, lufﬁacyclin and the peptide lufﬁn P1 displays antifungal activity\(^9\) and anti-HIV-1 activity,\(^2\) respectively.

Towards the end of December 2019, a novel coronavirus (2019-nCoV/SARS-CoV-2) with human to human transmission, originated in Wuhan, China, and caused several human infections and disorders in the respiratory system.\(^10,11\) This viral disease is a pandemic that has become a global challenge and the number of newly infected patients has been increasing day by day.\(^12\) The coronavirus group comprises of numerous species and induces respiratory tract and gastrointestinal infections in vertebrates; nevertheless, some CoVs such as SARS, MERS, and SARS-CoV-2 have been reported to be especially dangerous to humans. Since the SARS-CoV-2 outbreak, different traditional herbs with promising results have been used alone or in combination with conventional drugs for the treatment of infected patients.\(^5\) There exist numerous uncertainties surrounding the novel coronavirus behavior; thus, it is too early to conclude whether medicinal plants, spices, or isolated compounds and molecules could be used as preventive drugs or as appropriate therapeutic compounds against COVID-19.\(^14\) However, the novel coronavirus SARS-CoV-2 and the previously reported viruses, MERS-CoV and SARS-CoV exhibit high similarity in genome sequences. Analysis of the genome sequences of these three viruses has revealed that SARS-CoV-2 has a higher identity with SARS-CoV (89.1% nucleotide similarity) than with MERS-CoV.\(^15\) Hence, it is hypothesized that previous researches on phytomedicinal and herbal metabolites, which have been demonstrated to have anti-coronavirus properties, may be an appreciated guide to searching and discovering antiviral phytochemical extracts which may be effective against the SARS-CoV-2 virus.\(^14,16\) We herein recommend a solution for the preclusion and treatment of the novel coronavirus by the isolated compounds from *L. cylindrica*.

A total of seventeen compounds (1–17) were isolated from the air-dried fruits of *L. cylindrica*, including one novel, 3,5-dihydroxy-δ-valerolactone (1). The details of the new compound are discussed below, and
the chemical structures of all the compounds are shown in Fig. 1.

Compound 1 was obtained as a colorless oil. The molecular formula of 1 as C$_5$H$_8$O$_4$, consistent with two degrees of unsaturation, was deduced from the HRESIMS spectrum showing the molecule ion mass peak at m/z 155.0320 [M + Na]$^{+}$ (calcld. for 155.0315). The $^{13}$C NMR spectrum of 1 showed the signal at $\delta$C 178.6, suggesting the presence of ester carbonyl moiety, which accounts for one degree of unsaturation. Besides that, two oxygenated methine carbons ($\delta$C 90.1 and 69.6) together with two methylene carbons ($\delta$C 62.4 and 39.1) were present in the $^{13}$C NMR and DEPT spectra. Integration of the resonances in the $^1$H NMR spectrum of 1 showed the presence of two oxymethine protons at $\delta$H 4.52 (1H, td, J = 2.5, 6.5 Hz, H-3) and 4.47 (1H, dd, J = 3.5, 5.5 Hz, H-5), and two methylene groups at $\delta$H 3.84 (1H, dd, J = 3.5, 12.5 Hz, H-10).

Table 1

| No. | $^1$H (J in Hz) | $^{13}$C |
|-----|-----------------|---------|
| 1   | 2.47 dd (6.5, 18.0) | 39.1    |
| 2   | 3.01 dd (6.5, 18.0) |         |
| 3   | 4.52 dd (6.5, 2.5) | 62.4    |
| 4   | 3.84 dd (3.5, 12.5) |         |
| 5   | 4.47 dd (3.5, 5.5) | 90.1    |

N3 inhibitor

Fig. 1. Chemical structures of isolated compounds (1–17) from L. cylindria and N3 inhibitor.
4a), 3.78 (1H, dd, J = 3.5, 12.5 Hz, H-4b), 3.01 (1H, dd, J = 6.5, 18.0 Hz, H-2a), and 2.47 (1H, dd, J = 2.5, 18.0 Hz, H-2b) (Table 1). These data indicated 1 to be a δ-valerolactone.\textsuperscript{17} Analysis of HMQC and HMBC spectra, along with the comparison of the NMR data of 1 with those of 3-hydroxy-δ-valerolactone\textsuperscript{17} suggested close structural similarity between the two compounds, except for the presence of a hydroxyl group in 1. The location of the hydroxyl group at C-5 was supported by the key observation of HMBC correlations from H-5 to C-1, and H-3 and H-4 to C-5 (Fig. 2). Based on these evidences, the planar structure of 1 was elucidated as 3,5-dihydroxy-δ-valerolactone.

