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Potential of fibrinolytic protease enzyme from tissue of sand sea cucumber (*Holothuria scabra*) as thrombolysis agent

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Abstract. Cardiovascular diseases (CVD) are still the main cause of death in modern society with thrombosis as their most common underlying pathology. The occurrence of thrombosis is characterized by thrombus formation in the circulatory system of the body causing blood clot blockage. Thrombus blocking blood vessels can be destroyed through fibrinolysis by plasmin. Commonly administered fibrinolytic drugs activate plasminogen and convert plasminogen to plasmin which degrades fibrin. However, these agents have limitations such as higher costs, shorter half-lives, allergic reactions and intravenous administration-caused bleeding. Hence, more effective and safer antithrombotic drugs are needed in anti-thrombosis therapy. This paper was intended to assess the ability of *Holothuria scabra* tissue fibrinolytic proteases as a thrombolytic agent based on literature studies. Summary of recent studies showed that various strong fibrinolytic enzymes could be produced from various animal tissues such as snake and earthworm, yet from sea organism' tissues are barely reported. In this literature review, the potential of protease enzymes from tissue of *H. scabra* as thrombolysis agent is summarized and discussed. Among the findings is that *H. scabra* has the highest protein content among other sea cucumbers in Indonesia, yet the isolation of protease enzymes from *H. scabra* tissue has not been reported. Hence, there is high possibility in finding novel proteases, which may include serine and metallo-protease known for their thrombolysis activities.

1. Introduction

Cardiovascular disease (CVD), venous and arterial thrombosis are multifactorial diseases characterized by the formation of excessive clots in blood vessels [1]. The occurrence of thrombosis is characterized by thrombus formation in the body's circulatory system due to failure of homeostasis.



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This can cause blood vessels to become blocked and recovery can lead to blockage of the heart muscle or brain, and can end in death [2]. Blood clots have a major protein component, fibrin. Blood clots that block blood vessels can be destroyed through the process of fibrinolysis by plasmin. The main process of fibrinolysis is by activating plasminogen into the plasmin proteolytic enzyme. The enzyme will change the shape of the thrombus by destroying fibrin in blood clots so that the development of thrombosis is inhibited [3].

Urokinase, plasminogen activator (t-PA), streptokinase, which activates plasminogen and converts plasminogens into plasmin that decomposes fibrin, are the normal enzymes used as medicines. However, these agents have limitations such as higher costs, shorter half-lives, allergic reactions and intravenous administration of these agents are also known to cause bleeding. This prompted researchers to continue to look for sources of new fibrinolytic enzymes that are safer and cheaper for handling thrombolysis [4-5].

Protease is a large group of hydrolytic enzymes with the ability of degrading large protein molecules into smaller pieces through hydrolysis reaction. Numerous bacterial proteases had been reported, yet not all were tested for their fibrinolytic activities [6-9]. On the other hand, sea cucumbers and starfish are marine organisms that have been widely known to produce fibrinolytic protease enzymes with antithrombotic activity. This is likely because the protein content of these organisms is high [10]. Among the various sea cucumbers in Indonesia, sand sea cucumbers (*H. scabra*) have the highest protein content [11].

Proteolytic activity is known to occur in sea cucumber that could be correlated with the character and regeneration of the organism's autolytic system. The presence of highly active endogenous proteases is considered to have autolytic characteristics [12]. It has been stated that serine, cysteine and metallo-protease are involved in the regeneration process [13].

This article aims to evaluate the prospects for studying fibrinolytic proteases from the *H. scabra* tissue as antithrombotic agents based on literature studies. Practically and eventually the study is intended to obtain pure enzyme fibrinolytic enzymes from *H. scabra* tissue. Due to the increasing number of cardiovascular cases, the main problem of this paper is to determine the importance of *H. scabra* tissue as source of serine protease fibrinolytic enzymes with good activities used for CVD treatments.

2. Research Methods

A systematic literature search had been conducted from the Pubmed database published between 2010-2020 discussing the role of thrombolysis and its mechanism in the management of acute stroke. We searched for the Medical Subject Title Headings (MeSH) in several combinations including brain ischemia/ drug therapy, CVD therapy, fibrinolytic agents, CVD guide lines, platelet aggregation, protein levels in cucumber/ sea cucumber. The search was limited to humans by reviewing additional references from studies that included 13 articles.

