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Structure-based virtual screening to predict *Loxosceles* spider venom natural inhibitors

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ABSTRACT

Objective *Loxosceles* spider bite accidents are rising in wide areas of the world which necessitates the exploration of natural inhibitors to inhibit the most significant enzymes, namely sphingomyelinase D (Smase D) and hyaluronidase.

Methods Virtual screening using traditional Chinese medicine (TCM) against Smase D (PDB ID: 2F9R) and hyaluronidase was performed by the DrugRep server. Absorption, distribution, metabolism, and excretion (ADME) parameters were predicted via the same server. In addition, molecular dynamics (MD) simulation was conducted using CABS-flex 2.0 tool to prioritize the best potential natural inhibitors.

Results Tiliroside and Digitoxin were the best natural inhibitors from TCM to Smase D and hyaluronidase in terms of molecular docking and ADME parameters, while Digitoxin and *β*-carotene were the most potent inhibitors against hyaluronidase. MD simulations demonstrated the stability of the docked complexes.

Conclusion *In-silico* inhibition of *Loxosceles* spidervenom enzymes through TCM was demonstrated, which deserves wet-lab experimentation.

1 Introduction

Several animals have independently evolved an apparatus capable of secreting toxic products. The products are either proteins, peptides, enzymes (majority), or small molecules. These toxins are secreted to deter potential predators, but they can also be employed to fight off invaders [1]. A venom is composed of dozens of toxic products, the majority of which act as neurotoxins via targeting specific receptors or ion channels in the central nervous system (CNS). Moreover, some enzymes breakdown the main components of CNS such as myelin sheath [2]. Among the secreting animals, spiders represent a substantial threat to tropical regions involving South, Central, and North Americas, Central Africa, Middle-East, and South-East Asia. Fatal accidents were recorded in those areas owing to the prevalence of spiders from the *Loxosceles* genus (Family Sicariidae) [3, 4]. Although the protein content in the venom barely exceeds 300 μg/bite, serious skin lesions, kidney necrosis, and systematic complications were recorded [5]. Sphingomyelinase D (Smase D) is an essential component of the *Loxosceles* spider and it may attack the myelin sheath, leading to serious neurological symptoms, so blocking of Smase D would relieve the toxic effects of the venom [6]. Indeed, the severity of venom consequencesis correlated with the activity of Smase D [7]; therefore, its inhibition would detoxify *Loxosceles* spider venom as demonstrated

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by the action of benzene sulphonate compounds [8].

Loxosceles spider venom also contains another critical enzyme for toxicity, namely hyaluronidase. This enzyme acts as a spreading factor that degrades hyaluronic acid and chondroitin sulfate, and thus facilitates extracellular matrix damage. This enhances the dermatonecrotic action of the venom [9]. Antiserum as well as inhibitors of spider hyaluronidase are potential agents to attenuate both local and systemic effects of Loxoscelism, as was already demonstrated. Hyaluronidase blockage at least would prolong the time between bite accident and the medical intervention in addition to acting synergistically with antivenom serum [10].

Traditional Chinese medicine (TCM) is a healthcare and therapeutics collection based on experimental and scientific assay and regulation. Its popularity can be traced back more than 4 000 years. TCM involves small molecules (< 1 kDa), polysaccharides, proteins, polysaccharide-protein complexes, and miRNA [11]. TCM demonstrated many therapeutic effects against various illnesses [12]. TCM components are also considered as antidotes against venoms of insects and different snake species [13, 14]. Thus, this study aimed to virtually screen the TCM library for Smase D inhibitors of *Loxosceles* spider as a potential option to manage spider bites. The findings of the present study will suggest whether or not the theoretical usefulness of TCM is deemed effective in managing spider venoms.

