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SHORT COMMUNICATION

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Virtual screening, ADMET profiling, PASS prediction, and bioactivity studies of potential inhibitory roles of alkaloids, phytosterols, and flavonoids against COVID-19 main protease (M^{pro})

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ABSTRACT

The current research used a virtual screening method to study 57 isolated phytochemicals (alkaloids, phytosterols, and flavonoids) against the SARS-CoV-2 main protease (M^{pro}). The absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the selected compounds were analysed using admetSAR tool while SwissADME and Molinspiration chemoinformatics tools were used to examine the oral bioavailability and drug-likeness properties. Parameters such as physicochemical properties, activity spectra for substances (PASS) prediction, bioactivity, binding mode, and molecular interactions were also analysed. Our results favoured Lupeol (–8.6 kcal/mol), Lupenone (–7.7 kcal/mol), Hesperetin (–7.4 kcal/mol), Apigenin (–7.3 kcal/mol) and Castasterone (–7.3 kcal/mol) as probable inhibitors of SARS-CoV-2. This is because of their good binding affinities, bioactivities, drug-likeness, ADMET properties, PASS properties, oral bioavailability, binding mode and their interactions with the active site of the target receptor compared to Remdesivir and Azithromycin. Therefore, these compounds could be explored towards the development of new therapeutic agents against SARS-CoV-2.

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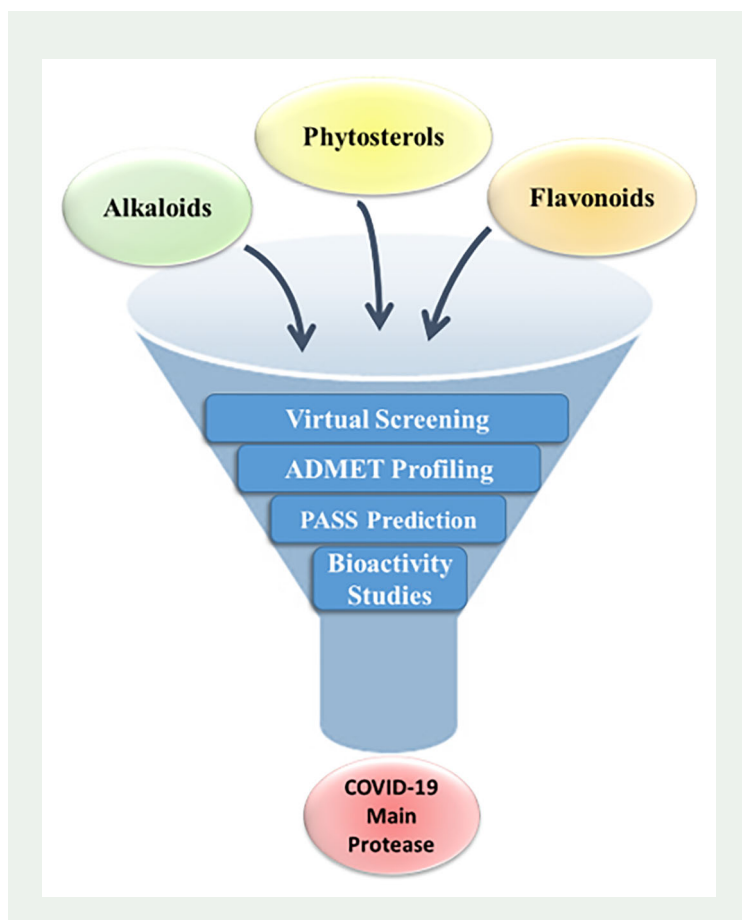
1. Introduction

The novel Coronavirus Disease (COVID-19) is the newly pandemic ravaging the world, causing global threat and has led to the death of people in thousands (https://corona-virus.jhu.edu/map.html). No drug has been officially approved for it cures so far, thereby, putting researchers on the verge of identifying a new therapeutic agent. SARS-CoV-2 main protease (M^{pro}) complexed with (N3-(N-[(5-meth-ylisoxazol-3-Yl)carbonyl]alanyl-l-valyl-N∼1∼-((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-Yl]methyl}but-2-enyl)-l-leucinamide) inhibitor (PDB ID: 6LU7) is the enzyme responsible for umpiring the replication and transcription of the virus (Xue et al. 2008), making it a target for inhibitors towards the design and development of new COVID-19 drugs (Anand et al. 2002).

