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In silico evaluation of the inhibitory property of *Holothuria scabra* (sea cucumber) with the catalytic domain of matrix metalloproteinase-1 for collagen degradation via interaction of triterpenoid saponins

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Abstract
Matrix metalloproteinase-1 (MMP-1) is a collagenase that cleaves the collagen present in the extracellular matrix (ECM), which results in skin wrinkling. In this *in silico* study, the triterpenoid saponins present from *Holothuria scabra* (desholothurin A, holothurinoside C, pervicoside C), were studied to determine its inhibitory properties against MMP-1. The approach was done via determining its dermatologic activity, its binding affinity against MMP-1 via molecular docking, followed by its pharmacokinetic properties. Dermatologic activity assessment of each triterpenoid saponins gave a Pa value of 0.408 for desholothurin A, 0.481 for holothurinoside C, and 0.297 for pervicoside C. In molecular docking, all triterpenoid saponins exhibited strong (such as conventional hydrogen bonding) and weak molecular interactions (such as van der Waals forces and carbon-carbon interactions). These molecular interactions was described by binding affinity giving desholothurin A a binding energy of -6.03 kcal/mol (37.79 uM), following holothurinoside C with -5.46 kcal/mol (99.14 uM), and pervicoside C with -3.05 kcal/mol (5480 uM). Lastly, *in silico* pharmacokinetic studies were done, which demonstrated poor drug-like properties of the compounds.

Keywords: *Holothuria scabra*; triterpenoid saponins; matrix metalloproteinase-1, dermatologic activity, molecular docking, pharmacokinetics

Introduction
Holothuria scabra (also known as sandfish), are species of sea cucumbers that are commercially-valuable in the Philippine archipelago belonging to the family of *Holothuriidae* [1]. The family of *Holothuriidae* are classes of sea cucumbers that may vary based on their sizes and vary in different places such as below rocks and rubbles, buried in sand, or lying exposed [2]. Specifically, they are found in shallow waters in about twenty (20) meter deep on the inner reef and seagrass beds with muddy sandy substrates near mangroves [2]. These species consist of six (6) orders, which are nearly 1,400 species commonly found in the Pacific Ocean, Southeast Asia and Indian Ocean [3]. Characteristics of *H. scabra* found in the Pacific Ocean and Southeast Asia are black to grey, or light brownish green that is sometimes with greyish-black lines, while *H. scabra* found in the Indian Ocean are usually dark grey with white, beige, or yellow traverse stripes [3]. Sizes also vary depending on the country they are found with the average length of twenty-four (24) centimeters which can grow in a maximum of forty (40) centimeters. Their weight depends on their location ranging from 380 grams to 580 grams freshwater weight, which can weigh up to two (2) kilograms as its maximum [3].
Sea cucumbers are an important source of traditional medicine, due to the presence of the bioactive compounds that are present, such as vitamins and triterpenoid glycoside saponins [4, 5]. Based on their structure, saponins are divided into three (3) classes: triterpenoid, steroid, and alkaloid, wherein the research focuses on triterpenoid saponins. Based on its structure, these saponins contain steroid triterpenoid aglycone (sapogenin) linked to several oligosaccharide moieties [5, 6] Moreover, *Holothuriidae* families differ among their sulfate groups whether attached to the carbohydrate chain of their saponins [3]. Some constituents of triterpenoid saponins found in *H. scabra* include desholothurin A, holothurinoside C, and pervicoside C in the eluate of *H. scabra* [4]. In the current study, the triterpenoid saponins seen in *H. scabra* were utilized to test their anti-wrinkling property. One of the ways of determining the anti-wrinkling property of a compound is by means of targeting specific proteins that

degrades the collagen. An example of this is a protein called matrix metalloproteinase-1 (MMP-1), a collagenase that degrades the collagen contributing to skin aging.

Skin aging is where the collagen scaffold loses its strength and stability, resulting to collagen degradation [7-10]. During collagen degradation, ultraviolet (UV) radiation being stimulated in the ECM's collagen within the human fibroblasts excretes a particular transcription factor called NF-κB. This factor releases MMPs such as MMP-1, which are responsible for disrupting the collagen structure [11]. In humans, MMP-1 is an interstitial collagenase responsible for beginning collagen fragmentation and alteration of collagen fibrils that triggers skin aging [12].

It was revealed that expression of MMPs such as MMP-1 was inhibited, and collagen synthesis of fibroblast was increased when the saponin extract was used to treat skin tissue [13]. Furthermore, there were studies that have been reported that saponin extracts increase collagen production [14]. The saponin extract was said to increase collagen production in skin fibroblast cells by means of phosphorylation of Smad 2 protein which results in the assumption that at the site of a skin wound, this phytochemical extract will promote the re-synthesis of the matrix [15]. However, there are still no studies reported regarding the inhibitory properties of triterpenoid saponins from *H. scabra* against MMP-1. With this, the study aims to evaluate the constituents, desholothurin A, holothurinoside C, and pervicoside C from *H. scabra* (Sea cucumber), study its effects on inhibiting collagen degradation via inhibition of MMP-1 expression *in silico* by means of molecular docking, and evaluate its pharmacokinetic profile.

Materials and Methods

Determination of Relative Abundance of Triterpenoid Saponins from *Holothuria Scabra*

The presence of triterpenoid saponins from *H. scabra* were obtained based from the study of Mitu's group (2017). This was done by determining their relative abundance eluate and mass-to-charge ratio. Their relative abundance eluate was expressed in their arbitrary values, while their mass-to-charge ratio were expressed in m/z.