2 Methods

2.1 Molecular docking prediction, and absorption, distribution, metabolism, and excretion (ADME) prediction

The crystal structure of Smase D was downloaded from the protein data bank (PDB ID: 2F9R). Hetero-molecules as well as water molecules were eliminated and polar hydrogens were added. The prepared receptor was uploaded to the DrugRep webserver [15] to conduct receptor-based virtual screening. Pocket 1 was selected to be docked to (center coordinates: 14.9, 42.8, − 8.2, while size values: 21, 18, 16). The ligand library selected for virtual screening was TCM. TCM library contains 2 390 monomer compounds from nearly 800 TCMs, including various structural types such as flavonoids, alkaloids, terpenoids, and glycosides. Selection of TCM as a potential therapeutic option was based on the explored antidote against several types of snakes [14]. The ADME parameters of the best ligands were also calculated via the DrugRep webserver. Smase D-best ligands interaction was analyzed through the LigPlot + program v.2.2 [16]. In addition to Smase D, the hyaluronidase three dimensional (3D) structure was retrieved from the Alphafold database (https://alphafold.ebi.ac.uk/) (ID: R4J7Z9), and the same protocol was followed. The active site was predicted through the FTSite tool 2012 [17].

2.2 Molecular dynamics (MD) simulations

MD simulations of the best two complexes and the native enzymes (Smase D and hyaluronidase) were conducted via CABS-Flex 2.0 server which depends on coarse-grained motions of the protein [18]. Over 50 cycles and 50 trajectory frames within 10 ns each with some additional distance restraints including a global weight of 1.0 were applied. Root-mean square fluctuations (RMSF) were used to express the complexes' motion.

3 Results

3.1 Molecular docking output

Molecular docking scores of the tested natural ligands from TCM demonstrated strong binding affinity of many natural ligands toward Smase D of *Loxosceles* spider (Table 1). Tiliroside was the best potential natural ligand capable of inhibiting the enzyme at its active site (docking score − 9.6 kcal/mol). This is followed by Picroside I whose binding score was – 9.5 kcal/mol. Concerning ADME parameters, Picroside I is better than Tiliroside as the latter has 1 violation of Lipinski's rule of five (molecular weight > 500 Da). ADME parameters of the rest of the ligands showed good pharmacokinetic profiles, indicating the richness of TCM in terms of its wide therapeutic efficacy.

Table 1 Molecular docking score and ADME parameters of best potential ligands to Smase D

Ligand	Score	MW	HBD	HBA	RB	Ring	LogP
Tiliroside	– 9.6	594.52	7	9	15	5	1.8
Picroside I	– 9.5	492.47	5	6	13	5	– 1.1
Chicoric acid	– 9.2	474.37	6	10	17	2	2.0
Hecogenin	– 9.1	430.62	1	2	1	6	4.8
Picroside III	– 9.0	538.50	6	7	15	5	– 1.4
Mudanpioside C	– 9.0	600.57	5	7	15	7	0.3
Narirutin	– 8.9	580.53	8	9	14	5	– 1.0
Beta-Carotene	– 8.8	536.87	0	0	10	2	13.6
N-(p-Coumaroyl) serotonin	– 8.8	322.40	3	3	8	3	3.0
Yamogenin	– 8.8	414.62	1	1	1	6	5.6

MW: molecular weight. HBD: hydrogen bond donor. HBA: hydrogen bond acceptor. RB: rotatable bonds.

For hyaluronidase, FTSite prediction unveiled the involvement of Phe 252, Gly 303, Tyr 199, Leu 192, Glu 120, Asp 118, Thr 61, Tyr 64, Trp 307, Asn 178, Tyr 273, Leu 16, and Ile 234 in the binding site (Figure 1). Given that no predicted pocket was found to involve those amino acids, custom grid box was selected. Center values of *X*, *Y*, and *Z*

were 0.889 789, 6.465 22, and 1.825 65, respectively; whereas the size dimensions were 33, 24, and 29, respectively.

