Phytochemical and in silico studies for potential constituents from Centaurium spicatum as candidates against the SARS-CoV-2 main protease and RNA-dependent RNA polymerase

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ABSTRACT

In the present study, a new secoiridoid glycoside lisianthoside II 1, along with seven known compounds 2–8, were isolated from Centaurium spicatum L. In-silico molecular docking and molecular dynamic simulation against SARS-CoV-2 Main protease (Mₚᵣᵒ) and RNA-dependent RNA polymerase (RdRp) were conducted. The affinity docking scores revealed that 8 is the best bound ligand to Mₚᵣᵒ active site with binding energy of −14.9877 kcal/mol (RSMD = 1.16 Å), while 6 was the highest against RdRp (−16.9572 kcal/mol, RMSD = 1.01 Å). Moreover, the molecular dynamic simulation revealed that 8 with a (ΔG) of −7.9 kcal/mol (RMSD value of 2.6 Å) and 6 (RMSD value of 1.6 Å) and binding free energy (ΔG) of −7.1 kcal/mol achieved the highest stability over 50 ns of MDS inside the Mₚᵣᵒ and RdRp enzyme’s active site, respectively. Hence, the isolated compounds could be a good lead for development of new leads targeting COVID-19.

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1. Introduction

Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) is a global rapid-spreading virus implying beyond the COVID-19 disease with high risk of transmission, increased rate of complications and mortality (Caly et al. 2020; Ferraz et al. 2020; Hossam et al. 2020). According to the world health organization (WHO), from December 2019 to now, there are about 216 million confirmed cases and about 4.5 million deaths. The infection and replication cycle of SARS-CoV-2 begins with the binding of its S protein to the angiotensin-converting enzyme 2 (ACE2) receptor on a human cell surface, followed by a structural change of the S protein that enables the fusion of the viral membrane and the cell membrane. The viral genes can enter the host cell to be replicated producing more viruses for further viral shedding (El Hawary et al. 2020; Luan et al. 2020; Ahmed et al. 2021; Samira et al. 2021).

The involved mechanism of antiviral drugs may include the inhibition of viral entry, replication, assembly, and release or even the targeting of virus–host-specific interactions. Proteases plays an essential role in viral replication and transcription functions through extensive proteolysis and cleavage of the pp1a and pp1ab replicase polyproteins to generate individual functional proteins, including RNA-dependent RNA polymerase, helicase, an exoribonuclease, an endoribonuclease and others (Khan et al. 2020).

Main protease (M^{PRO}) is one of the most important viral protease enzymes for SARS-CoV-2 replication, which is responsible for proteolysis, viral replication, and infection process. In addition, RNA-dependent RNA polymerase (RdRp) is essential for viral replication and transcription of positive-strand RNA viruses thereby they are considered as an ideal target for SARS-CoV-2 inhibitors discovery for COVID-19 treatment. (Boozari and Hosseinzadeh 2021, Ertl et al. 2000, Zahran et al. 2020)

Searching for new compounds from natural sources known for their high safety and applicability will be a good avenue to treat SARS-COV-2 (Allam et al. 2020). It was reported that xanthones and flavonoids have potential anti-SARS-CoV-2 using computational methods such as molecular docking. (Da Silva Antonio, Wiedemann and Veiga-Junior 2020; Huang et al. 2020).
Centaurium is a genus of 20 species of family (Gentianaceae). C. spicatum (L.) Fritsch is the reported name in (Mitt. Naturwiss. Vereins Univ. Wien 5:97, 1907). This name has been modified as a part of procedures used to resolve data conflicts detected in the supplied data sets. From 2012 until now, a new classification based on the analysis of several DNA sequence regions has been reported for Schenkia spicata (L.) as an accepted name, and the old name C. spicatum (L.) Fritsch as its synonym (Mansio). Phytochemical profile of Centaurium revealed the presence of iridoids, secoiridoids and xanthones as major phytochemical components. (Valentão et al. 2000; Bibi et al. 2006; Mihaylova et al. 2019).