2.1 Eligibility Criteria of the Study

A review of the use of fibrinolytic proteases as antithrombotic agents has been performed that may suggest the ability to use fibrinolytic proteases as CVDs. For this purpose, the selection of studies was carried out based on the following inclusion criteria: [i] evaluation studies using knowledge and practice of using anti-thrombosis drugs in the treatment of CVD as an outcome measure. [ii] subjects related to the importance of fibrinolytic enzymes in CVD therapy applied to CVD; [iii] published in both Indonesian and English; [iv] observational studies. As part of the search strategy, the year of publication is also limited to the last 10 years.

2.2 Identification of Relevant Studies

This literature review was carried out in both ways, manually and non-manually. It was done based on previously used protocol and guidelines [14]. A search for relevant literature was initially carried (by NH, HF and ARS) out in two categories: 1. The importance of fibrinolytic enzymes in CVD therapy. 2. The highest protein content in sea cucumber and its species. 3. Thrombosis therapeutic drugs in CVD.

For the first category, the initial electronic and manual literature search through Pubmed and Science Direct as databases as well as manual searches regardless of the date of publication is carried out using the term MeSH-enzyme fibrinolytic enzyme; enzymes; *H. scabra*; sea cucumber; protein content of sea cucumber, CVD therapy guide line. This for thrombolysis agents, treatment studies or fibrinolytic protease enzymes worldwide yields 50 references and only 20 were retained.

Full-text of all articles were extracted by electronics and manual searches from Pubmed and Science Direct. The studies excluded from this review were: [i] studies conducted before 2010 [ii] studies of the importance of fibrinolytic enzymes in CVD therapy; [iii] reviews; [iv] study of protein content in various species of sea cucumber [v] study of CVD treatment guide lines throughout the world [vi] study of fibrinolytic enzyme produced by sea cucumber, or other animals such as earthworms, fungi, and food. For the second category, computerized literature searches through Google Scholar and the Garuda Portal and manual searches regardless of the date of publication were carried out (by HM and DSZ) using the MeSH Requirements for the treatment of thrombolysis or fibrinolytic protease enzymes. We identified 12 papers with this method. A variety of keywords were used in the search strategy including thrombolysis therapy, fibrinolytic protease enzymes, fibrinolytic protease-producing enzymes. Various keyword combinations were created using 'and', 'or' as Boolean operators. The coherent subject experts and sorted study authors were also contacted to clarify unclear or missing data whenever required. The studies excluded from this review were: [i] studies conducted before 2010 [ii] importance of fibrinolytic enzymes in CVD therapy; [iii] reviews; [iv] The highest protein content in various sea cucumber species; [iv] review; (v) study of CVD therapy guidelines.

2.3 Study Selection

Two authors (NH and SNE) identified independently studies, which met the inclusion criteria in this review. Initially, both the title and abstract of the notes produced by the search were evaluated to determine inappropriate studies that should be excluded based on the exclusion criteria. The review articles were included. Articles (full text) from the remaining studies were taken to fulfill the inclusion criteria. Research was screened using the STROBE checklist for observational studies, (by N and ARS).

2.4 Research Bias Control

The following questions have been included in this mini analysis in the risk of prejudice or quality evaluation: (i) completeness of reporting information on the importance of fibrinolytic enzymes in CVD therapy, (ii) selective outcome reporting, (iii) selection of outcome measures (practice test of the ability of thrombosis agents using fibrinolytic protease tissue was applied in cardiovascular treatment in Indonesia), (iv) research design, and (v) inter-conflict in conducting research. When the overall criteria are met, the risk of a reasonable bias was considered low [15]

3. Literature Review

3.1. Types of thrombosis therapy drugs available

Table 1 summarizes available major therapy drugs studied in the last decades (2009-2019). As seen on Table 1, drugs used in thrombosis therapy are mainly categorized as antiplatelet, anticoagulant and fibrinolytic. In antiplatelet group, most drugs have mechanism of action by blocking of adenosine diphosphate (ADP) binding with platelets. On the other hand, the actions of fibrinolytic group are dominated by formation of plasmin that will lead to fibrin degradation. Data from Table 1 showed that despite having been long discovered (for example Urokinase in 1968 [16]), fibrinolytic drugs are still intensively studied as so as other group of drugs.