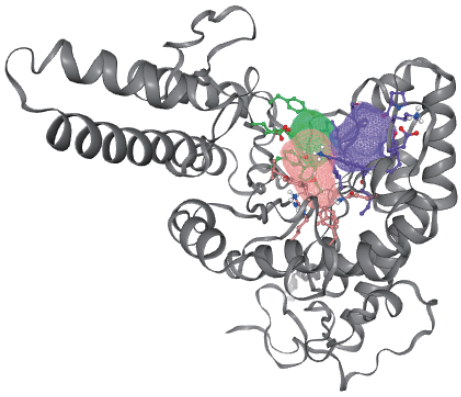

Figure 1 Predicted active site of hyaluronidase
The catalytic residues of the active site are colored in magenta, pink, and green along with the corresponding space filled within the active site.

The binding affinity of the top 10 ligands through molecular docking of the TCM library against hyaluronidase ranked ranged from – 9.8 to – 7.9 kcal/mol (Table 2). It is worth noting that the majority of them belong to the terpenes family of compounds with few exceptions like digitoxin, which is a well-known cardiac glycoside. Digitoxin was the best potential natural inhibitor to hyaluronidase with a binding affinity of – 9.8 kcal/mol. Besides, some violations (at least in the molecular weight) of the ligand candidates to Lipinski's rule of five were observed. However, this should not undervalue their pharmacokinetics or druglikeness since these ligands are already present in the market as drugs of dietary supplements.

Table 2 Molecular docking scores of TCM library against spider hyaluronidase

Name	Score	MW	HBD	HBA	RB	Ring	LogP
Digitoxin	– 9.8	764.94	5	6	12	8	2.8
Beta-Carotene	– 9.6	536.87	0	0	10	2	13.6
Periplocin	– 8.8	696.83	6	7	13	7	0.7
Zeaxanthin	– 8.7	568.88	2	2	12	2	10.9
Khasianine	– 8.7	721.93	7	6	11	8	2.1
Itraconazole	– 8.6	705.63	0	4	11	7	7.2
Lutein	– 8.6	568.88	2	2	12	2	11.0
1,5-Dicaffeoylquinic acid	– 8.2	516.45	7	10	16	3	1.5
Lycopene	– 7.9	536.89	0	0	16	0	15.5

MW: molecular weight. HBD: hydrogen bond donor. HBA: hydrogen bond acceptor. RB: rotatable bonds.

The LigPlot+ program was adopted to figure out the detailed interactions between the best two ligands with Smase D. On one hand, the Tiliroside-Smase D complex unveiled the formation of six H-bonds through His 12, Asp 52, Lys 93, Ser 132, Ser 166, and Pro 174. On the other hand, Asp 34, His 47, Asp 91, Lys 93, Ser 166, and Tyr 169 contributed to the strong binding through the formation of H-bonds to Picroside I. Additionally, several residues constituted hydrophobic interactions between the two complexes (Figure 2).

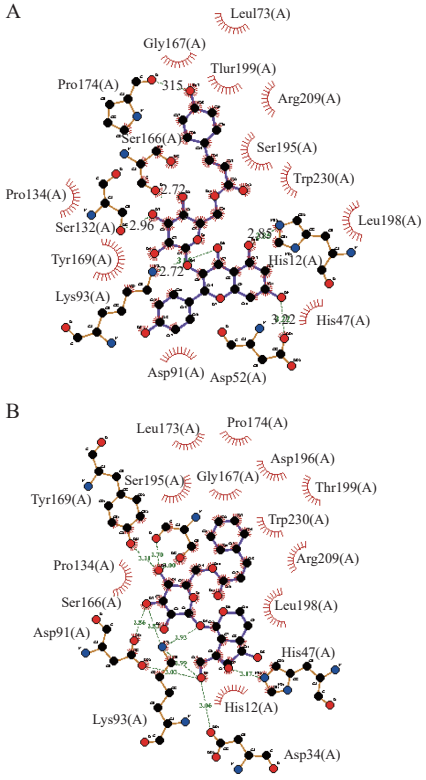

Figure 2 Protein-ligand interaction of the best two complexes as predicted by LigPlot +
A, Tiliroside-Smase D complex. B, Picroside I-Smase D complex.

