JSMARTech
Journal of Smart Bioprospecting and Technology **p-ISSN**: 2686-0805 **e-ISSN**: 2714-7894
October 2021, VOL 02 NO 03 **DOI**:https://doi.org/10.21776/ub.jsmartech.2021.002.03.96

Virtual Screening of *Mimosa pudica* Secondary Metabolites as Hyaluronidase B Potential Inhibitor to Prevent *Vespa velutina* Venom Spreading

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Submission: 01 June 2021; **Revised**: 07 July 2021; **Accepted**: 14 July 2021

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ABSTRACT. *Vespa velutina, also known as the Yellow-legged hornet, is a wasp species native to Asia with a large distribution area in Indonesia. Hyaluronidase B in a wasp venom acts as a "spreading factor", which is the key at the beginning of envenomation. Shameplant (Mimosa pudica), a common plant in Indonesia, has shown the potential to be a hyaluronidase B inhibitor. This study aimed to analyze the potential of secondary metabolites in Shameplant as an inhibitor of V. velutina Hyaluronidase B base on their molecular interactions and as a topical drug base on physicochemical characteristics. In silico computational studies is performed to predict the binding modes of M. pudica compounds and hyaluronidase B enzyme. The secondary metabolites were retrieved from the PubChem database and screened using SwissADME. The seven metabolite compounds were docked with Hyaluronidase B and hyaluronan by HEX Cuda 8.0.0 program. Hyaluronidase B was also docked with its native ligand (hyaluronan) to validate the docking study. Three dimensional and 2D views were then evaluated using Discovery Studio 2016. Results of this study are all compounds do not have the same molecular interaction with the control. It defines no inhibition of the interaction on the active side. Mimopudine is the most potent inhibitor of hyaluronidase B based on its binding energy. While, jasmonic acid is the only compound that meets the physicochemical parameter of the topical drug.*

Keywords: *Virtual screening, Hyaluronidase B, Mimosa pudica, Vespa velutina,*

INTRODUCTION

Case of hornet-stings-related deaths in Indonesia became a hot topic in early 2021. This “killer” hornet has been identified in the Vespa genus that is spread worldwide. One of the species is *Vespa velutina*, or commonly known as the Yellow-legged hornet, is a hornet species native to Asia with a distribution area in Indonesia and is known as an aggressive species [1]. *V. velutina* often builds nests in residential areas, where humans can attack. The attack occurred with a pheromone which triggered the colony to attack. Hornet stings are events that involve a complex component called venom [2]

Hyaluronidase B, commonly known as Ves v2, is an enzymatic component found in *V. velutina* venom. The structure of hyaluronidase has general similarities in various organisms, where these structures influence enzymatic activity. This enzyme acts as a "spreading factor" which is the key at the beginning of envenomation. Hyaluronidase in a hornet venom is classified as an allergen with a hyaluronidase catalytic mechanism based on the glycosaminoglycan degradation pathway [3-4]. Cleavage of β-1,4-glycosidic bonds occurs in the degradation process of hyaluronic acid [4]. The impact that occurs is a decrease in tissue viscosity, an increase in membrane permeability, and the exposed tissue becomes very permeable to venom components [5].

Shameplant (*Mimosa pudica*) originates from the Americas, but this plant is widespread in Indonesia. *Mimosa pudica* is classified as an invasive plant because its existence threatens the endemic plant [6]. A previous study [7] showed that the secondary metabolite compound Putri Malu could be a hyaluronidase inhibitor. However, this study still does not explain the molecular interactions that occur. Therefore, this research needs to be conducted to analyze the potential of secondary metabolites in Shameplant as an inhibitor of *V. velutina* Hyaluronidase B base on their molecular interactions and as a topical drug base on physicochemical characteristics, which is expected to be the first step in designing an anti-allergen. In addition, the use of Shameplant can also help to overcome ecological problems.

RESEARCH METHODS

Materials

Hyaluronan as native ligand (CID: 24759) and seven active compounds from *M. pudica* which used as ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) base on KNApSAcK database (https://www.knapsackfamily.com/) (Table 1). Crystallized structures of *V. velutina* Hyaluronidase B (UniProt entry: C0HLL5) were taken from UniProt (https://www.uniprot.org/). Druglikeness was defined based on SwissADME physicochemical properties.