As the research on COVID-19 is ongoing globally, drugs such as Remdesivir, Hydroxychloroquine, and Azithromycin, among others, have been reported as potential inhibitors of this virus (Kaddoura et al. 2020). However, the significance of phytochemicals in medicinal plants cannot be ignored in the quest of identifying new drugs (Kathleen 2010). Therefore, this study investigated the potential inhibiting roles of

alkaloids, phytosterols, and flavonoids against SARS-CoV-2 main protease (M^pro) through virtual screening, drug-likeness analysis, PASS prediction and ADMET profiling.

2. Results and discussion

2.1. Molecular docking analysis

A widely adopted and reliable modelling method for evaluating the inhibiting efficiency of compounds against target receptors is molecular docking (Sulaiman et al. 2019). Table S1 (supplementary materials) shows the docking results of the docked compounds and standards against the target receptor (SARS-CoV-2 M^pro) (Figure S1, supplementary materials). It can be observed from Table S1 that the binding affinities of sets of docked compounds (alkaloids, phytosterols, and flavonoids) against the target receptor range from −9.0 kcal/mol to −3.9 kcal/mol for alkaloids, −8.6 kcal/mol to −6.7 kcal/mol for phytosterols, and −7.9 kcal/mol to −6.9 kcal/mol for flavonoids, with Curarine (-9.0 kcal/mol), Lupeol (-8.6 kcal/mol), and Kaempferol (-7.9 kcal/mol) having the highest binding affinities in each set. A comparison of this result with values obtained for Remdesivir (−7.6 kcal/mol) and Azithromycin (−6.3 kcal/mol) indicates that some of the screened compounds in each set (Table S1) have better binding affinities as compared to the two standards. Furthermore, the binding affinity of a compound corresponds to the AutoDock Vina docking score and can be used to calculate the inhibition constant value (K_i) (Equation (1)) (Onawole 2017). The lower the K_i value of a compound, the more its inhibiting efficiency (Burlingham and Widlanski 2003). As observed in Table S1, many of the screened phytochemicals showed outstanding inhibitory values than Remdesivir and Azithromycin, and thus, could be probable inhibitors of the 6LU7 target receptor, but a careful check through their toxicity profile proved some to be unsafe as drug candidates owing to their severe acute oral toxicity and their ability to inhibit human either-a-go-go (hERG2), thereby flourishes only five (Table S2 and Figure S2) as safer and excellent drug candidates (see lmmd.ecust.edu.cn/admetsar2). Notably, despite good inhibition constant and toxicity profile shown by the identified five compounds for further analyses, Lupenone show no hydrogen bond interaction while Hesperetin does not form any electrostatic/hydrophobic interactions (Table S2). However, one of the aims of molecular docking simulation is to establish the ability of a ligand to interact with active site of the target receptor and form both hydrogen and other electrostatic/hydrophobic interactions with important amino residues in the active site. As observed in Table S2, Lupenone and Hesperetin interact effectively with the SARS-CoV-2 main protease forming both electrostatic/hydrophobic and hydrogen bond interactions with important amino acids (Tyr237, Tyr239, Leu272, Leu286, Leu287), and (Gly143, Ser144, Cys145, Leu141, Glu166, Phe140, Asn142), respectively. All the five ligands selected share the same pocket and interaction mode with both Remdesivir and Azithromycin whose randomised clinical trials against SARS-CoV-2 has been completed or interact with important amino acids in the main active site of the target receptor which falls between its domains II and III, thus justifying the good inhibition constant observed in Lupenone, Hesperitn and other selected ligands. However, the number and forms of interactions observed in the five selected ligands could be improved when the ligand is optimised during the

lead optimisation stage of drug discovery while their structures will be modified to improve their potency, efficacy, pharmacokinetics and reduce their toxicity level.

