Potential of Hydrolyzed Waste in Portunus sp. Non-Shell as Nutraceutical With Bioinformatics Analysis

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Key words: Hydrolyzate, *Portunus* sp., non-shell waste, nutraceutical, bioinformatics

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Abstract: This study aims to obtain a profile of the potential hydrolyzate of *Portunus* sp. with bioinformatics analysis. The material used in this study was *Portunus* sp. non-shell from the meat canning industry *Portunus* sp. Non-shell waste is hydrolyzed using papain enzyme to produce hydrolyzate. The hydrolyzate was screened with LC-HRMS and analyzed using swiss ADME, PASS and Pro Tox. LC-HRMS screening shows the results that there are twelve compounds, namely L-Phenylalanine, Tryptophan, trans-3-Indoleacrylic acid, Isoleucine, Betaine, Trigonelline, Adenosine, Tyrosine, Propionylcarnitine, Stachydrine, Maltose, Arginine. swiss ADME analysis shows that the hydrolyzate of *Portunus* sp. has potential as a pretty good drug ingredient based on the review of Lipinski's rules and pharmacokinetic property. The results of the PASS analysis to predict the potential for biological activity indicate that the hydrolyzate of *Portunus* sp. Non-shell has potential as NADPH peroxidase inhibitors, Omptin inhibitors, Membrane integrity agonists, Preneoplastic conditions treatment, Phobic disorders treatment, Antiseborrheic, Pseudolysin inhibitors, Saccharopepsin inhibitors. Toxicity analysis based on Pro Tox predictions showed no potential Carcinogenicity, Immunotoxiticity, Mutagenicity. These results indicate that the hydrolyzate of *Portunus* sp. potentially as a nutraceutical and safe for consumption.

INTRODUCTION

Increased production and export of *Portunus sp.* An increase will always follow the meat in industrial waste. *Portunus* sp. waste can reach 40-60% of the total weight of *Portunus* sp. Shell waste can be further processed into polysaccharides, chitosan and glucosamine. As for eggs, they can be transformed into culinary preparations. Not many wastes in the form of mustards and gills utilize it, so, efforts need to be made, so there is an added value. One effort to use fishery waste is to use hydrolysis technology. Hydrolysis technology is a process of breaking complex bonds into more direct relationships using enzymes, acids and bases[1]. The use of hydrolysis technology produces hydrolyzate products with better nutritional and functional properties[2]. Enzymatic hydrolysis is more beneficial because of its high product quality[3]. Thus, the non-shell waste of *Portunus* sp. can be made into a more useful hydrolyzate product.

Bioinformatics analysis began to be developed at this time. Bioinformatics analysis is a method for predicting the ability of an active ingredient to cause computational biological effects. Bioinformatics analysis is usually used in the context of finding new drugs. Bioinformatics analysis was developed to reduce costs and reduce the length of the process needed for an active ingredient to become a drug[4]. These factors are the low effectiveness of drugs, the emergence of toxic effects and obstacles in the marketing process itself[5]. Therefore, this research was conducted using a bioinformatics analysis approach. Bioinformatics analysis was used to obtain the potential profile of *Portunus* sp. hydrolyzate as a nutraceutical.

MATERIALS AND METHODS

The tools used in this study were hand blenders (Philips HR 1364), ovens (Memmert), centrifuges (Himac CR 21G), water bath shakers (Wisebath), beaker glass 50 ml, burette, pH meter, Liquid Chromatography-High Resolution Accurate Mass Mass Spectrometry (LC-HRMS) as well as a set of computers.

The materials used in this study were non-shell waste from *Portunus* sp., Papain enzyme, distilled water, NaOH (Merck), pH paper, formaldehyde (Merck) 35%. selenium, H_2SO_4 (Merck), NaOH, HCl (Merck), H_3BO_3 (Sigma) and hexane (Merck), HCl (Merck) 0.1 N solvent, 0.5 N NaOH, 0.5 N NaOH, phosphate buffer solution 0.2 M pH 8, HCl, 6 N and 0.01 N.