Description of Triterpenoid Saponins

Desholothurin A is a class of triterpenoid saponin that the *H. scabra* produces. The bioactive compound has a molecular formula of $C_{54}H_{86}O_{24}$ and a molecular weight of 1119.2 g/mol. Holothurinoside C is a class of triterpenoid saponin found in *H. scabra.* This molecule has a molecular formula of $C_{54}H_{88}O_{23}$ with a molecular weight of 1103.2 g/mol. Pervicoside C is a class of triterpenoid saponin found in *H. scabra.* This bioactive compound has a molecular formula of $C_{54}H_{88}NaO_{25}S$ and has a molecular weight of 1192.3 g/mol.

Preparation of Triterpenoid Saponins as Ligands

The structure of triterpenoid saponins were drawn using Chem Sketch software. These structures were optimized by cleaning the structure to standardize their bond lengths and angles. Afterward, *mol* files of the structures were obtained. For visualization of these ligands, the *mol* files were converted to *pdb* files. BIOVIA Discovery Studio Visualizer was utilized to have a clearer visualization of their 3D structure.

Determination of Dermatologic Activity of each Triterpenoid Saponins

After preparation, determination of the dermatologic activities of each triterpenoid saponin was conducted in the software *Way2Drug Predictive Services* (PASS Online (Mechanisms)). The dermatologic activity was based on the statistical data given by the software depending on the confidence interval, or the probability to be active (Pa) and probability to be inactive (Pi). An activity that has Pa > 0.71 was considered to show a specific biochemical activity. Moreover, a compound that has a Pa > Pi, but has a Pa < 0.71 was considered to still exhibit the said activity (approximately ≥ 50%) [16].

Structure Optimization of Ligands and Receptor

Structure Optimization of each Triterpenoid Saponin (Ligands)

UCSF Chimera, a software that optimizes structure, was used to minimize each ligand structure. This was done to attain a proper molecular arrangement, to detect any residues present from the structure, and to improve localized interactions before docking [17]. Optimizing of each ligand was set to 1000 descent steps which acts as a default for the minimization of the structure. Furthermore, heteroatoms, and hydrogen bonding present were removed to obtain a better conformation upon docking.

Preparation and Structure Optimization of MMP-1 (Receptor)

The structure of the receptor, MMP-1, was obtained from the Protein Data Bank (PDB). Specifically, the crystal structure of the CAT domain of human MMP-1 (PDB ID:3SHI) with an accession number - P03956 and a database name - UniProt was used. The structure is classified as a hydrolase (interstitial collagenase) that represents a trimeric assembly, having 3 chains present in various orientations. BIOVIA Discovery Studio Visualizer was utilized to visualize and analyze the receptor to have a clearer visualization of the 3D structure. Heteroatoms, and hydrogen bonding present were removed, and energy minimization was done with UCSF Chimera using 1000 decent steps and default parameters. Parameters used in the study was necessary to obtain an optimized conformation of ligand-receptor complex with dependent to position, orientation, and binding affinity assessment.

Molecular Docking Study

The molecular docking study was based on the method of Yasmeen & Gupta (2019), which they assessed the interaction of selected terpenoids from *Dalbergia sissoo* with the catalytic domain of MMP-1 via AutoDockTools (ADT) v1.5.6 (Scripps Research Institute, USA). Their method was utilized for molecular docking with some modifications and different software utilization. In the study, molecular docking via blind docking was performed to assess the binding affinity of the ligands to the CAT domain of human MMP-1. The Lamarckian genetic algorithm (LGA), which stimulates arbitrary modifications in the structural properties of the ligands, was used to determine the most energy favorable ligand-protein binding conformations. All parameters were set to default except for the genetic algorithm (GA) runs. GA runs was set to 200 runs prior to determination of the binding stability of each ligand-receptor complex. Throughout the method, the protein was kept rigid, and the ligands were kept flexible. BIOVIA Discovery Studio was then used to visualize and analyze docked structures. There are no studies yet that assessed the inhibitory property of desholothurin A against MMP-1 via molecular docking (*in silico* studies).

Evaluation of the Inhibitory Property of the Triterpenoid Saponins against MMP-1

Evaluation of the inhibitory property of each triterpenoid saponin against MMP-1 was determined based on their binding energy (kD) and inhibitory constant (kI) via ADT v1.5.6. The standard of the kD to demonstrate its efficiency is about ≤-6 kcal/mol [18]. This is a significant property to consider when evaluating the quality of screening hits, because larger compounds have more binding energy due to the greater number of interactions they make, but they may not be the most efficient binders as having heavier atoms may lead to false-positive results [18, 19]. Meanwhile, the smaller the kI, the higher the binding affinity and the lesser dose is required to inhibit the activity of that enzyme [20]. Moreover, validating the kD and kI of each ligand-receptor complex was done by means of visualizing their strong and weak molecular interactions via BIOVIA Discovery Studio. Strong molecular interactions such as hydrogen bonding, and weak molecular interactions such as Van der Waals forces and carbon-carbon interactions were usually determined in the software.

Assessment of Pharmacokinetic Properties of each Triterpenoid Saponin

The conversion of the *mol* files of each triterpenoid saponin to their *Simplified Molecular Input Line Entry System* (SMILES) format was done by using the SwissADME software. The generated SMILES were then subjected to ADMETLab 2.0 to determine their pharmacokinetic profiles such as physicochemical properties, medicinal chemistry, absorption, distribution, metabolic profile, excretion, and toxicology profile. The interpretation of each result were described in terms of the empirical decision generated by the software, wherein color green, yellow and red results were reported as excellent, medium, and poor, respectively.

Results and Discussion

Relative Abundance of Triterpenoid Saponins Present from *Holothuria scabra*

In relation to the current study, such studies have found that some triterpenoid saponins such as desholothurin A, holothurinoside C, and pervicoside C may be isolated from methanol extracts from *H. scabra* [4, 21, 22]. These were further proved by [4] with the use of liquid chromatography-mass spectrometry-based metabolomic (LC-MS) analysis. With this, their results served as the foundation for selecting specific triterpenoid saponins to be investigated in the study, shown in Supplementary Table 1. The relative abundance eluate of each triterpenoid saponin were described according to their arbitrary values.

