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Interference of Chaga mushroom terpenoids with the attachment of SARS-CoV-2; in silico perspective

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ABSTRACT

Finding a potent inhibitor to the pandemic SARS-CoV-2 is indispensable nowadays. Currently, in-silico methods work as expeditious investigators to screen drugs for possible repurposing or design new ones. Targeting one of the possible SARS-CoV-2 attachment and entry receptors, Glucose-regulated protein 78 (GRP78), is an approach of major interest. Recently, GRP78 was reported as a recognized representative in recognition of the latest variants of SARS-CoV-2. In this work, molecular docking and molecular dynamics simulations were performed on the host cell receptor GRP78. With its many terpenoid compounds, Chaga mushroom was tested as a potential therapeutic against the SARS-CoV-2 receptor, GRP78. Results revealed low binding energies (high affinities) toward the GRP78 substrate-binding domain β (SBDβ) of Chaga mushroom terpenoids. Even the highly specific cyclic peptide Pep42, which selectively targeted GRP78 over cancer cells in vivo, showed lower binding affinity against GRP78 SBDβ compared to the binding affinities of terpenoids. These are auspicious results that need to be tested experimentally. Intriguingly, terpenoids work as a double sword as they can be used to interfere with VUI 202,012/01, 501.V2, and B.1.1.248 variants of SARS-CoV-2 spike recognition.

1. Introduction

SARS-CoV-2, which appeared in the 21st century, has caused many drastic changes in the fabric of the world [1]. COVID-19 pandemic is still producing health and economic consequences, while great efforts are spent on finding possible antivirals [2]. Many host-cell receptors are identified by the coronaviruses, including heparan sulfate proteoglycans, Aminopeptidase N, Angiotensin-Converting Enzyme 2 (ACE2), furin, O-Acetylated Sialic Acid, and the Glucose Regulated Protein 78 (GRP78) [3, 13–9]. Directly after the entry of the virus, it kills the T lymphocyte cells, which leads to lymphopenia. Meanwhile, the inflammatory response activated via the virus also starts attacking the lymphocyte cells and leads to their apoptosis. Ultimately, when the viral particles have accumulated, some symptoms start to appear, such as destruction in the endothelial barrier, losing the capacity of oxygen diffusion, and in severe cases, the increase in inflammation caused by different cytokines can lead to death [10]. In addition, many studies have predicted the interaction between different molecules and the RBD of the spike protein [11–13]. Our main concern is targeting GRP78, accordingly, contradicting the SARS-CoV-2 entry.

GRP78, or binding immunoglobulin protein (BiP), is encoded by the heat shock protein A5 (HSPA5) gene and reside inside the endoplasmic reticulum (ER) of normal cells [14–16]. GRP78 functions as a chaperone protein that binds to unfolded proteins and directs them to the refolding or degradation machinery; hence it is described as the master of the unfolded protein response (UPR) mechanism [16]. Thus, stressed cells have elevated levels of GRP78 expression in order to overcome the massive number of unfolded proteins. In the dormant stable cell states, three transmembrane stress sensor proteins (residing in the ER)are bound to GRP78. These are the Activating transcription factor 6 (ATF6), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and Inositol-requiring enzyme 1 (IRE1) [17, 18]. When the stress signal sparks, the three proteins are released and become active in order to alleviate the stress inside the cell. Consequently, GRP78 is overexpressed and translocated to other cellular compartments, including the cell membrane, where the chaperone protein can carry out various functions [18–24]. Once exposed to the cell surface, GRP78 acts as a gate for pathogen recognition and entry [3, 21, 25–28]. We previously reported the possibility of recognizing SARS-CoV-2 spike by the cell-surface GRP78 and defined the spike region C480–C488 as the recognition site [3]. This recognition was validated experimentally by Lee et al., who identified the association of GRP78 with both human Angiotensin-converting enzyme 2 (ACE2) and SARS-CoV-2 spike protein.
2. Materials and methods

2.1. Structure retrieval

The structures of the terpenoid compounds (twenty-eight) were retrieved from the PubChem database [43]. Most of the terpenoids were found in the 3D structure-data files (SDF) on PubChem, so it was used to build the docking study’s input files (PDBQT) utilizing AutoDock Tools software [44]. Unfortunately, few compounds were available on the PubChem as 2D, therefore we generated the 3D structures using Avogadro software and optimized the geometry using the steepest descent algorithm with the universal force field (UFF) of Avogadro [45,46].