Optimization of non-shelly *Portunus* sp. hydrolyzate waste: The optimization of hydrolysis conditions is achieved by using the Response Surface Methodology (RSM) method. The optimized design is then validated through various random parameter combinations to evaluate the usefulness of the design[6]. *Portunus* sp. non-shell waste hydrolyzate was selected based on the best degree of hydrolysis, then carried out bioinformatics analysis to obtain a profile as a nutraceutical.

Hydrolyzed analysis using LC-HRMS: The screening of compounds was carried out with LC-HRMS Shimadzu (Shimadzu Corp, Kyoto, Japan). The HRMS LC used is Thermo Scientific Q Exactive with 70,000 resolutions for MS1 plus 17,500 resolutions for MS2 as well as the polarity used is positive. In contrast, the software used for reading compound names is mzCloud MS/MS Library with the latest update (May 2019).

Analysis of bioinformatics of hydrolyzed *Portunus* sp.: Bioinformatics analysis is done by accessing the PubChem server (https://pubchem.ncbi.nlm.nih.gov/) to obtain 2D structure and 3D structure information from bioactive compounds. The bioactive compounds were then analyzed to obtain the properties of absorption, distribution, metabolism and excretion with SwissADME (http://www.swissadme.ch/)[7]. Prediction of biological activity of hydrolyzed compounds was analyzed by online Prediction of Activity Specifications for Substances (PASS) (http://www.way2drug.com/PASSOnline/index.php)[8]. LD50 toxicity prediction was performed using Pro Tox's online prediction (http://tox.charite.de/protox_II/index.php)[9].

RESULTS AND DISCUSSION

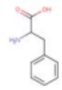
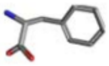
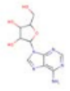
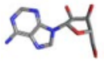
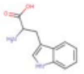
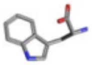
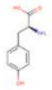
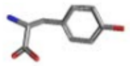
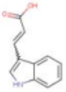
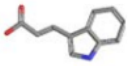
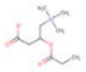
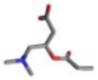
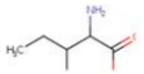
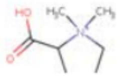
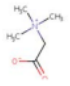
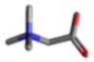
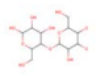
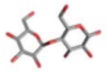
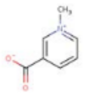
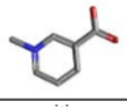
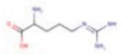
Bioactive compounds from LC-HRMS leaching to *Portunus* sp. hydrolyzate are as shown in Table 1. Pharmacokinetics properties analysis was performed to see whether the compound from *Portunus* sp. hydrolyzate could reach the target location in sufficient concentration. Besides, pharmacokinetics properties analysis can show whether these compounds can survive in a relatively long time and function as expected. The results of this Swiss ADME analysis are shown in Table 2. The non-shell *Portunus* sp. hydrolyzate was analyzed by Lipinsky's rule using Swiss ADME concerning potential as a drug candidate shown in Table 3 and 4.

Predictions for assessing biological activity are carried out by an online application (http://www.pharmaexpert.ru/passonline/) and estimates of probability values (Pa) are presented in Table 5. The LD50 toxicity prediction results use the Pro Tox online prediction (http://tox.charite.de/protox_II/index.php), as shown in the Table 6.

Prediction of potential medicinal ingredients, recently developed by the in silico method[10]. The analysis was carried out by looking at the level of Absorption, Distribution, Metabolism and Excretion (ADME) which can be assessed by a computer model by accessing the website: http://www.swissadme.ch[11]. LC-HRMS screening shows that there are twelve compounds, namely L-Phenylalanine, Tryptophan, trans-3-Indoleacrylic acid, Isoleucine, Betaine, Trigonelline, Adenosine, Tyrosine, Propionylcarnitine, Stachydrine, Maltose, Arginine.