Dermatologic Activity of Each Triterpenoid Saponin from *Holothuria scabra*

The dermatologic activities of triterpenoid saponins present from *H. scabra* were determined using Way2Drug software. Knowing the dermatologic activity of triterpenoid saponins may give rise to changing conformation of MMP-1, whereas this can result in inhibiting the enzyme's action. In determining the dermatologic activity, two (2) parameters were used to predict the dermatologic activity, the probability to be active (Pa) and the probability to be inactive (Pi). Higher Pa indicates higher chances for the same activity to occur in an actual set-up. The higher the Pa is, the higher the chance to confirm the predicted activity by experiment. As shown in Table 1, desholothurin A and holothurinoside C are more likely to inhibit MMP-1 and exhibit dermatologic activity with a value of 0.408 and 0.481 respectively whereas pervicoside C has a Pa value of 0.297 which has the least chance to exhibit similar activities. Furthermore, in the case of their Pa values having < 0.5, but their Pa > Pi, then these compounds were said to have a 50% chance to exhibit this activity.

Table 1: Dermatologic Activity of Each Triterpenoid Saponin from *H. scabra*

Type of Triterpenoid Saponin	Pa	Pi
Desholothurin A	0.408	0.052
Holothurinoside C	0.481	0.036
Pervicoside C	0.297	0.089

Desholothurin A and holothurinoside C are more likely to show dermatologic activity than pervicoside C based on their Pa value. Therefore, it is probable that desholothurin A and holothurinoside C present in *H. scabra*, has more potential to be active against MMP-1, thereby inhibiting its activity and preventing skin aging. Further proving of the activity of the stated triterpenoid saponins regarding inhibition of collagen degradation, molecular docking against MMP-1 was performed by determining the molecular interactions and the binding stability of the ligand and enzyme.

Molecular Docking of Each Triterpenoid Saponin from *Holothuria scabra*

To further support the result obtained from the dermatologic activity manifested by the compounds, each metabolite was subjected to molecular docking as ligands and MMP-1 (3-SHI) as the receptor using Autodock v1.5.6. This was done to study how each triterpenoid saponin can interact and inhibit MMP-1. Figure 1 and 2 shows the molecular surface and 3-dimensional (3D) structure of each interaction, respectively. This illustrates that all the triterpenoid saponins, desholutorin A (see Figure 1-A; 2-A), holothurinoside C (see Figure 1-B; 2B), and pervicoside C (see Figure 1-C; 2C) successfully docked with the MMP-1. Therefore, all triterpenoid saponins exhibited binding stability with MMP-1.

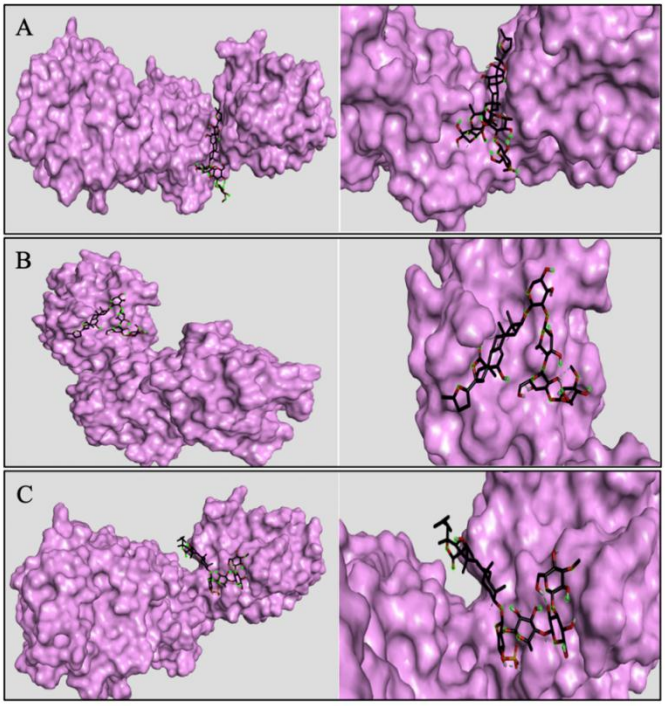

Fig 1: Image of Purple Molecular Surface of MMP-1 (3SHI; enzyme) and stick model of the ligands, A.) Desholothurin A, B.) Holothurinoside C, and C.) Pervicoside C with corresponding Enlarged Docked images.