On the other hand, the structure of the cyclic peptide Pep42 was built by comparative modeling from its amino acid sequence (CTVALPG-GYVRVC) with the aid of the I-TASSER web server (https://zhanggroup.org/I-TASSER/, accessed on December 6, 2021) [25,47,48]. First, the cyclic peptide structure was constructed by forming the S-S bond between C1 and C13 using Avogadro software. Then, Molecular Dynamics Simulation (MDS) for 200 ns was performed on the cyclic peptide using CHARMM 36 force field in the nanoscale molecular dynamics (NAMD) software [49,50]. Cluster analysis was performed through Maestro software on the cyclic peptide trajectories. Finally, four main clusters were extracted to get representative conformations for the Pep42 to be used in the docking experiments [51].

2.2. Protein preparation

GRP78 structure (PDB ID: 5E84) was downloaded from the protein data bank (PDB) [52]. It exhibited the wild-type open conformation of GRP78 since other structures such as 6HAB, 5F0X, 3LDQ, and 6ZYH were either in the closed conformation or missing some domains [52,53]. The structure was then prepared for the docking study by removing water molecules and ligands while missing Hydrogen atoms were added with the help of PyMOL software [54].

2.3. Molecular dynamics, docking, and MM-GBSA calculations

The different conformations of GRP78 after 50 ns MDS run were used in the docking experiments. As reported before, four different conformations of the protein resembled the four most popular clusters of GRP78 trajectories. The protein’s representative conformations were taken using Chimera software at 17.8, 26.2, 51.8, and 37.8 ns. [55]. The Pep42 cyclic peptide (selected conformations after MDS), EGCG, and the 28 terpenoids were tested against the four GRP78 conformations. AutoDock Vina software was used in the docking study, while AutoDock Tools and PyMOL were employed to generate the input files and analyze the output files [44,56]. All of the docking experiments followed a flexible ligand in a flexible active site protocol. The grid boxes were chosen to be of size $48 \times 48 \times 56 \text{ Å}^3$ centered at 52, 22, and 38 Å (minor differences existed between the four different conformations of GRP78) with a default grid spacing of 0.375 Å. The searching box covered all of the active residues (I426, T428, V429, V432, T434, F434, F451, S452, V457, and 1459) [17,57].

For further analysis, the docking complexes were examined using the discovery studio visualizer software [58]. First, data were tabulated and

List of abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ACE2 | Angiotensin-Converting Enzyme 2 |
| ADMET | Absorption, Distribution, Metabolism, Excretion, and Toxicity |
| ATF6 | Activating transcription factor 6 |
| BBM | Blood Brain Barrier |
| BiP | binding immunoglobulin protein |
| CHARMM | Chemistry at Harvard Macromolecular Mechanics |
| CS-GRP78 | cell-surface GRP78 |
| EGCG | (−)Epigallocatechin gallate |
| ER | endoplasmic reticulum |
| GRP78 | Glucose-regulated protein 78 |
| HSFA5 | heat shock protein A5 |
| IRE1 | Inositol-requiring enzyme 1 |
| MDS | Molecular Dynamic Simulation |
| MM-GBSA | Molecular Mechanics Generalized Born and Surface Area |
| NAMD | nanoscale molecular dynamics |
| PERK | protein kinase RNA-like endoplasmic reticulum kinase |
| RBD | receptor-binding domain |
| RMSD | Root Mean Square Deviation |
| RMSF | Root Mean Square Fluctuations |
| RoG | Radius of Gyration |
| SASA | surface Accessible Surface Area |
| SDBα | substrate-binding domain α |
| SDBβ | substrate-binding domain β |
| SDF | structure-data file |
| UFF | universal force field |
| UPR | unfolded protein response |