The inhibition of viral Mpro protease and RNA-dependent RNA polymerase (RdRp) enzymes are two of the most crucial molecular targets for SARS-COV-2 treatment discovery due to their role in blocking viral replication. Therefore, they have been chosen in our study to evaluate the effect of the isolated compounds from C. spicatum for discovery of a potential inhibitor targeting SARS-CoV-2.

2. Results and discussion

2.1. Compounds isolated from C. spicatum

Phytochemical investigation of C. spicatum (L.) Fritsch afforded a new secoiridoid glycoside lisianthoside II 1 as well as seven known compounds lisianthoside I 2 (Hamburger et al. 1990), gentiopicroside 3 (Kumarasamy et al. 2003), catalpol 4 (Piątczak et al. 2015), 1-Hydroxy-3,5,6-trimethoxyxanthone 5 (Peres, Nagem and de Oliveira 2000), 8-hydroxy-3,5-dimethoxy-1-β-D-glucopyranosyl-xanthone 6 (Hajimehdipoor et al. 2006), Demethylstemonin 7 (Peres, Nagem and de Oliveira 2000) and 8-hydroxy-3,5-dimethoxy-1-O-gentiobiosyl xanthone 8 (Hajimehdipoor et al. 2006) (Figure 1).

2.2. Structure elucidation of compound 1

Compound 1 was obtained as yellow amorphous powder (14 mg) with [α]D31.8 8D-87.4° (c = 0.333, MeOH). The molecular weight of 1 was 730 with a molecular formula of C33H46O18, which was evident from [M − H]− peak at m/z 729 in the negative ion FAB-MS and also from positive ion HR-FAB + MS [M + H]þ Mass at 731.2767. (calcd 731.2762). 1H-, 13C- NMR, and 13C-multiplicities (from DEPT spectra) of compound 1 (Table S1) are nearly the same as that of 2; Lisianthioside I, a reported symmetric dimeric secoiridoid glycoside (Hamburger et al. 1990) except for the presence of an additional –CH2– group at position C-6 of part (a) of the original symmetric dimeric structure Lisianthioside I (compound 2) at δH 1.71 (2H, m, C-6 of part a) which was confirmed from 1H-NMR spectrum and HMBC correlations where there are two sets of protons at δH 1.71 attached to C-6 (δC 25.9) of part (a) and at δH 1.61 attached to C-6 (δC 25.9) of part (b) with total integration equals to four protons (Cf. compound 2 where there is only one set of protons at δH 1.61 attached to C-6 with δC 25.9 of part (b)) Site of attachment of the additional –CH2– moiety was decided to be between C-5 and C-6 of part (a) from H–H COSY spectrum where there is a strong correlation between (δH 1.71 H-6) of part (a) and both (δH 3.11, H-5 and δH 3.08 H-7) of part (a).
long-range correlation observed in HMBC experiment between protons of both (H-5 and H-7) with C-6 ($\delta_H$ 3.11, and 3.08, respectively) on one side and the long-range correlation observed between ($\delta_C$ 25.9, C-6) of part (b) and between protons of both (H-5 and H-7) with C-6; ($\delta_H$ 3.31 and 4.28, respectively) on the other side confirmed the previous suggestion (Figure S1). In addition, a long-range correlation observed in HMBC experiment between anomeric protons of the glucose units of both part (a) and part (b) ($\delta_H$ 4.61 and 4.51, respectively) with C-1 of each part (a) and part (b) ($\delta_C$ 98.0 and 97.0, respectively) (Figure S1) which confirmed that this was the site of glycosylation.

The configuration of H-1 was decided to be of $\alpha$ configuration from the reported naturally occurring secoiridoids (Takagi et al. 1982), and from chemical shift of C-1 of reported data, while that of H-5 is of $\beta$ type and those of H-10 in part (a) or H-9 in part (b) are of $\beta$ type from coupling constants ($J$) and from the reported data. (Takagi et al. 1982) It can be concluded that, the configuration of the chiral centers of compound 1, was the same as known compound 2.