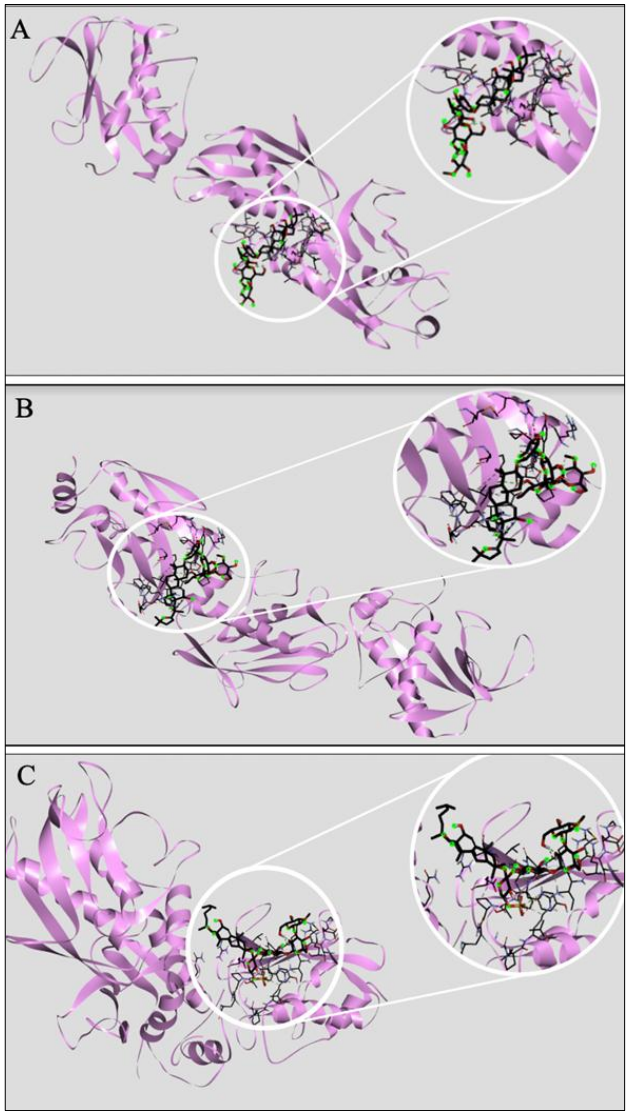

Fig 2: Docked 3D-Structure of Triterpenoid Saponins (encircled structures) against MMP-1 (purple structure; 3-SHI; enzyme) containing alpha helices, beta sheets and loops. (A) Desholothurin A; (B) Holothurinoside C; and (C) Pervicoside C.

Binding Stability of Each Triterpenoid Saponin

The binding stability of desholothurin A, holothurinoside C, and pervicoside C were described by means of their binding energy (kD), and inhibition constant (kI). Shown in Table 2, desholothurin A exhibited the lowest kD and kI (-6.03 kcal/mol and 37.79 uM), followed by holothurinoside C (-5.46 kcal/mol and 99.14 uM), and pervicoside C (-3.05 kcal/mol and 5480 uM).

Table 2: Binding Stability of Triterpenoid Saponins According to their Binding Energy (kD) and Inhibition Constant

Triterpenoid Saponins	kD	kI
Desholothurin A	-6.03 kcal/mol	37.79 uM
Holothurinoside C	-5.46 kcal/mol	99.14 uM
Pervicoside C	-3.05 kcal/mol	5480 uM

Based on the obtained kD and kI, desholothurin A exhibited the most stable binding with MMP-1 among the other two triterpenoid saponins, thus, a lower concentration of it is needed to inhibit collagen degradation. Meanwhile, holothurinoside C and pervicoside C may exhibit unusual binding with MMP-1, considering that a standard binding stability requires at least ≤ -6.0 kcal/mol. The binding energy of each triterpenoid saponins was further validated by determining its molecular interaction against MMP-1.

Molecular Interaction of Triterpenoid Saponins against MMP-1

Figure 3 demonstrates each specific molecular interaction with the amino acids of MMP-1. Desholothurin A (Figure 3 A-1), holothurinoside C (Figure 3 B-1), and pervicoside C (Figure 3 C-1) exhibited unbounded weak molecular interaction such as Van der Waals forces (unbounded light green), which are indicated as the pocket atoms in the 2D structure of docked figures. Desholothurin A displayed Van der Waals forces with amino acids Ala128, Ala234, Ala249, Asn120, Asp245, Leu125, Leu235, Leu248, Pro126, Thr121, Thr122, and Val246 of MMP-1. For holothurinoside C, Arg127, Arg165, Arg169, Pro123, Thr122, Ser162, His168, Asp167, and Gly166 are the amino acid positions that showed Van der Waals interaction. Lastly, pervicoside C exhibited Van der Waals forces to specific amino acids of MMP-1 such as Gly155, Arg117, Glu154, Ile118, Thr122, Glu199, and Arg165.

Aside from Van der Waals forces, bounded strong molecular interactions such as conventional and carbon hydrogen bonding, and bounded weak molecular interactions such as carbon-carbon interactions (pi-alkyl interactions) are also seen in each triterpenoid saponin. These molecular interactions are specifically described in both glycoside portion (seen in Figure 3A-2, 3B-2, 3C-2) and triterpene portion (seen in Figure 3A-3, 3B-3, 3C-3) of each triterpenoid saponin.

For the glycoside portion of each triterpenoid saponin, conventional hydrogen bonding was found in the interaction of desholothurin A with the amino acid residue Gly233, holothurinoside C with the amino acid residues Tyr121, Phe163, Glu199, and Glu119, and pervicoside C with the amino acid residues Glu119, Ser153, and Tyr121. Additionally, carbon hydrogen bonding was demonstrated in both holothurinoside C and pervicoside C shown with the amino acid residue Asn120. However, holothurinoside C displayed unfavorable donor-donor interaction with Arg117, while attractive charges with Arg127 in pervicoside C.

For the triterpene portion of each triterpenoid saponin, conventional hydrogen bonding was only found in desholothurin A, interacting with the amino acids Gln247 and Arg127. Meanwhile, pi-alkyl interactions were found in all triterpenoid saponins, wherein desholothurin A and pervicoside C interacted with the amino acid residue Pro123, and holothurinoside C interacted with Val164.