[9]. Elevated levels of GRP78 were reported in COVID-19 patients [29]. Moreover, increased severity of COVID-19 (patient needs ICU or died) was reported in cancer patients compared to normal individuals [30]. A number of natural remedies were suggested to be fighting against COVID-19 [31–33]. Various naturally-derived compounds such as terpenoids were able to block the site of the cell-surface GRP78 (CS-GRP78) recognition, the receptor-binding domain β, and to compete for pathogen recognition [34,35]. Terpenoids from Chaga mushroom (Inonotus obliquus) were reportedly anti-cancerous effects [36,37]. Furthermore, we previously assessed the effectiveness of terpenoids in binding to SARS-CoV-2 spike protein, where twenty-eight terpenoid compounds were docked to the SARS-CoV-2 spike receptor-binding domain (RBD) [38]. Most of the tested terpenoids showed excellent binding affinities against the spike RBD (−5.6 down to −7.8 kcal/mol). At the same time, two of the terpenoids, betulinic acid, and inonotusane C, bonded near to the spike’s ACE2 binding interface [38]. The rationale for testing the same group of terpenoids against GRP78 was that CS-GRP78 could recognize viral particles and hence might be a suitable target of the Mushroom terpenoids. Dual targeting of a viral protein and one of its host-cell receptors is promising to be tested against the mushroom terpenoids. It was not important to know the exact target of each of the investigated terpenoids. In fact, their combined affinity would make them a possible maestro in preventing infection.

In the present study, we predicted the binding potency of the same terpenoid compounds against the host-cell receptor GRP78 SBDb, substrate-binding domain β, the same recognition site for CS-GRP78 by SARS-CoV-2 spike, juxtaposed with the peptide Pep42 and the (−)Epigallocatechin gallate (EGCG) as positive controls. Other domains of GRP78 may also be available for terpenoids but are yet to be explored. The study was based on molecular docking and molecular dynamics simulation to mimic the terpenoids-GRP78 system in physiological conditions. These computational methods successfully suggested new drug candidates against COVID-19 [39–41]. After that, Molecular Mechanics Generalized Born and Surface Area (MM-GBSA) for the best two complexes (Oleanolic acid and Inonotusulide A) in addition to the residual contribution to the binding was calculated using MMPBSA.py implemented in AmberTools 17 [42].
Fig. 1. 2D structures of the terpenoids ranked according to their binding affinities to GRP78 RBD.
The whole trajectory with a stride of 1 was used in the calculation of binding energy, and the method of generalized born (igb) was used.

Some complexes are represented graphically in Fig. 3. Fig. 3B depicts betulinic acid, and inonotusane C docked into the SBD (3b-Hydroxycinnamolide). The peptide Pep42 and EGCG (red >) are positive controls due to their specificity in binding HSPA5. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 2 represents the average binding energies of the terpenoid compounds against the four different conformations of GRP78 (colored columns), compared to Pep42, and (−)-Epigallocatechin gallate (EGCG) as positive controls (red columns), while the error bars represent the standard deviation (SD) [63]. Surprisingly, all of the twenty-eight terpenoid compounds (blue columns) exhibit lower (better) binding affinities to the GRP78 SBD compared to Pep42, yet still in the same range as EGCG. The average binding energy values ranged from −8.48 ± 1.29 kcal/mol (Oleanolic acid) to −6.75 ± 0.30 kcal/mol (3b-Hydroxycinnamolide).

Table 1 shows the interactions established upon docking of the 28 terpenoids against GRP78 SBDβ from the top left (best compound) to the right bottom (worst in binding). Each terpenoid molecule was docked to the four different conformations of the GRP78 using AutoDock Vina.

3. Results

Fig. 1 demonstrates the 2D structures of the terpenoids ranked according to their average binding affinities against GRP78 SBDβ from the top left (best compound) to the right bottom (worst in binding). Each terpenoid molecule was docked to the four different conformations of GRP78 using AutoDock Vina.

3.1. Terpenoids binding energies against GRP78 SBDβ

Fig. 2 represents the average binding energies of the terpenoid compounds against the four different conformations of GRP78 (colored columns), compared to Pep42, and (−)-Epigallocatechin gallate (EGCG) as positive controls (red columns), while the error bars represent the standard deviation (SD) [63]. Surprisingly, all of the twenty-eight terpenoid compounds (blue columns) exhibit lower (better) binding affinities to the GRP78 SBDβ compared to Pep42, yet still in the same range as EGCG. The average binding energy values ranged from −8.48 ± 1.29 kcal/mol (Oleanolic acid) to −6.75 ± 0.30 kcal/mol (3b-Hydroxycinnamolide).