All previous data was in agreement with the suggested structure and hence, the most reasonable structure hypothesis of 1 was that of an asymmetric dimer secoiridoid glycoside; Lisianthioside II which was suggested as being a new compound.
2.3. NMR data of compound 1

Yellow amorphous powder, [α]D 31.8 D = 87.4° (c = 0.333, MeOH), 1H-NMR (600 MHz, CD3OD): Part (a);  \( \delta_H \): 1.71 (2H, m, H-6), 2.58 (1H, ddd, 6.5, 6.5, 6.0, H-10), 3.08 (2H, m 2.17, H-7), 3.11 (1H, dd, 6.5, 6.5, H-5), 4.28 (2H, t, 12.0 4.36, d, 12.0, H-8), 5.14 (2H, m, H-11), 5.32 (1H, brs, H-1), 5.47 (1H, dd, 9.9, 18.0, H-9), 7.50 (1H, brs, H-3). Glu.; 4.61 (1H, d, 7.2, H-1'), 3.25 (1H, m, H-2'), 3.29 (1H, m, H-3'), 3.41 (H-4'), 3.41 (H-5'), 4.05 (1H, dd, 4.5, 11.6, H-6a'), 4.52 (1H, d, 11.6, H-6b'). Part (b);  \( \delta_H \): 1.61 (2H, m 1.68, m, H-6), 2.87 (1H, ddd, 6.5, 6.5, 6.0, H-9), 3.31 (1H, dd, 6.5, 6.5, H-5), 4.28 (2H, t, 12.0 4.36, d, 12.0, H-7), 5.22 (1H, m, H-10), 5.32 (1H, brs, H-1), 5.55 (2H, dd, 9.9, 18.0, H-8), 7.20 (1H, brs, H-3). Glu.; 4.51 (1H, d, 7.2, H-1'), 3.41 (1H, m, H-2'), 3.25 (1H, m, H-3'), 3.26 (1H, m, H-4'), 3.30 (1H, m, H-5'), 4.05 (1H, dd, 4.5, 11.6, H-6a'), 4.52 (1H, d, 11.6, H-6b').

13C-NMR (150 MHz, CD3OD): Part (a);  \( \delta_C \): 25.9 (C-6), 28.3 (C-5), 35.4 (C-7), 43.9 (C-10), 69.7 (C-8), 98.0 (C-1), 105.9 (C-4), 120.7 (C-11), 133.2 (C-9), 153.9 (C-3), 168.4 (C-12). Glu.; 99.8 (C-1'), 74.4 (C-2'), 77.5 (C-3'), 71.0 (C-4'), 78.2 (C-5'), 63.5 (C-6'). Part (b);  \( \delta_C \): 25.9 (C-6), 28.7 (C-5), 44.6 (C-9), 69.7 (C-7), 97.1 (C-1), 111.5 (C-4), 121.0 (C-11), 134.7 (C-8), 151.9 (C-3), 173.8 (C-11). Glu.; 99.8 (C-1'), 74.2 (C-2'), 75.6 (C-3'), 71.0 (C-4'), 78.0 (C-5'), 62.3 (C-6').

2.4. Molecular docking of isolated compounds against SARS COV-2 main protease (M<sub>pro</sub>)

The active site of SARS COV-2 (M<sub>pro</sub>) main protease (PDB ID: 6LU7) was recognized by the site finder function. The molecular simulation of interactions between the identified compounds and the M<sub>pro</sub> active site was conducted and the binding affinities pose scores and binding interactions are listed in (Table S2, Figure S3). The binding affinity values of the compounds with the active site showed high affinities ranging from −14.987719 to −9.22552 kcal/mol in comparison to −11.0603 kcal/mol for N3 (benzyl (3S,6R,9S,E)-9-isobutyl-6-isopropyl-3-methyl-1-(5-methylisoxazol-3-yl)-1,4,7,10-tetraoxo-12-((2-oxopyrrolidin-3-yl) methyl)-2,5,8,11-tetraazapentadec-13-en-15-oate) that possess strong antiviral activity at 10 μM concentration in SARS-CoV-2 infected Vero cells. (Mengist et al. 2021).