Unsurprisingly, digitoxin is a stronger inhibitor than β-carotene as reflected by the H-bonds as well as hydrophobic interactions between digitoxin and hyaluronidase whist β-carotene which forms only hydrophobic attractions as illustrated in Figure 3. This can be traced to the absence of polar groups capable of donating/accepting H-bonds with the active site residues of the enzyme. Digitoxin formed seven H-bonds with spider hyaluronidase which enables it to act as a superior inhibitor to that enzyme.

3.2 MD simulations

Limited molecular motion of Smase D in response to binding Tiliroside and Picroside I was observed. This implies that the docked complexes showed high stability and low fluctuations as revealed by the RMSF output in comparison with the native enzyme. Figure 4 depicts the RMSF of the best two complexes as well as the native enzyme. This emphasizes the potential druggability of

Tiliroside and Picroside I in the active site of Smase D of the *Loxosceles* spider.

MD simulations of hyaluronidase alone and together with the best two ligands (Digitoxin and β-Carotene) were also performed. It turned out that the Digitoxin-enzyme complex exhibited a relatively high RMSF pattern compared to the native enzyme, particularly residues 71-81 (Figure 5). This might be interpreted by the high stability of the complex as there were no marked fluctuations observed in protein motion between the docked and native protein. On the flip side, β-Carotene had even less motion compared with native hyaluronidase, emphasizing the greater stability of the docked complexes. The greater stability exhibited by the two ligands confirms their suitability as natural inhibitors to the enzyme. The high fluctuations observed in the first residues are traced to the long loop presented in the N-terminal region of the enzyme.

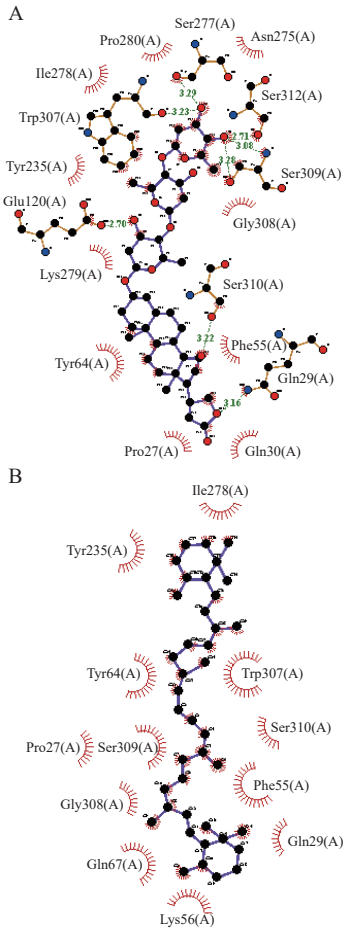

Figure 3 Hyaluronidase-Digitoxin (A) and β-Carotene (B) interactions as predicted by LigPlot +