Table 1 shows the interactions established upon docking of the 28 terpenoids against GRP78 substrate-binding domain β, where the number and type of the interactions are listed. The selected complexes are ranked according to the average binding affinity as in Figs. 1 and 2. The complex selection was based on the binding affinity values, where the complex with binding affinity close to that of the mean value was selected for the analysis. The selected complexes were examined using the Discovery studio visualizer to analyze the data further.

Some complexes are represented graphically in Fig. 3. Fig. 3B depicts betulinic acid, and inonotusane C docked into the SBD of GRP78 to quantify their binding behavior and compare it to a previous study [38] where it is tightly bound to the spike of SARS-CoV-2 (Table 1).

3.2. Molecular dynamic simulations and binding free energy calculations for the best two complexes

Molecular dynamics simulations (50 ns) for the best two complexes (Oleanolic acid and Inonotsulide A) were performed using NAMD software, then MM-GBSA was calculated using amber tools. In Table 2, the residuals’ contribution to GRP78 binding of the best two compounds


Equilibrated for 50 ns (flattened RMSD after about 8 ns (Fig. 4 A)). (Oleanolic acid and Inonotsulide A) are listed.

The interactions were established on selected ligand-GRP78 complexes based on binding affinity values. Bold residues are the most repeated interactions in most complexes (orange line) showed more stable RoG values compared to the latter complex (RMSD around 8 Å). This might indicate the stability of the first complex relative to the other one. Fig. 4 B shows the per-residue Root Mean Square Fluctuations (RMSF) in Å for the apo-GRP78 (blue line) and the two complexes (GRP78-Oleanolic acid and GRP78-Inonotsulide A). The apo-GRP78 and the GRP78-Oleanolic acid complex were almost the same, showing fluctuations (RMSF < 8 Å) at the SBDx (residues 565–600) and the N and C termini. On the other hand, GRP78-Inonotsulide A complex showed higher fluctuations in SBDx (residues 565–600) and SBDj (residues 428–491) and even in the nucleotide-binding domain (residues 133–138) of the GRP78. This reflected the complex’s stability in the case of Oleanolic acid compared to GRP78-Inonotsulide A complex.

3.3. ADMET properties of Chaga mushroom terpenoids

Table 3 shows the properties of each compound and whether they agree with the Lipinski’s rules of five (green) or not (red). Nearly all of the studied terpenoids are druggable according to the rule of five except for the values of LogP of Inonotusol G, and Inonotusic acid compounds (shown in red in Table 3). Additionally, the pkCSM webserver was used to check the ADMET properties as tabulated in Table 4.

4. Discussion

We previously reported in silico the Inonotus obliquus terpenoids’ effectiveness (Chaga mushrooms) in binding the receptor-binding domain of SARS-CoV-2 spike protein [38]. Most of the terpenoid compounds were reported to be tightly bound to the spike protein at the receptor-binding domain at the ACE2 binding surface. At the same time, betulinic acid (~7.5 kcal/mol) and inonotusane C (~7.4 kcal/mol) were the best two compounds in binding the spike. On the other hand, Beta glycinar, betulinic acid, and galactomannan show high affinity against the S1 (~7.4 to ~8.6 kcal/mol) of the spike as reported in another prediction study [64].

According to our previous work, the 50 ns MDS was enough to equilibrate the GRP78 system [35]. Meanwhile, the cyclic peptide Pep42 was simulated for 200 ns MDS at the same physiological conditions of salt, water, and temperature. The protein (GRP78) and the cyclic peptide Pep42 systems were equilibrated during the first 20 ns of the simulation as reflected from the Root Mean Square Deviation (RMSD) and the Radius of Gyration (RoG) curves (in Å) versus the simulation time (ns) shown in the Supplementary Fig. S1. Pep42 was proved to be the distinctive docking element of GRP78 SBDj [65,66], giving average binding energy of ~6.23 ± 0.50 kcal/mol, while for EGCG, it gave a value of ~7.97 ± 0.40 kcal/mol.