Protons H-4 and H-5 displayed a small coupling constant (\(^{1}J_{H4,H5} = 3.5\) Hz), which is consistent with a gauche conformation\textsuperscript{18} (Fig. S1). The NOESY spectrum of 1 displayed no spatial correlation between oxymethylene protons H-3 and H-5, indicating two cases for configuration of (3S,5R)-1 and (3R,5S)-1 (Fig. S1). Furthermore, the electronic circular dichroism (ECD) spectra calculations for both 1 and its enantiomer were carried out using the time-dependent density functional theory (TDDFT) method.\textsuperscript{19} The experimental ECD curves of 1 correlated well with that calculated for (3S,5R)-1 in the range of 200 to 242 nm (Fig. 3). Consequently, the structure of 1 was conclusively determined to be (3S,5R)-dihydroxy-δ-valerolactone.

The other compounds were identified as phenanthrene (2),\textsuperscript{20} (S)-dehyrovomifoliol (3),\textsuperscript{21} 1,2-naphthoquinone (4),\textsuperscript{22} cinnamic acid (5),\textsuperscript{23} 2,6-dimethyl-1,4-benzenediol (6),\textsuperscript{24} phthalic acid (7),\textsuperscript{25} 4-(hydroxymethyl)benzene-1,2-diol (8),\textsuperscript{26} litchiol B (9),\textsuperscript{27} pinoresinol (10),\textsuperscript{28} apigenin (11),\textsuperscript{29} tridecan-7-one (12),\textsuperscript{30} henicosan-11-one (13),\textsuperscript{30} 3-O-β-D-glucopyranosyl-spinasterol (14),\textsuperscript{31} 3-O-β-D-glucopyranosyl-oleanolic acid (15),\textsuperscript{32} lucyoside F (16),\textsuperscript{33} and lucyoside H (17),\textsuperscript{34} by comparison of their spectral data with values reported in the literature. To the best of our knowledge, compounds 3 and 9 were reported the first time from \textit{Luffa} species. Compound 14 was isolated from \textit{L. cylindrica} for the first time.

Along with various structural proteins, all the CoV genomes encode for a critical viral component called Main Protease (M\textsuperscript{pro}).\textsuperscript{34} The latter is a 306 amino acids long enzyme that mainly helps in the replication of the virus through proteolytic processing of its RNA replicase machinery. M\textsuperscript{pro} from different human and animal CoVs have been shown to possess high similarity in terms of the primary amino acid sequence as well as the functional tertiary conformation of the enzyme.\textsuperscript{35} The recently discovered SARS-CoV-2 also shares the homology in its M\textsuperscript{pro} enzyme.\textsuperscript{36} Thus, we have employed M\textsuperscript{pro} as a target. Also, N3 holds the potential to specifically inhibit M\textsuperscript{pro} from multiple coronaviruses and has previously displayed potent antiviral activity against infectious bronchitis virus in an animal model.\textsuperscript{37} The structure of the N3 inhibitor co-crystalized with the M\textsuperscript{pro} of SARS-CoV-2, which was recently released in Protein Data Bank (PDB) (6LU7),\textsuperscript{38} gave us insights into the molecular mechanism of N3 inhibitor action against the new coronavirus.\textsuperscript{39}

In this study, we demonstrate docking molecules of the isolated compounds (1–17) and N3 inhibitor into the PDB6LU7 protein (Fig. 4) using PyRx 0.9.4 virtual screening software,\textsuperscript{40} to contribute to the orientation and encourage the use of natural metabolites for SARS-CoV-
The docking was successful in fifteen compounds. The docking simulation results with docking score energy (DS) and root-mean-square deviation (RMSD) between isolated compounds (1, 3–11, and 14–17) and the PDBeLUL7 protein is shown in Table 2.