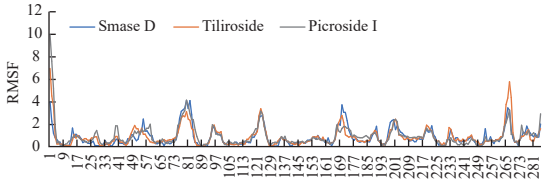

Figure 4 RMSF of the best two ligands as well as native Smase D

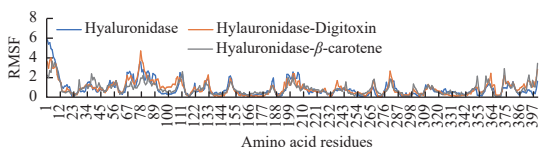

Figure 5 RMSF of the best two ligands as well as native hyaluronidase enzyme

4 Discussion

Owing to the serious complications of *Loxosceles* spider venom, medical management as well as interventions have been proposed and adopted. This includes the use of colchicine to inhibit Smase D in addition to the coupling of hemodialysis to filter the venom out of the blood. However, these interventions remain unclear about their efficacy [19]. Therefore, the exploitation of TCM as a natural source to manage *Loxosceles* spider venom serves as an important alternative. The results of the present study unveiled the potential inhibitory action of Tiliroside and Picroside I toward Smase D. The protein-ligand interaction confirmed that the binding of those natural ligands is typically in the active pocket of the enzyme. Moreover, ADME parameters were found to fall within Lipinski's rule of five, thus indicating good pharmacokinetic and druglikeness profiles. Also, the MD simulations emphasized the stability of the docked complexes compared to unbound Smase D as evidenced by the restricted RMSF findings [20]. A high amount of tiliroside can be found in the herb of *Potentilla grandiflora* (197.7 mg/g) and flowers of *Potentilla nepalensis* (187.4 mg/g) [21]. Meanwhile, *Picrorhiza kurroa* represents a significant medicinal plant due to its rich source of active constituents, Picroside I and Picroside II [22]. In conclusion, the application of Tiliroside and Picroside I as inhibitors to Smase D proved superior potential enzyme inhibitions. As a natural cardiac glycoside, Digitoxin can be used as a drug for cardiac disorders, including congestive heart failure, atrial fibrillation or flutter, and certain cardiac arrhythmias. With a very narrow therapeutic window, Digoxin is a two-edged sword since it is powerful in blocking *Ca* channels in the myocardium and, at the same time, has a cardiotoxic influence [23]. Then again, carotene is the principal carotenoid found in the highest amounts in carrots, responsible for carrot orange-yellow pigment. It is a very powerful antioxidant that demonstrated anti-cancer and other bioactivities. Given its liposolubility, it needs to be formulated in a special form to improve its absorption [24]. Hylaronidase in the venom of spiders was investigated to pave the way for Smase D action through degrading connective tissue matrix at the site of the bite [9]. Therefore,

blocking the two enzymes would be theoretically an antidote to spider venom. Nevertheless, it is advisable to validate the results of the current virtual screening using *in vitro* assays either in the form of pure drugs or herbal sources.

5 Conclusion

According to the results of the present study, TCM is a rich source having multiple activities against a wide variety of infestations, including *Loxosceles* spider Smase D. Inhibition of this enzyme would cancel much of the toxicity of spider venom. Tiliroside and Picroside I were the best natural inhibitors to Smase D whereas Digitoxin and *β*-Carotene are superior inhibitors to hyaluronidase in terms of molecular docking, ADME and MD simulations; hence, further laboratory validation is warranted. The present study coined the potential application of TCM in treating spider venom accidents, which would benefit the affected regions with those risky spiders.

Competing interests

The authors declare no conflict of interest.

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基于结构的虚拟筛选预测斜蛛属蜘蛛毒液天然抑制剂

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【摘要】目的 世界范围内斜蛛属蜘蛛咬伤事故不断增多，急需探索天然抑制剂来抑制最显著的鞘磷脂酶 D 和透明质酸酶。**方法** 通过 DrugRep 服务器使用中药抗鞘磷脂酶 D（PDB ID: 2F9R）和透明质酸酶进行虚拟筛选。通过同一服务器预测吸收、分布、代谢和排泄（ADME）参数。此外，使用 CABS-flex 2.0 工具进行分子动力学模拟，确定潜在最佳天然抑制剂的优先次序。**结果** 从分子对接和 ADME 参数来看，中药抑制抗鞘磷脂酶 D 和透明质酸酶的天然抑制剂中，Tiliroside 和 Digitoxin 表现最佳。而洋地黄毒苷和 β-胡萝卜素是对透明质酸酶最有效的抑制剂。分子动力学模拟证明了对接复合物的稳定性。**结论** 通过中药对斜蛛属蜘蛛毒液酶的计算机模拟抑制得到证明，值得开展湿实验分析。

【关键词】斜蛛属蜘蛛；鞘磷脂酶 D；中医药；分子对接；分子动力学模拟；吸收、分布、代谢与排泄（ADME）