Up on docking, the main types of established interactions were: the formation of H-bonds and hydrophobic contacts with some π-sigma, π-alkyl, and π-π stacked interactions in some complexes as shown in Table 1, where the most repeated interactions are in bold. The F451 residue in GRP78 was the most frequent in forming contacts (hydrophobic) with terpenoids with a total of 74 interactions (π-sigma and π-alkyl hydrophobic contacts). The V453, I459, V429, and I426 residues...
Fig. 3. A) The interaction pattern of Pep42 cyclic peptide (white cartoon and sticks) with GRP78 SBDβ (wires). The protein surface is calculated and represented by the hydrophobicity according to the color scheme at the figure’s lower-left corner. (B) The interaction pattern of Betulinic acid and Inonotsane C (sticks) against GRP78 SBDβ (wires). The H-bonds and hydrophobic contacts are depicted by dashed-green lines and dashed-iris lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
formed 37, 34, 27, and 19 hydrophobic contacts with terpenoids, respectively. These hydrophobic patches of the substrate-binding domain of GRP78 were the docking platform of the unfolded proteins in stressed cells [25, 57]. On the other hand, Pep42 formed three H-bonds with Q449(2) and Q492, and four hydrophobic contacts with I450 & V453 (Alkyl contacts), I426 (π-sigma) and F451 (π-π stacked) of GRP78 (Fig. 3A). This is in excellent agreement with previous in vivo studies, where Pep42 was reported to selectively recognize and bind GRP78 over cancer cells [65, 66]. Interestingly, the V453 residue, which resembles the substrate-binding defective mutant of GRP78, is reported here to bind to the Pep42 and 17 terpenoid compounds against GRP78. V453 was described as a crucial residue in spike and ACE2 recognition of GRP78 [9]. Whereas EGCG formed one H-bond to E427, one π-σ stacking with F451, one σ-π interaction with I459, two π-Alkyl interactions with F451 & K460, and two Alkyl interactions with I426 & I459.

Inonotusane C formed ten hydrophobic contacts (dashed-gray lines) with GRP78 residues I426(2), V429, F451(6), and V495. In comparison, betulinic acid interacted with both H-bonds (dashed-green lines) of E427 and I450 and formed four hydrophobic contacts (I426, F451, and I459(2)) with GRP78. It seems that F451 is very important in recognizing Inonotusane C by GRP78. It was involved in 6 hydrophobic interactions with almost every part of the molecule. Hydrophobic interactions were also a landmark of terpenoids interactions with SARS-CoV-2 spike (three in the case of inonotusane C and four in the case of betulinic acid), where K458 and Y473 were the most reported residues from the spike that formed these hydrophobic contacts with terpenoids [38].

The residues from GRP78 SBDβ, defined as the docking platform of the substrates I426, T428, V429, V432, T434, F451, S452, V457, and I459, are shown in bold and underlined in Table 2. For the GRP78-Oleanolic acid complex, I426, V429, V457, and I459 were the main contributors to binding (−1.26, −1.01, −1.43, and −1.47 kcal/mol, respectively), while for the GRP78-Inonotsulide A complex, R488 was the main contributor (−1.21 kcal/mol). The contribution of the substrate-binding site (bold and underlined) of GRP78 in the binding of Oleanolic acid to the protein is clear from Table 2 (−7.1 kcal/mol). In comparison, a lower contribution of these residues was reported in the case of the GRP78-Inonotsulide A complex (−0.63 kcal/mol). E427 (red-colored) negatively contributed to binding (having positive energy difference) in both complexes (+1.12 and +0.52 kcal/mol for Oleanolic acid and Inonotsulide A, respectively). F478, G454, and D511 showed negative contributions to the binding. The total binding energy for the Oleanolic acid was lower (−22.51 kcal/mol) than for Inonotsulide A (−16.51 kcal/mol); hence Oleanolic acid was the best-suggested compound that could bind to GRP78 SBDβ.