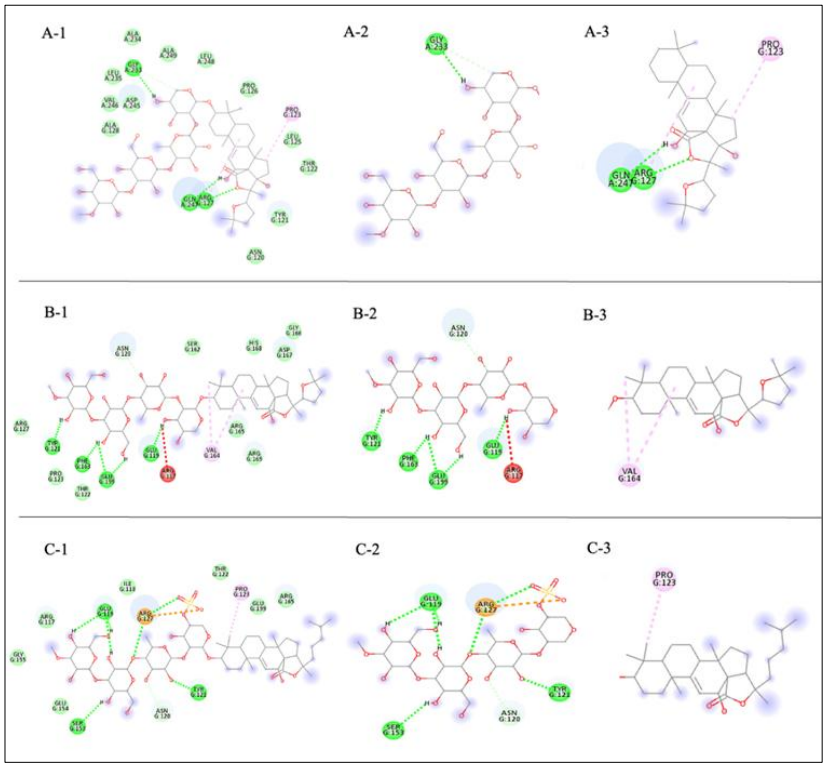

Fig 3: 2-Dimensional (2D) Interactions of Each Triterpenoid Saponins with the Amino Acids of MMP-1. (A-1) Desholothurin A, (A-2) Glycoside portion of Desholothurin A, (A-3) Triterpene portion of Desholothurin A; (B-1) Holothurinoside C, (B-2) Glycoside portion of Holothurinoside C, (B-3) Triterpene portion of Holothurinoside C; (C-1) Pervicoside C, (C-2) Glycoside portion of Pervicoside C, (C-3) Triterpene portion of Pervicoside C.

Legends: Dark Green – Conventional Hydrogen Bonding, Pink - Pi-alkyl Interactions, Red – Unfavorable Donor-Donor, Orange – Attractive Charges, Unbounded Light Green – Van der Waals Interaction, and Bounded Light Green – Carbon Hydrogen Bonding.

Generally, the pocket atoms shown in each triterpenoid saponin (see Figure 3A-1, 3B-1, and 3C-1) are Van der Waals interactions described as weak molecular interactions. These weak interactions are known to create high functionalities of materials and distinguish fine structures of the compound. Also, these are known to be the determinants for the formation of the protein-ligand complex. These are nonspecific interactions that can occur between any two molecules, independent of their chemical structure. They are short-lived but useful for binding high-molecular-weight organic materials to surfaces, such as protein [23]. Although weaker than ionic interaction, hydrophobic attraction, and hydrogen bonding, it plays a significant role in stabilizing the protein complexes. This refers to the group of interactions between individual molecules or atoms that took place due to the interaction of electron clouds surrounding two polar systems. Individually, Van der Waals interactions are weak interactions between molecules that are in close proximity to one another. The attraction between two atoms increases as they get closer until the Van der Waals contact distance separates them. When two molecules are too close to each other, the potential energy of repulsion increases dramatically, causing the assembly to become unstable and causing repulsion even when the molecules are neutral. Thus, the potential energy of repulsion reduces as the molecules move more apart [24].

Regarding the glycoside portion of each triterpenoid saponin (see Figure 3A-2, 3B-2, 3C-2), most of the interaction present in desholothurin A, holothurinoside C, and pervicoside C are conventional hydrogen bonds by which this bond promotes ligand-binding affinity. This is significant in terms of the stabilization of the ligand-protein complex. A functional group's ability to produce them is determined by the position of its hydrogen atoms [25].

In the glycoside portion of desholothurin A, it only exhibited conventional hydrogen bonding as it interacts with Gly233 (see Figure 3A-2). This amino acid is a nonpolar and nonchiral natural amino acid, which its specific side group is so small that hydrogen bonding between the peptide group and external molecules is not hampered. Hydrogen bonds have a positive correlation to protein-ligand stability which makes the desholothurin A to have greater binding stability than the two (2) saponins [26]. In addition, electronegativity also gives significance when it comes to the binding affinity of a molecule. Since the interacting atom present from the amino acid (O) have a high range of electronegativity, they tend to hold electrons tightly making the interaction more stable [27].

On the other hand, focusing on the glycoside portion of holothurinoside C, there is an unfavorable donor-donor present from the interaction of holothurinoside C that may decrease the stability of interaction with MMP-1 (see Figure 3B-2). The unfavorable donor-donor interaction is specifically displayed in the amino acid residue Arg117**.** It shows that the protein-ligand interaction reduces the stability of the enzyme-ligand complex. This signifies that there is a repulsion occurring between the two molecules [28]. In nature, arginine (Arg) residues were known to have positively charged side chains, making it to have an ability to transfer protons to other compounds. Arg117 unfavorably donates protons with its guanidinium group to the oxygen of holothurinoside C, which makes it an unusual interaction because the oxygen present is also bonded with an amino acid (Glu119). Thus, this made the binding of holothurinoside C to become unstable, as both ligand and enzyme tend to donate protons with each other.

For the glycoside portion of pervicoside C, there is a presence of an attractive charge interaction between Arg127's guanidinium group (specifically N) to the oxygen of pervicoside C (see Figure 3-C2). This may be described as salt bridge interaction, since between the presence of positively charged, protonated amine from Arg and the negatively charged, deprotonated carboxylate of pervicoside C, there is a salt bridge interaction occurring that destabilizes the enzyme-ligand complex. Arg127 donates protons with its guanidinium group to the oxygen of pervicoside C [29], which makes it an attractive interaction because the oxygen present attaches with the nitrogen of Arg. Furthermore, Arg127 also exhibits hydrogen bonding with another oxygen atom using its hydroxyl group, making it a stronger interaction but at the same time unstable as it employs attractive interaction with other molecules. These interactions made the pervicoside C to have the lowest binding stability among the other saponins mentioned.