Table 1. List of *M. pudica* active compounds

Compounds	CID
Jasmonic acid (**JA**)	5281166
L-Mimosine (**LM**)	440473
Noradrenaline (**NA**)	439260
Turgorin (**TG**)	442991
2"-O-alpha-L-Rhamnosyl-6-C-fucosyl-luteolin (**LT**)	44257957
Cassiaoccidentalin B (**CB**)	70698280
Mimopudine (**MP**)	100927206

Docking Simulations

Protein (Hyaluronidase B) were prepared using Discovery Studio 2016 to remove water molecule and previous attached ligand. To minimise its energy, ligands (Native ligand and active compounds) were prepared using PyRx 8.0 [8]. Molecular docking has been run using Hex 8.0.0 CUDA software [9] with Shape+Electro+DARS for correlation type, while other parameters remain default. Preparation and Docking results saved in .Pdb format to be visualized and analyzed using Discovery Studio 2016 [10].

RESULTS AND DISCUSSIONS

Topical Druglikeness

Physicochemical properties of a compound have a close correlation with topical drug-likeness. One of the seven secondary metabolites from *M. pudica* has good potential as a candidate for topical drug constituents (Table 2). Jasmonic acid meets the criteria for parameters MW <400, LogP between -1.0 and 4, safe skin reaction, and TPSA <140 Å² [11-12]. Furthermore, this compound has the highest skin permeation coefficient.

Molecular Docking

Docking results between control and compound can be seen in Figure 1. Hyaluronidase-hyaluronan interaction was used as a negative control with a binding energy value of -350.3 kJ/mol. This interaction shows the presence of 5 residues, including Lys155, Lys166, Asn213, Glu162, and Asn214. All amino acid residues that bind to the hyaluronan have this type of hydrogen bond interaction. Five of the seven secondary metabolites of *M. pudica* showed higher binding energy values than the controls, including JA, MP, CB, NA, and TG. The interaction between JA and Hyaluronidase occurred at Glu204 and Arg158 with a binding energy of -348.24 kJ / mol. CB has a binding energy value of -349.04 kJ/mol bound to the residues Ser189, Val199, Trp119, and Phe186. NA has a binding energy value of -348.61 kJ /mol, which is bound to the Trp119 residue. TG has a binding energy value of -348, 93 kJ / mol, which is bound to Arg158, Glu204, Thr200, Glu154, and Lys207. MP has a binding energy value of -347.37 kJ / mol bound to the Glu204 and Glu154 residues. MP is a secondary metabolite compound with the largest binding energy as a complex, thus allowing inhibition of the hyaluronidase-hyaluronan interaction. However, none of the compounds had the same amino acid residues with the control (Table 3). It defines no inhibition of the interaction on the active site. Differences in amino acid residues indicate the binding site changes in hyaluronan after docking using JA, MP, CB, NA, and TG compounds. Conformation changes can affect catalytic activity, be it induction or inhibition. Induction is characterized by decreased binding energy and inhibition with an increase in binding energy [13]. *V. velutina* stings cause reactions by injecting venom via their ovipositors into their target [11]. Wasps and hornets mainly have three groups of components in their venom: small molecular weight peptides, high molecular weight proteins and other components that act as enzymes, toxins and allergens [1]. Hyaluronidases are enzymes that hydrolyze hyaluronan in human skin to increase tissue permeability to fluids. Hyaluronidase in snake and insect venom functions as a "spreading factor" by degrading host hyaluronic acid, thus allowing the spread of toxin [12].

MP is a compound with the most significant potential as a hyaluronidase B enzyme inhibitor of hyaluronidase-hyaluronan interactions. Inhibition of this interaction is expected to be a preventive step in the distribution of *V. velutina* venom due to the activity of the enzyme hyaluronidase B. Unfortunately, the use of MP as a topical drug still requires further treatment to increase its lipophilicity [14]. Furthermore, research related to topical drug design still requires several further steps as validation steps to determine the efficacy of these compounds. Primary molecular candidates need to be identified and analyzed first through in vitro or in vivo studies using physicochemical parameters and [13]. Moreover, a molecular dynamic analysis to determine the stability period of jasmonic acid compounds binds to hyaluronan as a reference for topical drug applications.