According to Lipinski’s rule of five, a compound is considered to be druggable if the number of hydron bond acceptors and donors is less than or equal to 10 and 5, respectively, its solubility (LogP) ≤ 5 and its molecular weight <500 Da [62, 67].

For the ADMET properties, Table 4 shows the prediction of the pkGSM webserver. The absorption of the compounds can be predicted (according to Table 4) through three values (water solubility in log(mol/L), Caco2 permeability, and intestinal absorption). Compounds with more negative water solubility values, Caco2 permeability >0.9, and intestinal absorption >30% indicate that the server predicted them as soluble. Nearly all compounds achieved good Caco2 permeability and intestinal absorption values, and all compounds had a negative value less than −3.02 log mol/L. The next prediction is for the distribution of the terpenoids, which can be known through the fraction of compounds that are not bound to serum proteins and Blood-Brain Barrier (BBB) permeability. The higher the unbound fraction and the more negative the values of BBB permeability indicate a good distribution. Inhibitors of Cytochrome P450 can activate the drug metabolism and, therefore, be removed from the market. The compounds were used to predict whether they were inhibitors of different isozymes (CYP1A2, CYP2C19, CYP2C9,

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**Table 2**
The MM-GBSA calculations for the best two complexes after 50 ns MDS. Red-colored residues represent the residues that have a negative contribution to binding (positive binding energies). The average binding free energies and their terms are shown at the bottom of the table for each complex with its standard deviations.

| COMPLEX     | GRP78 - Oleanolic acid complex | GRP78 - Inonotsulide A complex |
|-------------|--------------------------------|--------------------------------|
| RESIDUAL CONTRIBUTION TO BINDING | Residue | Binding energy (kcal/mol) | Residue | Binding energy (kcal/mol) |
| I459        | −1.47                          | R488                          | −1.21                          |
| V427        | −1.43                          | I483                          | −0.72                          |
| F453        | −1.35                          | V453                          | −0.68                          |
| I426        | −1.26                          | E460                          | −0.58                          |
| V429        | −1.01                          | I493                          | −0.40                          |
| V453        | −0.98                          | T458                          | −0.37                          |
| Q449        | −0.77                          | I459                          | −0.34                          |
| T428        | −0.58                          | V490                          | −0.30                          |
| V495        | −0.47                          | V457                          | −0.29                          |
| T477        | −0.26                          | A486                          | −0.26                          |
| T456        | −0.25                          | P484                          | −0.24                          |
| S448        | −0.25                          | P487                          | −0.21                          |
| G425        | −0.19                          | P491                          | −0.20                          |
| T458        | −0.16                          | Q492                          | −0.19                          |
| V492        | −0.16                          | I520                          | −0.19                          |
| F478        | +0.21                          | I522                          | −0.18                          |
| G454        | +0.24                          | E427                          | +0.52                          |
| E427        | +1.12                          | D511                          | +0.73                          |

ΔE_DOB (kcal/mol): −31.05 ± 4.8
ΔE_ELE (kcal/mol): −5.13 ± 4.9
ΔE_GAS (kcal/mol): −8.07 ± 4.7
ΔE_GAS (kcal/mol): +0.43 ± 0.6
ΔG_GAS (kcal/mol): −36.19 ± 5.9
ΔG_SOLV (kcal/mol): 13.68 ± 4.6
ΔG TOTAL (kcal/mol): −22.51 ± 4.7
ΔG TOTAL (kcal/mol): −16.51 ± 10.4
Fig. 4. (A) Root mean square deviation (RMSD) in Å and Radius of Gyration (RoG) in Å, Surface Accessible Scheme 2 and number of H-bonds versus time in ns for the MD simulation of GRP78-Oleanolic acid (orange) and GRP78-Inonotsulide A (gray) complexes. (B) Root Mean Square Fluctuations (RMSF) versus residue number of apo-GRP78 (blue), GRP78- Oleanolic acid (orange), and GRP78-Inonotsulide A (gray) complexes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
CYP2D6, and CYP3A4). All compounds were predicted not to inhibit the last three isoforms, and only Inonotusic acid was predicted to be an inhibitor of CYP1A2 and CYP2C19. The model predicts whether a compound is a renal organic cation transporter 2 substrates for excretion. Since interaction with this transporter helps in the clearance of the compound and may produce adverse interactions, negative values are considered good. Only one compound (3b Hydroxycinnamolide) showed a positive prediction. Finally, toxicity is predicted through four indicators. Ames toxicity is a test that indicates whether the compound is a carcinogen. Inhibition of hERG I/II is the principal cause of fatal ventricular arrhythmia and has resulted in the withdrawal of many substances. As its name implies, hepatotoxicity indicates whether the compound may disrupt the liver’s normal function. None of the studied terpenoids was predicted to have a carcinogenic effect or act as an inhibitor for hERG I. On the other hand, three compounds (Ergosterol peroxide, Ergosterol, and Lanosterol) were predicted to be inhibitors of hERG II. Five compounds (Trametenolic acid, Oleanolic acid, inonotusol E, inonotusol D, and inonotusol C) were predicted to cause hepatotoxicity to the liver.