With regards to the triterpene portions of the triterpenoid saponins mentioned, minimal interactions occur compared with the glycoside portions of these compounds (see Figure 3A-3, 3B-3, 3C-3). Desholothurin A exhibited weak and strong interactions such as pi-alkyl interactions and hydrogen binding. Holothurinoside C and pervicoside C only exhibited pi-alkyl interactions which is a weak interaction. For desholothurin A, the presence of glutamine (Gln247) and arginine (Arg127) promotes ligand-binding affinity due to the conventional hydrogen bonding (see Figure 3A-3). The arginine's amide group (Arg127) manifests a great electronegativity within the oxygen of desholothurin A, which shows a strong bond interaction. Meanwhile, the amino group of proline (Pro123) shows a Van der Waals interaction that manifests pi-alkyl interactions. For holothurinoside C, the amino group of valine (Val164) participates in pi-alkyl interaction with its methyl group (see Figure 3B-3). Lastly, in pervicoside C, proline (Pro123) binds weakly with its methyl group of the ligand by means of pi-alkyl interactions (see Figure 3C-3). From these, it can be inferred that desholothurin A is more stabilized than the two (2) triterpenoid saponins since it has stronger molecular interactions.

Physicochemical Properties of each Triterpenoid Saponin from *Holothuria scabra*

Shown in Table 3 is the analysis of the physicochemical properties of desholothurin A, holothurinoside C, and pervicoside C. Based on the results, each triterpenoid saponins has a large Van der Waals volume present, giving them a volume > 1000. This means that this compound is very large, being impenetrable to other molecules at ordinary temperatures. Their nHA was obtained to have values > 12, meaning its accepting of hydrogen bonds is not optimal. For the nHD, it obtained a value > 7, higher than its optimal value making them difficult to receive hydrogen bonded atoms. For nHet, they also obtained values higher than the optimal value that is > 15, as well as for TPSA that is > 140. For logS, holothurinoside did not qualified in the range of optimal value, wherein desholothurin A and pervicoside C considered to have optimal values. In logP, the three triterpenoid saponins were able to meet the acceptable range for both

properties. Lastly, results for logD7.4 showed that the three saponins obtained values < 1, lower than its optimal value.

Table 3: Analysis of the Physicochemical Properties of each Triterpenoid Saponin from *H. scabra*

Description	Desholothurin A	Holothurinoside C	Pervicoside C
	Results Interpretation	Results Interpretation	Results Interpretation
Volume	1062.668	1053.877	1098.523
nHA	24	23	25
nHD	12	11	11
nHet	24	23	26
TPSA	361.36	341.13	375.27
logS	-3.792	-4.018	-2.781
logP	1.29	1.633	1.154
logD7.4	0.778	0.93	0.815

Starting the pharmacokinetic parameters of the triterpernoid saponins, physicochemical properties such volume, nHD, nHet, and TPSA, the triterpenoid saponins showed values greater than their optimal values. This shows that having a greater number of atoms present, there's an increasing amount in volume and TPSA. In nHet, pervicoside C has the highest nHet results because only it has a heteroatom due to the presence of a compound known as sodium sulfate. High TPSA of the three triterpenoid saponins indicates that when entering and passing through the low polarity interior of cell membranes, a high polar surface area confers a high desolvation energy barrier which makes the molecules to be poorly soluble [30]. For logS and logP, the three triterpenoid saponins were all considered proper compounds as they were able to meet the acceptable range for both properties. For logD7.4, however, having values lower than its optimal value indicates an inability to cross the hydrophobic barrier.

Medicinal Chemistry of each Triterpenoid Saponin from *Holothuria scabra*

As seen in Table 4, desholothurin A, holothurinoside C, and pervicoside C has almost the same results for all the tests for its medicinal chemistry. Desholothurin A, holothurinoside C, and pervicoside C, has a SAscore of 7.557, 7.527, and 7.535, respectively. These shows a red empirical decision, wherein it exhibits poor results, as a score near than 1 must be obtained for easier production. The NPscore were almost the same value: 2.396, 2.399, and 2.275, respectively. The NPscore of the triterpenoid saponins is within the range of -5 to 5, which describes them as natural products. Same results were also observed when it comes to the Lipinski Rule, GSK Rule, and Golden triangle whereas, all the triterpenoid saponins were rejected and having a red empirical decision. Lastly, for the Pfizer Rule, all the saponins were accepted giving a green empirical decision.

Table 4: Analysis of Medicinal Chemistry of each Triterpenoid Saponin from *H. scabra*

Description	Desholothurin A		Holothurinoside C		Pervicoside C	
	Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision
SAscore	7.557		7.527		7.535	
NPscore	2.396		2.399		2.275	
Lipinski Rule	Rejected		Rejected		Rejected	
Pfizer Rule	Accepted		Accepted		Accepted	
GSK Rule	Rejected		Rejected		Rejected	
Golden Triangle	Rejected		Rejected		Rejected	

Legend: Green = accepted; Red = rejected

Based on the SAscore, desholothurin A, holothurinoside C, and pervicoside C were all difficult to synthesize. This is because the triterpenoid saponins has a high molecular weight, thus, this increases the viscosity. Also, they have a longer chain, which is more difficult for them to flow because of the entanglement in their structure [31]. Meanwhile, all exhibited an excellent NP score which makes them to be natural product. In definition, natural products are chemical compounds that are produced or came from a living organism found in nature. This practically makes sense, wherein these saponins can be found from sea cucumbers specifically from *H. scabra* [4]. For the Lipinski Rule, all obtained poor results that make the compounds to be poorly absorbed and less permeable to biological membranes. Lipinski rule results are also consistent in terms of the GSK Rule and Golden Triangle Rule. Based on the results, the content of the triterpenoid saponins did not satisfy the rules wherein all of the triterpenoid saponins were rejected having a red empirical decision meaning that they do not have a favorable ADMET profile. Lastly, Pfizer rule described that all the compounds are toxic.