We re-docked the N3 inhibitor in the same configuration to get a docking score for the natural binding. The docking score was found to be $-7.51$ kcal.mol$^{-1}$ and was used to compare the binding of the isolated compounds with the $M_{\text{pro}}$ of SAR-CoV-2. The two main residues that formed polar interaction with the inhibitor were Arg105 and Gln110 (Fig. 5A). A grid was generated around the conserved residues of the substrate-binding pocket with the main emphasis on the residues making polar contacts with the N3 inhibitor. Subsequently, the isolated compounds were then docked using the same grid. From the docking results, it was observed that lucyoside H (17), an oleanane saponin demonstrated $-7.54$ kcal.mol$^{-1}$ binding energy with eight hydrogens bonds and interaction with five residues, Ala285, Lys137, Asn277, Met276, and Leu287 (Fig. 5E), thereby providing dramatic and approximate effect on the anti-SAR-CoV-2 activity along with N3 inhibitor. The other oleanane saponin, lucyoside F (16) displayed a slightly lower effect on the protein than compound 17 and N3 inhibitor with a binding energy value of $-7.47$ kcal.mol$^{-1}$ with four hydrogen bonds and interacted with three residues, Asr289, Thr199, and Leu272 (Fig. 5D). Saponins 14 and 15 showed a significant effect on the protein of SARS-CoV-2 with the binding energy of $-7.13$ and $-7.29$ kcal.mol$^{-1}$, respectively. As illustrated in Fig. 5B, the corresponding ligand interactions of 14 with the virus protein were five hydrogen-bonding interactions with the Thr199, Lys137, Asr289, Arg131, and Asr197 residues with the bond distances of 2.6, 2.3, 2.2, 2.2, and 2.1 Å, respectively. Compound 15 displayed the effect by three hydrogen binding bonds and three interacting residues of Asn238, Lys137, and Thr199 with the bond distances of 2.6, 2.5, and 1.9 Å, respectively (Fig. 5C). These results showed that compounds 14 and 15 shared the same residues (Thr199 and Lys137) via the interaction with the SARS-CoV-2 protein. Besides that, compounds 10 and 11 revealed a noticeable effect on the PDBeLUL7 protein with the binding energy values of $-6.76$ and $-6.77$ kcal.mol$^{-1}$ (Table 2). Compound 10 exhibited the activity by only one hydrogen binding bond and an interacting residue Asn151 with the bonding distance of 2.5 Å. Meanwhile, the activity of compound 11 was deciphered by binding to four hydrogen bonds as well as interacting with four residues Gln192, Ser144, His163, and Glu166 with the bond distance values of 2.6, 2.6, 2.5, and 2.3 Å, respectively (Table 2). The other compounds 1 and 3–9 showed a moderate effect on the protein of SARS-CoV-2 with the binding energy values of $-4.68$, $-5.08$, $-5.37$, $-4.83$, $-4.89$, $-5.52$, $-4.78$, and $-5.09$ kcal.mol$^{-1}$, respectively (Table 2).

The above-mentioned data showed that saponins had the most potent effect on the SARS-CoV-2 inhibition. Interestingly, all of the corresponding ligand interactions of saponins 14–17 with PDBeLUL7 protein were the hydrogen-bonding interactions between the enzyme residues and the hydroxyl groups in the sugar rings of these compounds (Fig. 5). Consequently, the key role of the glycosyl group in the SARS-CoV-2 inhibition was indicated. Among the isolated saponins, compound 14 is a sterol saponin, that demonstrated a lower effect than oleanane saponins 15–17, thereby suggesting that the nature of aglycone in saponins might be a crucial factor in mediating the inhibition of SARS-CoV-2. Saponins are widely distributed in the plant kingdom and have been reported to possess a large number of biological activities, including anti-inflammation, anti-cancer, antioxidant, anti-HIV, as well as anti-viral. Additionally, compound 17 that differs from compound 16 by a methyl group at the position of C-23, exhibited higher activity against COVID-19 protein than compound 16. This result indicated that the presence of the methyl group at C-23 can increase SARS-CoV-2 inhibitory activities. Furthermore, the glycosyl group at C-28 in compound 17 that was changed by the carboxyl group in compound 15 demonstrated a lower effect on the PDBeLUL7 protein. Thus, it is proposed that the number of glycosyl groups in the saponins structure might have an impact on the SARS-CoV-2 inhibitory effect of saponins. On the other hand, compounds 10 and 11 that are a lignan and a flavonoid, respectively, displayed quite similar inhibitory activity, thereby demonstrating a similar ability to inhibit the SARS-CoV-2 protein of lignans and flavonoids. Consequently, the above-mentioned evidences might support for the crucial role of saponins in the anti-coronavirus drug studies.

In conclude, we isolated a new δ-valerolactone (1), together with sixteen known compounds (2–17) from the extract of L. cylindrica fruits. The isolated compounds were compared for the SARS-CoV-2 inhibitory ability through PDBeLUL7 protein to N3 inhibitor by using the molecular docking technique. All the isolated saponins (14–17) displayed the remarkable binding abilities into the pocket of SARS-CoV-2 $M_{\text{pro}}$ with docking energy scores of $-7.13$, $-7.29$, $-7.47$, and $-7.54$ kcal.mol$^{-1}$, respectively. Consequently, these findings provide the direction of research and application of the natural products in general and components isolated from L. cylindrica isolated components in particular, in the prevention and treatment of 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.
Fig. 5. Docking simulation of the interactions between N3 inhibitor, and compounds 14–17 and the PDB6LU7 protein of SARS-CoV-2 (A–E).
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Appendix A. Supplementary data

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