Overall, most compounds showed excellent absorption, metabolism and excretion, good toxicity, and moderate distribution prediction, and most of them were considered druggable according to Lipinski’s rule of five.

Targeting the cell-surface GRP78 is safe as this protein functions as its normal function. None of the studied terpenoids might

### Table 3

| Compounds names    | Number of H-donors | number of H-acceptors | LogP       | Molecular weight |
|--------------------|--------------------|-----------------------|-----------|-----------------|
| Inonotutsiol D     | 3                  | 3                     | 1.58314   | 411.351         |
| Inonotutsiol B     | 3                  | 3                     | 0.91655   | 411.351         |
| Inonotsuxiodiol A  | 2                  | 3                     | 1.24775   | 410.343         |
| Inonotsuxoxide A   | 2                  | 3                     | 1.40515   | 410.343         |
| Inonotsulide C     | 2                  | 4                     | 0.51276   | 426.342         |
| Inonotsulide A     | 2                  | 4                     | 0.51276   | 426.342         |
| Inonotsudiol A     | 2                  | 2                     | 1.75994   | 394.344         |
| Ergosterol peroxide| 1                  | 3                     | 1.22716   | 385.313         |
| Ergosterol         | 1                  | 1                     | 1.93674   | 353.315         |
| Betulinic acid     | 2                  | 2                     | 1.35196   | 410.343         |
| Betulin            | 2                  | 2                     | 1.67865   | 394.344         |
| 3b Hydroxycinnamolide | 1                 | 3                     | 0.29938   | 229.17          |
| Trametenolic acid  | 2                  | 2                     | 1.43325   | 410.343         |
| Spirinoonotsuxolidol| 2                 | 3                     | 1.16646   | 410.343         |
| Oleanolic acid     | 2                  | 2                     | 1.27067   | 410.343         |
| Lanosterol         | 1                  | 1                     | 1.93674   | 377.337         |
| Inotodiol          | 2                  | 2                     | 1.75994   | 394.344         |
| Inonotusol G       | 2                  | 3                     | 6.33366   | 456.711         |
| Inonotusol F       | 1                  | 3                     | 1.43744   | 421.346         |
| Inonotusol E       | 4                  | 5                     | 0.22756   | 444.357         |
| Inonotusol D       | 5                  | 5                     | 0.56295   | 445.365         |
| Inonotusol C       | 5                  | 5                     | 0.56295   | 445.365         |
| Inonotusol B       | 5                  | 6                     | 0.05076   | 461.364         |
| Inonotusol A       | 5                  | 6                     | 0.05076   | 461.364         |
| Inonotusolic acid  | 0                  | 2                     | 5.0495    | 312.453         |
| Inonotusane C      | 1                  | 1                     | 1.57407   | 357.303         |
| Inonotusane B      | 3                  | 3                     | 0.91655   | 411.351         |
| Inonotsutrold E    | 3                  | 3                     | 1.58314   | 411.351         |