Table 2. Physicochemical characteristic of seven *M. pudica* secondary metabolites

Compounds	Molecular weight (g/mol)	LogP	Skin Permeation (cm/h)	Skin reaction	TPSA (Å²)
JA	210.27	2.01	-23.22×10^{-3}	No	54.37
LM	196.18	-1.96	-38.16×10^{-3}	Irritant	105.55
NA	169.18	-0.17	-29.55×10^{-3}	Acute toxic	86.71
TG	411.32	-10.36	-37.72×10^{-3}	No	231.72
LT	578.52	-0.71	-37.29×10^{-3}	Irritant	239.97
CB	576.50	-0.53	-36.14×10^{-3}	No	236.81
MP	337.33	-2.64	-37.04×10^{-3}	No	159.97

Table 3. Interacting residues in hyaluronidase B protein and hyaluronan with *M. pudica* bioactive compounds

Interaction	Amino acid residue	Category	Type	Binding energy (kj/mol)
Hyaluronidase B X HA (Control)	A:LYS155:HZ1 - :LIG1:O	Hydrogen Bond	Conventional	-350
	A:LYS166:HZ1 - :LIG1:O		Conventional	
	A:LYS166:HZ1 - :LIG1:O		Conventional	
	A:ASN213:HD21 - :LIG1:O		Conventional	
	A:ASN213:HD21 - :LIG1:O		Conventional	
	:LIG1:H - :A:GLU162:OE2		Conventional	
	:LIG1:H - :A:GLU162:OE1		Carbon	
	:LIG1:H - :A:ASN214:OD1		Carbon	
Hyaluronidase-HA x JA	A:LIG1:H - A:GLU204:O	Hydrogen Bond	Conventional	-348,24
	A:ARG158:CD - A:LIG1:O		Carbon	
Hyaluronidase-HA x LM	A:LIG1:H - A:PRO255:O	Hydrogen Bond	Conventional	-353,43
	A:LIG1:HA - A:ASP291:OD1		Carbon	
	A:LIG1:H - A:SER254:O		Carbon	
	A:ASP291:OD2 - A:LIG1	Electrostatic	Pi-Anion	
	A:LIG1 - A:VAL245	Hydrophobic	Pi-Alkyl	
Hyaluronidase-HA x NA	A:TRP119 - A:LIG1	Hydrophobic	Pi-Pi Stacking	-348,61
	A:TRP119 - A:LIG1			
Hyaluronidase-HA x TG	A:ARG158:HH12 - A:LIG1:O	Hydrogen Bond	Conventional	-348,93
	A:LIG1:H - A:GLU204:O		Conventional	
	A:LIG1:H - A:THR200:O		Conventional	
	A:LIG1:H - A:GLU154:OE1		Carbon	
	A:LIG1 - A:LYS207	Hydrophobic	Pi-Alkyl	
Hyaluronidase-HA x LT	A:PHE42:HN - A:LIG1:O	Hydrogen Bond	Conventional	-357,8
	A:LIG1:H - A:SER299:O		Conventional	
	A:LIG1:C - A:MET18	Hydrophobic	Alkyl	
	A:PHE17 - A:LIG1:C		Pi-Alkyl	
	A:TYR51 - A:LIG1:C		Pi-Alkyl	
	A:LIG1 - A:ILE94		Pi-Alkyl	
	A:LIG1 - A:PRO96		Pi-Alkyl	
Hyaluronidase-HA x MP	A:LIG1:H - A:GLU204:OE2	Hydrogen Bond	Conventional	-347,37
	A:LIG1:H - A:GLU204:OE2	Hydrogen Bond	Carbon	
	A:GLU154:OE1 - A:LIG1	Electrostatic	Pi-Anion	