Absorption, Distribution, and Excretion of each Triterpenoid Saponin from *Holothuria scabra*

Shown in Table 5 is the analysis of the absorption of each triterpenoid saponin in a body system. In permeability screening, all obtained an empirical decision of red in Caco-2 permeability. An excellent result of the Caco-2 permeability must have a predicted value of >5.15log cm/s. Therefore, the results indicate that the triterpenoid saponins does not have a proper Caco-2 permeability, since all have a predicted value of <5.15log cm/s. HIA is associated with Caco- permeability since it is a property that is evaluated to also assess if the saponins can be absorbed from the gastrointestinal system into the bloodstream of the body. For HIA, all triterpenoid saponin also obtained an empirical decision of red. This implicates that each of the triterpenoid saponin are poorly absorbed. For the oral bioavailability, all obtained an empirical decision of red in $F_{30\%}$. Triterpenoid saponin's oral bioavailability at 30% does not have the capability to reach systemic circulation.

Presented also in Table 5 is the analysis of the distribution of each triterpenoid saponin in a body system. In PPB, desholothurin A, holothurinoside C, obtained an empirical decision of a color green, wherein it describes an excellent result for all triterpenoid saponins. This specifically means that they have a proper PPB (≤ 90%), being high protein-bound with a low therapeutic index. For VD, each triterpenoid saponin also obtained an empirical decision of a color green, having an excellent VD across the body fluid and tissues (a value within the range of 0.04-20 = optimal). Lastly, for BBB penetration, all triterpenoid saponin obtained an empirical decision of color green (- = excellent results; + = poor

results), which they have the ability to cross the blood-brain barrier, which may give CNS side effects.

In Table 5, the analysis of the excretion of each triterpenoid saponin were also shown. The clearance penetration of all triterpenoid saponin resulted to an empirical decision of a color red, which means it has a poor clearance penetration (≥ 5 = excellent; < 5 = poor). Meanwhile, $T_{1/2}$ of desholothurin A and holothurinoside C have an empirical decision of yellow. This means that both triterpenoid saponin have a slightly reliable estimate of clearance and volume of distribution. Lastly, pervicoside C obtained an empirical decision of a color green, which means it has a relatively reliable estimate of clearance penetration and volume of distribution. Analyzing of $T_{1/2}$ follows these specific ranges: 0-0.3 = excellent (green); 0.3-0.7 = medium (yellow); 0.7-1.0 = poor (red).

Table 5: Analysis of Absorption, Distribution, and Excretion of each Triterpenoid Saponin from *H. scabra*

	Description	Desholothurin A		Holothurinoside C		Pervicoside C	
		Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision
Absorption	Caco-2 Permeability	-6.29		-6.054		-6.084	
	HIA	+++		+++		+++	
	F30%	+++		+++		+++	
Distribution	Plasma Protein Binding (PPB)	41.003%		43.000%		68.310%	
	Volume Distribution (VD)	0.044		0.094		0.218	
	Blood-brain Barrier (BBB) Penetration	--		--		--	
Excretion	Clearance (CL)	0.446		0.250		0.522	
	Half-life ($T_{1/2}$)	0.465		0.551		0.284	

Legend: Green = excellent; Yellow = medium; Red = poor

The data showed that all triterpenoid saponins have a low value of Caco-2 permeability; hence, the compounds are predicted to not have a proper permeability. Moreover, Caco-2 permeability is associated with HIA, wherein it is evaluated to identify if the triterpenoid saponins have a high HIA. In this case, a poor Caco-2 permeability correlates with low HIA, suggesting that all triterpenoid saponins does not have a proper intestinal absorption. Furthermore, human oral bioavailability is one of the most important pharmacokinetic parameters. The results showed that at 30%, each of the triterpenoid saponin is unable to reach the therapeutic site of action due to the poor absorption of each compound. With this, it can be deduced that desholothurin A, holothurinoside C, and pervicoside C have a poor absorption property due to low Caco-2 permeability, HIA, and $F_{30\%}$.

Furthermore, the PPB of triterpenoid saponins showed excellent results, wherein they can participate along with plasma proteins which enables them to control the free drug concentration in plasma and in equilibrium with plasma, which efficiently displays drug potency *in vivo* [32]. For VD, it gave excellent and high results, wherein it has a proper distribution along the body, specifically in other tissue compartments. The only adverse effect of having a proper VD is that they have the ability to leave the plasma, which may require a higher dose of the drug to maintain a given plasma concentration [33]. However, precautionary measures are required wherein time and dose must be well-calculated in order to have a safe effect in a body system. Lastly, for BBB penetration, triterpenoid saponins have the ability to cross the BBB, which results in CNS side effects. A controlled dose is required to avoid severe side effects influenced by these compounds [34].

For the excretion of these triterpenoid saponins as drug compounds, their clearance and half-life were observed. All triterpenoid saponins employed a poor clearance in a body system. Each drug was suggested to be removed rapidly from the body to avoid adverse effects. However, the compounds were said to be slowly removed from the body. These are problematic with persons having the renal or hepatic disease [35]. Proper control of dosage must be done for an appropriate excretion of these compounds outside the body system. $T_{1/2}$, in contrast, has better results than the clearance of a drug. Desholothurin A and holothurinoside C obtained a medium result wherein it can take more time to be eliminated in the body by exactly 50%. In comparison with pervicoside C, it obtained an excellent result which takes less time to be eliminated in the body. Generally, pervicoside C has fewer withdrawal problems than desholothurin A and holothurinoside C.