### 5. Conclusion

Terpenoids, found in the Chaga mushroom, have been reported to bind to the spike of SARS-CoV-2 with acceptable binding affinity. Furthermore, in the current study, we report the binding affinity of terpenoids to one of the host-cell entry routes of SARS-CoV-2, GRP78. All of the 28 terpenoid compounds have comparable binding affinities with the positive control EGCG. At the same time, they are better (lower) than the Pep42 cyclic peptide that is reported to be specific for COVID-19. Thus, the present study suggests that terpenoids are robust candidates in influencing the spread of SARS-CoV-2 new variants. Hence, Chaga mushroom’s terpenoids might be used as prophylactic agents for high-risk persons such as elders, the front-line medical staff, and diabetic & cancer patients.
Table 4
The Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties of the tested terpenoids as calculated using pkCSM webserver.

| Compound name         | Water solubility (log (mol/L)) | Caco2 permeability | Intestinal absorption (human) | Fraction unbound (human) | BBB permeability | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | Renal OCT2 substrate | Ames toxicity | hERG I inhibitor | hERG II inhibitor | Hepatotoxicity |
|-----------------------|--------------------------------|--------------------|-------------------------------|--------------------------|-------------------|-----------------|-------------------|------------------|------------------|-----------------|---------------------|----------------|----------------|----------------|--------------|
| Inonotriol D          | -4.32                          | 1.373              | 100                           | 0.095                    | -0.275            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotriol B          | -4.251                         | 1.2                | 49.745                        | 0.094                    | 0.569             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonosuxoxidol A      | -4.593                         | 1.384              | 59.213                        | 0.118                    | 0.543             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonosuxoxide A       | -4.546                         | 1.204              | 100                           | 0.062                    | 0.611             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotulide C         | -4.456                         | 0.313              | 60.549                        | 0.084                    | 0.464             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotulide A         | -4.363                         | 0.438              | 60.385                        | 0.11                     | 0.535             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotriol D          | -4.345                         | 1.276              | 100                           | 0                         | -0.336            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Ergosterol peroxide   | -3.642                         | 1.251              | 80.472                        | 0.139                    | 0.497             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Ergosterol            | -4.927                         | 1.255              | 100                           | 0.025                    | 1.159             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Betulinic acid        | -3.151                         | 1.316              | 100                           | 0.144                    | 0.746             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Betulin              | -4.341                         | 1.331              | 100                           | 0.127                    | -0.29             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| 3b Hydroxyxannamolide | -2.295                         | 1.211              | 85.275                        | 0.433                    | -0.004            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Trametenolic acid     | -3.329                         | 1.203              | 100                           | 0.124                    | 0.735             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | Yes          |
| Spirononotuxoxidol    | -4.248                         | 1.358              | 62.479                        | 0.101                    | 0.575             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Oleanolic acid        | -3.02                          | 1.252              | 53.696                        | 0.131                    | 0.747             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | Yes          |
| Lanosterol            | -4.795                         | 1.273              | 100                           | 0.012                    | 1.176             | No              | No                | No               | No               | No               | No                  | No            | Yes            | Yes            | No           |
| Inotriol              | -4.253                         | 1.416              | 100                           | 0.068                    | -0.291            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inontusol G           | -5.974                         | 1.373              | 94.283                        | 0                        | -0.196            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inontusol F           | -4.511                         | 1.418              | 100                           | 0.003                    | 0.678             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inontusol E           | -3.74                          | 0.576              | 63.728                        | 0.25                     | -0.165            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inontusol D           | -3.799                         | 0.687              | 62.548                        | 0.149                    | -0.198            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | Yes          |
| Inontusol C           | -3.799                         | 0.687              | 62.548                        | 0.149                    | -0.198            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | Yes          |
| Inontusol B           | -3.957                         | 0.631              | 64.612                        | 0.308                    | -0.377            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inontusol A           | -3.957                         | 0.631              | 64.612                        | 0.308                    | -0.377            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotuscin           | -5.861                         | 1.725              | 96.964                        | 0                        | 0.054             | Yes             | Yes               | No               | No               | No               | No                  | No            | Yes            | Yes            | No           |
| Inonotusane C         | -4.597                         | 1.251              | 100                           | 0.026                    | -0.213            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotusane B         | -4.251                         | 1.2                | 49.745                        | 0.094                    | 0.569             | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
| Inonotriol E          | -4.32                          | 1.373              | 100                           | 0.095                    | -0.275            | No              | No                | No               | No               | No               | No                  | No            | No             | No             | No           |
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