The predicted pharmacokinetic analysis of the non-shell *Portunus* sp. hydrolyzate is shown in Table 2. Pharmacokinetics is a mathematical description of the ADME process rate and concentration-time relationship. Many pharmacologically active compounds are chosen

Table 1: Twelve compounds from non-shell Portunus sp. hydrolyzate using LC-HRMS

Name/Formula (Relative level)	2D	3D	Name/Formula (Relative level)	2D	3D
L-Phenylalanine $C_9H_{11}NO_2$; 19.29%			Adenosine $C_{10}H_{13}N_5O$; 2.53%		
DL-Tryptophan $C_9H_{11}NO_3$; 2.50%			L-Tyrosine $C_{11}H_{12}N_2O$; 15.68%		
Trans-3-Indoleacrylic acid $C_{11}H_9NO_2$; 15.54%			Propionylcarnitine $C_{10}H_{19}NO_4$ 2.46%		
Isoleucine $C_6H_{13}NO_2$; 11.04%			DL-Stachydrine $C_7H_{13}NO_2$; 2.29%		
Betaine $C_5H_{11}NO_2$; 7.53%			D-(+)-Maltose $C_{12}H_{22}O_{11}$; 2.23%		
Trigonelline $C_7H_7NO_2$; 4.93%			DL-Arginine $C_6H_{14}N_4O_2$; 1.91%		

Number sequences are based on the most relative composition

Table 2: Pharmacokinetics properties of non-shell *Portunus* sp. hydrolysates using SwissADME

Compounds	GI absorption	BBB permeant	Pgp substrate	log Kp (cm sec^{-1})
L-Phenylalanine	High	No	No	-8.39
DL-Tryptophan	High	No	No	-8.30
trans-3-Indoleacrylic acid	High	Yes	No	-6.09
Isoleucine	High	No	No	-8.32
Betaine	Low	No	No	-7.11
Trigonelline	High	No	No	-6.77
Adenosine	Low	No	No	-8.68
L-Tyrosine	High	No	No	-9.01
Propionylcarnitine	High	No	No	-7.48
DL-Stachydrine	High	No	No	-6.90
D-(+)-Maltose	Low	No	Yes	-10.92
DL-Arginine	High	Low	No	-10.34

Table 3: Physicochemicals of the non-shell *Portunus* sp. hydrolyzate using SwissADME

Compounds	MW (g.mol^{-1})	HA	AHA	RB	HBA	HBD	MR	TPSA	L
L-Phenylalanine	165.19	12	6	3	3	2	45.50	63.32	0.64
DL-Tryptophan	204.23	15	9	3	3	3	57.36	79.11	1.12
trans-3-Indoleacrylic acid	187.19	14	9	2	2	2	54.97	53.09	2.16
Isoleucin	131.17	9	0	3	3	2	35.44	63.32	0.44
Betaine	117.15	8	0	2	2	0	28.35	40.13	-1.56
Trigonelline	137.14	10	6	1	2	0	35.05	44.01	-1.13
Adenosine	267.24	19	9	2	7	4	62.67	139.54	-2.30
L-Tyrosine	181.19	13	6	3	4	3	47.52	83.55	0.35
Propionylcarnitine	217.26	15	0	7	4	0	53.67	66.43	-0.85
DL-Stachydrine	144.19	10	0	1	2	1	43.29	37.30	-0.07
D-(+)-Maltose	342.30	23	0	4	11	8	68.12	189.53	-5.40
DL-Arginine	174.20	12	0	5	4	4	44.54	127.72	-1.55

MW: Molecular Weight; HA; Heavy Atoms; AHA; Aromatic Heavy Atoms; RB; Rotatable Bonds ; HBA; Hydrogen Bond Acceptor; HBD; Hydrogen Bound Donor; MR; Molar Refractivity; TPSA; Topology Polar Surface Area (Å²); L; Lipophilicity