Metabolism of each Triterpenoid Saponin from *Holothuria scabra*

The metabolism of each triterpenoid saponin obtained from *H. scabra* was done by assessing whether these compounds have the ability to act as CYP gene inhibitor and as a CYP gene substrate. In assessment of metabolism results, CYP gene inhibitor and CYP gene substrates are divided among the following; 1A2, 2C19, 2C9, 2D6, and 3A4. Metabolism results showed that all triterpenoid saponin has the ability to become a substrate of CYP and an CYP inhibitor.

Table 6: Analysis of Metabolism of each Triterpenoid Saponin from *H. scabra*

Description	Desholothurin A	Holothurinoside C	Pervicoside C
	Results Interpretation	Results Interpretation	Results Interpretation
CYP1A2 inhibitor	YES	YES	YES
CYP1A2 substrate	YES	YES	YES
CYP2C19 inhibitor	YES	YES	YES
CYP2C19 substrate	YES	YES	YES
CYP2C9 inhibitor	YES	YES	YES
CYP2C9 substrate	YES	YES	YES
CYP2D6 inhibitor	YES	YES	YES
CYP2D6 substrate	YES	YES	YES
CYP3A4 inhibitor	YES	YES	YES
CYP3A4 substrate	YES	YES	YES

Legend: YES = probable to be an inhibitor or substrate; NO = not probable to be an inhibitor or substrate

The results depend primarily on how they interact with CYP gene families. Triterpenoid saponins showed that they have the ability to be a substrate and an inhibitor interacting with CYP enzymes. Being a substrate of CYP, they can be easily metabolized in the liver wherein they are converted to active metabolites. Meanwhile, being inhibitors of CYP, they can slow down drug metabolism, which increases drug effects in specific peripheral targets [36].

Toxicology of each Triterpenoid Saponin from *Holothuria scabra*

The toxicology profile of desholothurin A gave an empirical decision of color green (see Table 7), wherein this may possibly cause adverse effects related to cardiac and resting potential. Meanwhile, holothurinoside C, and pervicoside C illustrated to have a yellow empirical decision in terms of their hERG (human ether-a-go-go) blockers, as they can slightly have adverse effects on cardiac and resting potential (see Table 7). On the other hand, their H-HT also illustrated a green empirical decision, which may cause adverse hepatic effects (see Table 7). Lastly, all triterpenoid saponins gave a green empirical decision for skin sensitization (see Table 7), wherein it may cause adverse effects, dermatologically.

Table 7: Analysis of Toxicology of each Triterpenoid Saponin from *H. scabra*

	Description	Desholothurin A		Holothurinoside C		Pervicoside C	
		Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision	Results Interpretation	Empirical Decision
Toxicology	hERG Blockers	--		--		--	
	Human Hepatotoxicity (H-HT)	--		--		--	
	Skin Sensitization	--		--		--	

Legend: Results are described as: --- to -- = green, - to + = yellow, and ++ to +++ = red.

A voltage-gated potassium channel encoded by hERG plays a key function in the control of the exchange of cardiac action potential and resting potential during cardiac depolarization and repolarization. Long QT syndrome (LQTS), arrhythmia, and Torsade de Pointes (TdP) are all possible side effects of hERG blocking, and can result in palpitations, fainting, or even death. Based on the results shown in ADMETLab screening, all triterpenoid saponins gave a green empirical decision resulting to a blockage of the hERG gene. This means that the saponins may cause unnecessary side effects when ingested. Furthermore, another toxicology result indicated that all triterpenoid saponins present in the study may have adverse hepatic effects according to their H-HT results. This result signifies that there is a possibility that once desholothurin A, holothurinoside C, and pervicoside C are ingested as a form of medication, it may cause liver damage.

Conclusion

Triterpenoid saponins from *H. scabra* such as desholothurin A, holothurinoside C, and pervicoside C showed a good dermatologic activity, which may contribute to anti-wrinkling properties of becoming a drug. This is further validated by molecular docking, wherein all triterpenoid saponins had the ability to bind with MMP-1 via strong and weak molecular interactions. However, only desholothurin A showed no unusual interactions, while holothurinoside C and pervicoside C exhibited unusual interaction which affect its binding stability with MMP-1. Hence, it can be concluded that desholothurin A has the best inhibitory activity from the three (3) triterpenoid saponins used.

After molecular docking, the triterpenoid saponins' pharmacokinetic parameters and druglikeness properties was assessed to determine its ADMET profile, whether it can be used further to clinical studies and can be used as a candidate drug for further research. It had showed that these compounds are poorly absorbed, poorly excreted, and may exhibit toxicity to a body system. However, the compounds had showed excellent distribution and metabolism. To summarize, all triterpenoid saponins displayed a poor absorption, excretion, and toxicology profile, and have a good distribution and metabolic profile. Generally, it may be concluded that all triterpenoid saponins have an unfavorable ADMET profile.

This *in silico* findings supports that all triterpenoid saponins have the ability to inhibit MMP-1, although holothurinoside C and pervicoside C have unusual interactions. Desholothurin A may be recommended to topical formulations to prevent skin wrinkling caused by MMP-1. However, having the ability to inhibit MMP-1, these compounds somehow exhibit an unfavorable ADMET profile. Hence, molecular engineering is recommended for all triterpenoid saponins to further study and manipulate its composition, which may enhance its ADMET profile. Furthermore, molecular engineering may be

also used to control the interactions of these compounds against MMP-1 by transforming its molecular properties. Also, further studies are required by assessing their activity against MMP-1 *in vitro*.

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