Table 1. Reported thrombosis therapy drugs in the last decade

Types of Drugs	Drug Category	Function and Mechanism of Action	Source and Year
Clopidogrel	Antiplatelet	Inhibits adenosine diphosphate (ADP) binding to its platelet receptor	Del Brutto, <i>et al.</i> , 2019 [17]
Aspirin	Antiplatelet	Induces functional defect in platelets	Del Brutto, <i>et al.</i> , 2019 [17]
Dipyridamol	Antiplatelet	Inhibits ADP uptake by platelets	Rollini <i>et al.</i> , 2016 [18]
Aspirindipridamol	Antiplatelet	Inhibits platelet activation	Rollini <i>et al.</i> , 2016 [18]
Cilostazole	Antiplatelet	Inhibit the action of specific phosphodiesterase in platelets	Rollini <i>et al.</i> , 2016 [18]
Pasugrel	Antiplatelet	Blocks ADP-dependent activation of platelets	Rollini <i>et al.</i> , 2016 [18]
Ticagrelor	Antiplatelet	Blocks ADP-dependent activation of platelets	Del Brutto <i>et al.</i> , 2019 [17]
Wafarin	Anticoagulant	Inhibits synthesis of active clotting factors	Del Brutto <i>et al.</i> , 2019 [17]
Dabigatran	Anticoagulant	Prevents thrombin-mediated activation of coagulation factors	Del Brutto <i>et al.</i> , 2019 [17]
Api-, Rifaro-, Edo-xaban	Anticoagulant	Inhibits free and clot-bound factor Xa	Del Brutto <i>et al.</i> , 2019 [17]
Thienopyridines	Antiplatelet	Inhibits, irreversible ADP receptor	Phillips <i>et al.</i> , 2009 [19]
Glikoprotein IIb-IIIa blockers	Antiplatelet & anticoagulant	Prevents the binding of fibrinogen to glycoprotein IIb/IIIa receptors	Rollini <i>et al.</i> , 2016 [18] Phillips <i>et al.</i> , 2009 [19]
Streptokinase	Fibrinolytic & anticoagulant	Cleavages Arg/Val bond in plasminogen to form plasmin, which degrades fibrin clots	Adzerikho <i>et al.</i> , 2019 [20]
Urokinase	Fibrinolytic & anticoagulant	Keeps the plasminogen Arg-Val bond to create active plasmin to degrade fibrin clots	Fuentes <i>et al.</i> , 2016 [21]
PA (Plasminogen Activator)	Fibrinolytic & anticoagulant,	Converts plasminogen into plasmin to dissolve clots	Fan <i>et al.</i> , 2017 [22]

3.2. Importance of Fibrinolytic Enzymes in Cardiovascular (CVD) Therapy

Table 2 shows various important roles of CVD therapy fibrinolytic enzymes recorded from various sources over the past decades. As seen in Table 2, fibrinolytic enzymes that have been used in existing CVD therapy are dominated by those from bacteria of foods or their fermented products, rarely are from plant or animal tissues. Despite various reports about the presence of fibrinolytic enzymes in several sea cucumber species [23-26] those with proven use in CVD therapy are still behind. This implies that studies related with fibrinolytic enzymes sourced from sea cucumber should be encouraged to be produced and clinically tested for CVD therapy.

Table 2. Roles of fibrinolytic enzyme isolated from food in CVD therapy in the last decade

No.	CVD therapy Utilizing Fibrinolytic enzymes	Sources of Fibrinolytic Enzim	Author/s	Country
1	Myocardial infarction	Fungus <i>Cordyceps militaris</i>	Liu <i>et al.</i> , 2016 [26]	China
2	Acute myocardial infarction, ischemic heart disease, & high blood pressure	Bacterium from Cheonggukjang	Jeong <i>et al.</i> , 2015 [27]	Korea
3	Thrombosis & cerebrovascular	Bacterium from Doenjang	Yao <i>et al.</i> , 2017 [28]	Korea
4	Acute myocardial infarction, high blood pressure, ischemic heart, & stroke	Fungus <i>Pleurotus ostreatus</i>	Liu <i>et al.</i> , 2014 [29]	Jepang
5	Thrombosis	Fungus <i>Neurospora sitophila</i>	Liu <i>et al.</i> , 2016 [26]	China
6	Stroke & coronary artery disease	Bacterium from Kimchi	Anh <i>et al.</i> , 2015 [15]	Vietnam
7	Acute myocardial infarction & brain infarction	Bacterium from Cheonggukjang	Heo <i>et al.</i> , 2013 [30]	Korea
9	Lung emboli, cardiac myoinfarction, & deep vein thrombosis	Bacterium from Douchi	Zhang <i>et al.</i> , 2013 [31]	China
10	Myocardial infarction, ischemic heart disease, hypertension & stroke	Bacterium from rice	Vijayaraghavan & Vincent, 2014 [32]	India
11	Heart attack & thrombosis	Bacterium from soy	Devaraj <i>et al.</i> , 2018 [4]	India
12	Thrombosis	Bacterium from chickpeas	Wei <i>et al.</i> , 2011 [33]	China
13	Thrombosis	Bacterium from fermented fish	Prihanto <i>et al.</i> , 2013 [34]	Indonesia
14	Thrombosis	Bacterium from red bean Natto	Chang <i>et al.</i> , 2012 [35]	Taiwan
15	Heart disease & stroke	Bacterium from Tempeh Gembus	Afifah <i>et al.</i> , 2014 [36]	Indonesia
16	Hypertension, myocardial infarction, coronary heart disease, or stenocardia	Bacterium from Douchi	Hu <i>et al.</i> , 2019 [37]	China
17	Thrombosis	Bacterium from Cow Dung	Vijayaraghavan <i>et al.</i> , 2014 [38]	USA