The binding affinity of compound 8 exhibited the highest binding affinity with a binding energy of −14.9877 kcal/mol (RSMD = 1.16 Å). The interaction of compounds with the active receptor site is mainly supported by hydrogen bonds and hydrophobic interactions. The compound 8 interactions were formed by hydrogen bonding with MET 165 as H-donor (2 bonds), GLY 143 (H-acceptor) and GLN 189 (H-acceptor) together with H-π bond interaction with HIS 41 amino acid residues with a significant distance between the boundaries of the active site (Table S2, Figure S3). Some impressions of the hydrophobic interactions around the molecule have also occurred.

2.5. Molecular docking of isolated compounds against RNA-dependent RNA polymerase (RdRp)

Regarding RNA-dependent RNA polymerase, the pose score of compounds binding affinity was between −16.9572 and −11.744308 kcal/mol for the complex inhibitors'
compounds 6 and 2, respectively (Table S2, Fig. S4) in comparison to −17.562056 Kcal/mol for suramin; the complexed inhibitor ligand. It is apparent that compound 6 has the best docking binding energy scores with a value of −16.9572 kcal/mol, RMSD = 1.01 and shows hydrogen bond formation binding within the active site residue ASN 497 as H-donor, ARG 569, LYS 500 and ARG 569 with the hydroxyl group of the glycoside moiety and oxygen atom of the lactone ring in addition to H-π bond interaction between the compound and GLN 573 (Table S2, Figure S4).

Overall, scores of binding affinities of identified ligands suggest that the best binding ligands as inhibitors of SARS COV-2 (M\textsuperscript{pro}) main protease and RNA-dependent RNA polymerase (RdRp) mainly belong to xanthone class phytochemical compounds, which could be used as a potential treatment for COVID-19 or as a scaffold for developing new inhibitors for SARS COV-2.

### 2.6. Molecular dynamic simulation

Further computational validation was achieved through a number of MDS experiments and binding free energy (ΔG) calculations. In regard to M\textsuperscript{pro} (PDB: 6LU7), compound 8 was the best scoring compound. It also showed very good binding stability inside the enzyme’s active site over 50 ns of MDS with an average RMSD value of 2.6 Å and ΔG value of −7.9 kcal/mol (Figure S6).

On the other hand, compound 6 was the top-scoring compound with RdRP (PDB: 7D4F). It showed an interesting binding stability until the end of MDS with a low deviation from its starting binding pose by ~1.6 Å at the beginning of the simulation (at 4 ns) and remained slightly fluctuating around this value until the end of MDS. It got a ΔG value of −7.1 kcal/mol (Figure S7).

In conclusion, both compounds (8 and 6) can be considered good stable binders for M\textsuperscript{pro} and RdRP, respectively.

### 2.7. Physicochemical properties, accordance with drug-likeness rules and pharmacokinetics of the active compounds

SwissADME is a web tool that gives free access to predictive models for physicochemical properties, pharmacokinetics, and drug-likeness, in addition, the orally active compounds should obey to Lipinski’s rule of five (i.e. the drug-likeness). Lipinski’s rule of five parameters (molecular mass <500 daltons, no more than 5 hydrogen bond donors, no. of hydrogen bond acceptors less than 10 and octanol–water partition coefficient (logP) should not be greater than 5) indicates the drug likeliness of molecules (Antoine and Vincent 2017).

Physicochemical Properties, Lipophilicity, Pharmacokinetics and Drug likeness of the isolated compounds were studied and the results are recorded in (Table S3), where compounds 3, 5 and 7 are fully obeying Lipinski’s rule with high GIT absorption for compound 3 and 5 indicating its possibility to be orally effective drugs. All compounds have bioavailability score more than 0.00 so they suggested to be active at the receptor’s sites.
3. Experimental
See the Supplemental data

4. Conclusion
The phytochemical investigation of *C. spicatum* resulted in isolation of a new asymmetric dimeric secoiridoid glycoside, lisianthoside II, along with seven phytoconstituents. Furthermore, identified components were subjected to molecular docking, molecular dynamic and pharmacokinetic evaluation against SARS-CoV-2 Main protease (M\(^{\text{Prp}}\)) and RNA-dependent RNA polymerase (RdRp). The results revealed that compounds 6 and 8 showed a promising effect on binding with SARS-CoV-2 targets and could be an alternative source and scaffold for the development of new natural leads targeting COVID-19.

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