Table 4: Drug likeness hydrolyzate properties of *Portunus* sp. non-shell using Lipinski rule of five

Compounds	molecular mass >500 Dalton	High lipophilicity (expressed as LogP >5)	>5 hydrogen bond donors	>10 hydrogen bond acceptors	Molar refractivity should be between 40-130	Conclusion
L-Phenylalanine	Yes	Yes	Yes	Yes	Yes	Yes
DL-Tryptophan	Yes	Yes	Yes	Yes	Yes	Yes
trans-3-Indoleacrylic acid	Yes	Yes	Yes	Yes	Yes	Yes
Isoleucine	Yes	Yes	Yes	Yes	No	Yes
Betaine	Yes	Yes	Yes	Yes	No	Yes
Trigonelline	Yes	Yes	Yes	Yes	No	Yes
Adenosine	Yes	Yes	Yes	Yes	Yes	Yes
L-Tyrosine	Yes	Yes	Yes	Yes	Yes	Yes
Propionylcarnitine	Yes	Yes	Yes	Yes	Yes	Yes
DL-Stachydrine	Yes	Yes	Yes	Yes	Yes	Yes
D-(+)-Maltose	Yes	Yes	No	No	Yes	Yes
DL-Arginine	Yes	Yes	Yes	Yes	Yes	Yes

Table 5: Potential hydrolyzate properties of non-shell *Portunus* sp. using PASS Server

	Prediction of potential biological activities									
Compound	A	BC	D	E	F	G	H	I	J	
L-Phenylalanine	0.924	0.940	0.719	0.737	0.907	0.825	0.899	0.904	0.884	0.809
DL-Tryptophan	0.803	0.780	0.57	0.644	0.707	0.854	0.702	0.748	0.947	0.605
trans-3-Indoleacrylic acid	0.632	0.517	0.69	0.538	0.795	0.828	0.672	0.357	0.872	0.630
Isoleucine	0.891	0.863	0.593	0.718	0.855	0.833	0.925	0.925	0.898	0.925
Betaine	0.876	0.744	0.498	0.437	0.841	0.552	0.908	0.719	0.770	0.812
Trigonelline	0.681	0.573	0.436	0.503	0.595	0.453	0.746	0.656	0.702	0.634
Adenosine	0.482	- -	0.953	-	-	-	-	0.313	-	
L-Tyrosine	0.925	0.900	0.671	0.674	0.932	0.867	0.815	0.933	0.825	0.775
Propionylcarnitine	0.539	0.617	0.467	0.451	0.773	0.434	0.773	0.348	0.547	0.826
DL-Stachydrine	0.611	0.530	0.490	0.404	0.427	0.355	0.764	0.277	0.520	0.627
D-(+)-Maltose	0.607	0.317	0.697	0.684	0.922	-	0.324	-	0.814	0.635
DL-Arginine	0.882	0.935	0.486	0.432	0.804	0.726	0.842	0.817	0.828	0.782

A (NADPH peroxidase inhibitor); B (Omptin inhibitor (antibacterial)); C (Antihypox); D (Antiviral); E (Membrane integrity agonist (antimicrobial)); F (Preneoplastic conditions treatment); G (Phobic disorders treatment); H (Antiseborrheic);I (Pseudolysin inhibitor (pathogenic antimicrobials)); J (Saccharopepsin inhibitor (Antifungal))

Table 6: Oral toxicity prediction results from non-shell *Portunus* sp. hydrolysates using Pro Tox II