2.2. Drug-likeness and oral-bioavailability analysis

Drug-likeness analysis is very crucial in the early stage of drug discovery. Figure S3 shows the structures of the selected compounds. As stated in Lipinski's rule of five (RO5), a drug-like compound must have a molecular weight (MW) $\leq$ 500 Da, hydrogen bond donor (HBD's) $\leq$ 5, hydrogen bond acceptor (HBAs) $\leq$ 10 and (log P) $\leq$ 5 with only one violation allowed (Lipinski 2004). Interestingly, all the selected compounds (Table S3) obey the RO5 as compared to the two standards (Remdesivir and Azithromycin) with two violations each. SwissADME tool (http://www.swissadme.ch/) (Daina et al. 2017) was used to evaluate the oral-bioavailability of the selected compounds. An analysis of the bioavailability RADAR (Figure S4) gives the bioavailability properties of the selected compounds and standards. The pink area in the RADAR shows the most favourable zone for each of the bioavailability properties. As observed in Table S4, all the selected compounds fulfilled the 500 g/mol recommended (SIZE) by Lipinski for good drug candidates as compared to 602.58 and 748.98 g/mol obtained for the two standards respectively. The polarity (POLAR) was assessed using the Total Polarity Surface Area (TPSA) with recommended range of 20 to 130Å^2. Except for Azithromycin, all the selected compounds and standard fall within the acceptable TPSA values. The flexibility (FLEX) property was evaluated using the number of rotatable bonds whose value should not exceed nine. Obviously, all the selected compounds and Remdesivir fall within the recommended range. Lipophilicity (LIPO) and insolubility (INSOLU) were evaluated using xlogP3 and ESOL (log S) with the recommended range from -0.7 to $+5.0$, and from 0 to 6, respectively. Notably, all except Lupeol and Lupenone fall within the recommended values of xlogP3 and ESOL (log S). The Unsaturation (INSATU) determined using Fraction Csp3 falls with recommended range of 0.5 to 1. All the selected compounds have the same bioavailability score of (0.55) which is higher than (0.17) obtained for the two standards. In consequence, all the selected compounds possess outstanding oral-bioavailability properties compared to the two standards.

2.3. Assessment of (absorption, distribution, metabolism, excretion and toxicity (ADMET) properties

As observed in Table S5, the selected compounds and standards have positive human intestine absorption (HIA+), thus can easily be absorbed in the human intestine. Lupeol, Lupenone, Castasterone, and Remdesivir possess the ability to cross the blood-brain barrier (BBB+) while the aqueous solubility (log S) values of the selected compounds and standards fall within the recommended range of -1 to -5 (Tsaioun and Kates 2011), thus, the selected compounds and the standards have better absorption and distribution properties. Microsomal enzymes (Cytochrome P450 inhibitors) which catalyse reactions involved in metabolic activities of the drug were used to assess the metabolic activities of the selected compounds. As expected, most of the selected

compounds and standards are non-inhibitors of all the CYP450 inhibitors. The selected compounds and standards are non-AMES toxic, non-carcinogenic, and possessed type III oral toxicity (slightly toxic) which could be modified to Type IV (non-toxic) during the lead optimisation stage of drug discovery (Onawole et al. 2017). Human ether a-go-go (hERG2) is an important factor to consider in choosing excellent therapeutic drug candidates. hERG2 inhibition may block the potassium ion channel of the myocardium, thereby affecting the heart and can lead to serious health problems and loss of life (Sanguinetti and Tristani-firouzi 2006). Obviously, the selected compounds and standards are non-heRG2 inhibitors, with no eye corrosion. Summarily, all the selected drug candidates and standards show outstanding ADMET properties, and thus, reliable and safe potential inhibitors against the target receptor.

2.4. Prediction of activity spectra for substances (PASS)

The biological activity of the selected compounds and standard were predicted using the PASS online web-server, (Goel et al. 2011). This assessment reveals the biological activities of the compounds selected in the human body. Notably, Table S6 reveals that all the selected compounds possess inhibitory activity against SAR-CoV-2 with the probability to be active ($P_a{}^a$) is greater than probability to be inactive ($P_i{}^b$). Lupeol, Lupenone, Hesperetin, Apigenin, have the same biological effect, which entails the treatment of severe acute respiratory syndrome in the human body, possesses the ability to fight against influenza serving as antiviral respiratory treatment, and can also treat respiratory depression resulting into respiratory analeptic. In an interesting manner, all the selected compounds possessed positive biological activity than the standards.