3.3. Protein content in sea cucumbers

Protein-rich marine organisms such as starfish, sea finger, innkeeper worm, are known as potential sources of fibrinolytic (serine) protease enzymes with antithrombotic activity. Sea cucumber is a group marine organism known important source protein content [10]. There lies high potential of the group to also produce high protease enzymes from their muscle tissues. Table 3 lists various sea cucumbers in Indonesia with their protein content. As seen from Table 3, sand sea cucumbers (*H. scabra*) have the highest protein content [39].

Table 3. Protein Content of Sea Cucumber

Sea Cucumber Species	Protein Content (max. % b/b)	Source
<i>Holothuria scabra</i>	76.64	Karnila. <i>et al</i> , 2011 [39]
<i>H. fuscogilva</i>	58.21	Wen <i>et al</i> , 2010 [40]
<i>H. fuscopunctata</i>	50.48	Wen <i>et al</i> , 2010 [40]
<i>H. arenicola</i>	45.00	Haider <i>et al</i> , 2015 [41]
<i>H. nobilis</i>	42.54	Oedjoe, 2017 [11]
<i>H. atra</i>	42.32	Oedjoe, 2017 [11]
<i>H. edulis</i>	41.61	Oedjoe, 2017 [11]
<i>H. impatiens</i>	39.94	Oedjoe, 2017 [11]
<i>H. leucospilota</i>	39.87	Oedjoe, 2017 [11]
<i>H. arenicola</i>	26.30	Salarzadeh <i>et al</i> , 2012 [42]
<i>H. parva</i>	18.56	Salarzadeh <i>et al</i> , 2012 [42]
<i>H. polii</i>	9.86	Aydn <i>et al</i> , 2011 [43]
<i>H. tubulosa</i>	9.12	Aydn <i>et al</i> , 2011 [43]
<i>H. mammata</i>	8.18	Aydn <i>et al</i> , 2011 [43]
<i>Stichopus herrmanni</i>	47.36	Wen <i>et al</i> , 2010 [40]
<i>S. japonicus</i>	48.10	Yu <i>et al</i> . 2015 [44]
<i>Actinopyga mauritiana</i>	67.00	Haider <i>et al</i> , 2015 [41]
<i>A. mauritiana</i>	63.73	Wen <i>et al</i> , 2010 [40]
<i>A. caerulea</i>	57.26	Wen <i>et al</i> , 2010 [40]
<i>A. lecanora</i>	31.11	Oedjoe, 2017 [11]
<i>Basachia argus</i>	31.18	Oedjoe, 2017 [11]
<i>Bohadschia argus</i>	62.49	Wen <i>et al</i> , 2010 [40]
<i>Paracaudina australis</i>	20.22	Widianingsih <i>et al</i> , 2015 [45]
<i>Thelenota ananas</i>	55.58	Wen <i>et al</i> , 2010 [40]
<i>T. anax</i>	40.13	Wen <i>et al</i> , 2010 [40]

CVD is still a major premature death threat worldwide, which often involves thrombosis. Fibrinolytic drugs ranging from streptokinase, urokinase and plasminogen activator are still a promising way to treat thrombosis. However, these agents have limitations in costs, half-lives, allergic reactions and other side effects [4-5]. Hence, more effective and safer antithrombotic drugs are needed in anti-thrombosis therapy.