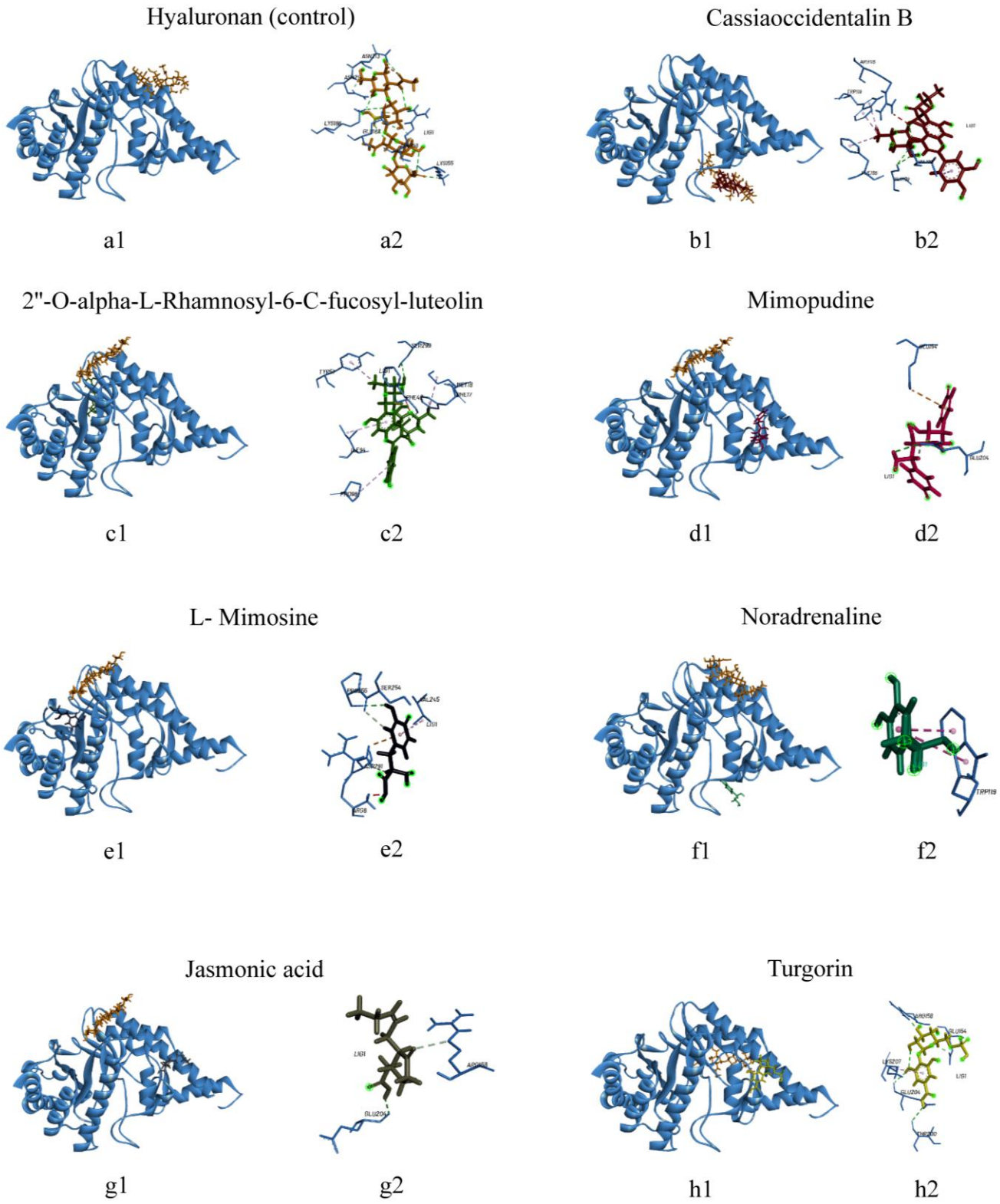

Figure 1. Docking results between complex of Hyaluronidase B- *M. pudica* secondary metabolite compounds with Hyaluronan, (a1) Hyaluronidase B-Hyaluronan (control), (b1) Hyaluronidase B-CB-Hyaluronan, (c1) Hyaluronidase B-LT-Hyaluronan, (d1) Hyaluronidase B-MP-Hyaluronan, (e1) Hyaluronidase B-LM-Hyaluronan, (f1) Hyaluronidase B-NA-Hyaluronan, (g1) Hyaluronidase B-JA-Hyaluronan, (h1) Hyaluronidase B-TG-Hyaluronan and 3D ligan-hyaluronidase B interaction (a2) Hyaluronan-Hyaluronidase B, (b2) CB-Hyaluronidase B, (c2). LT-Hyaluronidase B, (d2) MP-Hyaluronidase B, (e2) LM- Hyaluronidase B, (f2). NA-Hyaluronidase, (g2) JA-Hyaluronidase B, (h2) TG-Hyaluronidase B.

CONCLUSION

None of the compounds had the same amino acid residues as the control. It defines no inhibition of the interaction on the active site. Mimopudine is the most potential inhibitor of hyaluronidase B base on its binding energy though jasmonic acid is the only compound that meets physicochemical parameter of topical drug.

ACKNOWLEDGEMENT

The author would like to thank the bioinformatics lecturers and assistant for helping us to develop and finish this research article.

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