2.5. Evaluation of molecular interactions

Molecular interactions of the compound in the active site of the target receptor play a crucial role in drug design. It helps to improve the binding affinity of compounds in the lead optimisation stage. The molecular interactions of the selected studied compounds were discussed, due to their most encouraging properties and therefore compared with the active gauge of the target receptor. As shown in Figure S5, Hesperetin formed a conventional hydrogen bond with Leu141, Gly143, Ser144, Cys145, and Glu166 while Apigenin formed a conventional hydrogen bond with His41 and Tyr54, pi-cation with His41, pi-sulfur with Cys145 and pi-alkyl with Met49. However, Cys145 is the amino acid common in the binding mode of Hesperetin and Apigenin, thus, affirmed the similarity in the pocket and mode of interactions of the two compounds. Surprisingly, the 6LU7 receptor has three domains, and its active site is located in the cleft between domains I and II with Cysteine and Histidine residues forming catalytic dyad in its active site (Jin et al. 2020). It is obvious that Cys145 which is present in both Hesperetin and Apigenin is part of the catalytic subunit of the active site which would hinder the catalytic activity of the enzyme; therefore, both Hesperetin and Apigenin share the same pocket and interactions with the pocket of the target receptor, thus establishing their potency and efficacy against the target receptor. Similarly,

Lupeol and Lupenone (Figure S4) share the same pocket with the two standards used (Table S2). Lupeol formed alkyl and pi-alkyl interactions with Leu287, Tyr239, Leu273, Tyr237, and Leu286, while Lupenone formed the same interaction types with Leu272, Tyr239, Leu287, Leu286. Obviously, Leu286, Leu287, and Tyr287 are common to both, showing that they share the same pocket and mode of interaction. However, a careful check through the interactions of Remdesivir and Azithromycin with the target receptor (Table S2) validated the claim that the two standards share the same adjourning binding pocket to the active site which has been reported to be efficient in designing a therapeutic agents (Anand et al. 2002), with Lupoel and Lupenone. Remdesivir and Azithromycin had completed their randomised clinical trials as potential inhibitor of SARS-CoV-2 (Kaddoura et al. 2020). Therefore, the identified ligands could develop further through lead optimisation, molecular dynamics, preclinical studies, and clinical trials towards the development of new therapeutic agents to combat SARS-CoV-2 virus.

3. Conclusions

The need for a new therapeutic agent for COVID-19 is a necessity. Computer-aided drug design is an indispensable tool to hastening the discovery and development of new drug that can apprehend this global threat. This study used virtual screening via molecular docking to identify five compounds capable of inhibiting SARS-CoV-2 (M^{pro}). The binding energies of the identified compounds are Lupeol (–8.6 kcal/mol), Lupenone (–7.7 kcal/mol), Hesperetin (–7.4 kcal/mol), Apigenin (–7.3 kcal/mol) and Castasterone (–7.3 kcal/mol). Hesperetin and Apigenin shared the same pocket with the target receptor by interacting with Cys145, an important amino acid in the catalytic subunit of the SARS-CoV-2 (M^{pro}) active site, while Lupeol and Lupenone share the same adjourning pocket to the active site with Remdesivir and Azithromycin whose randomised clinical trials against SARS-CoV-2 had been completed. All the selected compounds obeyed Lipinski's (RO5) and show excellent oral-bioavailability and PASS properties. Similarly, the ADMET prediction shows that the selected compounds could easily be absorbed in the human intestine; are non-Ames toxic, non-hERG2 inhibitors and non-carcinogenic. Put together, all the selected compounds are probable inhibitors of SARS-CoV-2 M^{pro} and their properties could still be improved during the Hit-to-Lead optimisation process. We hope this work will be helpful to researchers especially drug design experts and the compounds identified could be explored further towards the development of a new drug against the deadly virus (SARS-CoV-2).

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