Data in Table 2 show that protein-rich fermented foods, such as Natto from Japan, Douches from China, Doenjang and Cheonggukjang from Korea, tempeh from Indonesia, and Kishk from Egypt are potential source microbial fibrinolytic enzymes. Production of fibrinolytic enzyme from protein-rich seafood or bacteria related to it is still scarce. Data in Table 3 shows that among protein-rich source in marine environment is sea cucumber. Sand sea cucumber (*Holothuria scabra*) have the highest protein content among other sea cucumbers in Indonesia [39]. The Holothurian is easy to find in the country exposing various beneficial properties as food and cosmetics commodities [45-47]. The presence of fibrinolytic proteases has been found quite abundant in sea cucumber including Holothurians [9,48-49]. Meanwhile, isolation of protease enzymes with fibrinolytic ability from *H. scabra* tissue has not been reported. Thus, development of antithrombotic agents using fibrinolytic protease from tissue of *H. scabra* intended for the treatment of CVDs is potential for novelty.

Data from these tables, and other studies assess prospects for the manufacture of fibrinolytic agents as fibrinolytic enzymes of protein-rich sea cucumber (H). Data from these tables are centered. Scabra could be made. The findings are a program showing the ability of fibrinolytic protease enzymes in the treatment of cardiovascular disease by Sand Sea Cumber (H-scabra) as antithrombotic agent (Figure 1).

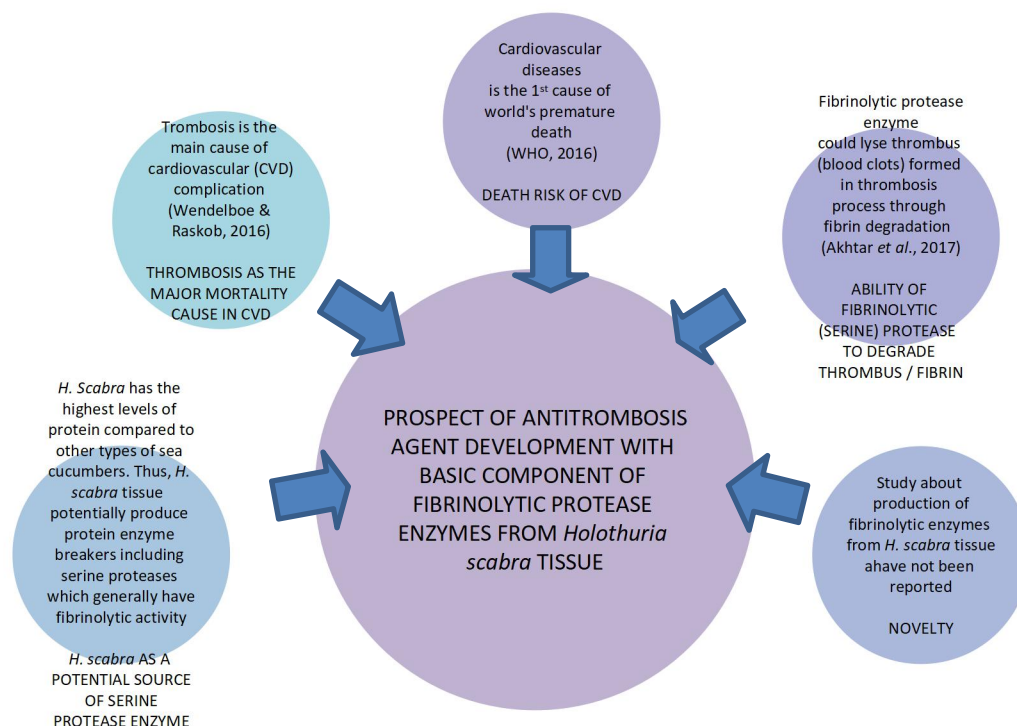


Figure 1. Prospects for antithrombotic agent development agents in the form of a fibrinolytic protease enzyme resulting from the secretion of proteolytic bacteria in *H. scabra* tissue

4. Conclusion

From this review, it could be concluded that:

- Sand sea cucumber (*H. scabra*) has the highest protein content, yet the isolation of protease enzymes with fibrinolytic ability from the organism's tissue has never been reported.
- The development of antithrombotic agents using fibrinolytic protease from tissues obtained from sand sea cucumber tissue is quite promising because the presence of fibrinolytic proteases is quite abundant.
- Development of antithrombotic agents using fibrinolytic protease from tissue of *H. scabra* offers novelty for the treatment of CVDs.

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