Compound	LD50 (mg kg^{-1})	Hepatotoxicity	Carcinogenicity	Immunotoxiticity	Mutagenicity	Cytotoxicity
L-Phenylalanine	2.400	Inactive	Inactive	Inactive	Inactive	Inactive
DL-Tryptophan	16.000	Inactive	Inactive	Inactive	Inactive	Inactive
trans-3-Indoleacrylic acid	2.500	active	Inactive	Inactive	Inactive	Inactive
Isoleucine	5.000	Inactive	Inactive	Inactive	Inactive	Inactive
Betaine	650	Inactive	Inactive	Inactive	Inactive	Inactive
Trigonelline	5.000	Inactive	Inactive	Inactive	Inactive	Inactive
Adenosine	8	Inactive	Inactive	Inactive	Inactive	Active
L-Tyrosine	1.460	Inactive	Inactive	Inactive	Inactive	Inactive
Propionylcarnitine	165	Inactive	Inactive	Inactive	Inactive	Inactive
DL-Stachydrine	2.078	Inactive	Inactive	Inactive	Inactive	Inactive
D-(+)-Maltose	19	Inactive	Inactive	Inactive	Inactive	Inactive
DL-Arginine	7.500	Inactive	Inactive	Inactive	Inactive	Inactive

which then fail to develop due to several factors such as poor bioavailability, high cleansing, low solubility and difficulty in the formulation. In Table 2, it is seen that all chemical components of the non-shell *Portunus* sp. hydrolyzate have high intestinal absorption ability. Nine of the twelve compounds of the non-shell *Portunus* sp. hydrolyzate have high GI absorption. This analysis shows that some compounds can spread well to all parts of the body to play an active role as a drug[12].

When we design an extract (in which there are compounds or peptides) to be a nutraceutical candidate, then there is what is called the Drug-likeness test. Drug-likeness is a term used to describe how the physicochemical properties of compounds affect molecular properties in vivo (Table 3 and 4). Most rules for testing drug-likeness use physiochemical properties obtained from molecular structures and match these properties with registered drugs. One rule that is widely used is the Lipinski rule[13] where a molecule must weigh ≤ 500 Da, Log $p \leq 5$, the number of hydrogen proton donor groups donor 5 and proton acceptor groups ≤ 10. The chemical composition of the non-shelly *Portunus* sp. hydrolyzate has excellent potential as a drug candidate. SwissADME analysis shows that *Portunus* sp.

hydrolyzate has potential as a pretty good drug ingredient based on a review of Lipinski's rules and pharmacokinetic properties.

PASS server is used to predict the potential for biological activity. PASS server can predict <300 pharmacological effects and biochemical mechanisms based on the structural formula of a substance and can be used efficiently to find new targets or mechanisms for several ligands. The nature or character of biological compounds can be predicted using the PASS activity spectrum predictions for substances that can be accessed online. Pa => 0.7 indicates that the compound is very likely to show activity in the experiment. However, it is possible that the substance has similarities with other known drug agents and the value of Pa => 0.5-<0.7 indicates that the substance tends to show activity, and may not be the same as other known drugs. The data in Table 5 shows that the non-shell *Portunus* sp. hydrolyzate has potential biological activity as an NADPH peroxidase inhibitor, Omptin inhibitor, Membrane integrity agonist, Preneoplastic conditions treatment, Phobic disorders treatment, Antiseborrheic, Pseudolysin inhibitor, Saccharopepsin inhibitor.

The toxicity analysis, based on the Pro Tox prediction in Table 6, shows that the hydrolyzate from *Portunus* sp. does not indicate the potential Carcinogenicity, Immunotoxiticity, Mutagenicity. Based on these results, the potential for the development of non-shell *Portunus* sp. hydrolysates can be further developed and these results can be used as a basis for testing in later stages in vitro and in vivo.

CONCLUSION

In conclusion, the analysis of the potential of non-shelly *Portunus* sp. hydrolyzate in the prediction of swiss ADME, PASS online and Pro Tox. These results indicate that *Portunus* sp. hydrolyzate has the potential to be a nutraceutical and safe for consumption. The prediction from the bioinformatics analysis shows that this research can be continued *in vitro* and *in vivo*.

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