Anti-Inflammatory Potential from Tilapia (Oreochromis niloticus) Viscera Hydrolysate with Bioinformatics Analysis (Prediction of Activity Spectra for Substances – PASS)

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Submission date: 21-Mar-2022 12:22PM (UTC+0700)
Submission ID: 1788968469
File name: Analysis_Prediction_of_Activity_Spectra_for_Substances_PASS.pdf (739.46K)
Word count: 3654
Character count: 18086

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Article *in* IOP Conference Series Earth and Environmental Science · May 2021

DOI: 10.1088/1755-1315/750/1/012044

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To cite this article: PH Riyadi *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **750** 012044

IOP Conf. Series: Earth and Environmental Science **750** (2021) 012044 doi:10.1088/1755-1315/750/1/012044

Anti-Inflammatory Potential from Tilapia (*Oreochromis niloticus*) Viscera Hydrolysate with Bioinformatics Analysis (Prediction of Activity Spectra for Substances – PASS)

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Abstract. Tilapia (*Oreochromis niloticus*) production in Indonesia for 5 (five) years has increased by 18%. Increased production of tilapia (*Oreochromis niloticus*) will result in an increase in fish waste and by-products, such as viscera, skin, bones, and scales. This waste can have an impact on environmental, health, social, and economic problems if not appropriately managed. Hydrolysis technology can turn viscera tilapia waste into more useful hydrolysate. This article aims to obtain a profile of the potential hydrolysates of tilapia viscera as an anti-inflammatory by bioinformatics analysis. The material used in this study was tilapia viscera waste. The waste is then hydrolyzed with alcalase enzyme to produce hydrolysate. LC-HRMS screening shows that there are 99 compounds and eight peptides. PASS analysis is used to predict the potential for biological activity. Most of the total hydrolysate content of tilapia viscera waste has potential biological activity as an anti-inflammatory. These results indicate that tilapia viscera waste hydrolysate has the potential as an anti-inflammatory.

1. Introduction

The volume of the production of tilapia (*Oreochromis niloticus*) was 1,114,156 tons in 2016 and 1,155,374 tons in 2017 [1]. The increase in production continued for the last five years to reach 11%. Increased tilapia production will result in increased fish waste. The fish waste consists of heads, skins, fins, tails, bones, viscera, and fish scales. Solid waste is the most significant contributor to the waste fisheries industry [2]. Viscera waste has high protein and unsaturated fat content [3]. Viscera waste can be utilized as a source of raw materials for hydrolysis proteins [4] and can minimize environmental and health problems and can reduce the economic impact [5].

One of the efforts to use fish waste is to use hydrolysis technology. Hydrolysis technology is a process of breaking down complex bonds into simple bonds. Termination of complex bonds using enzymes, acids, or bases [6]. The use of hydrolysis technology produces hydrolysis products with better nutritional and functional properties [7].

Since 2000, *Prediction of Activity Spectra for Biologically Active Substances* (PASS) has served as a freely accessible web resource for the prediction of biological spectral activity [8], [9]. Computer biological activity predictions can be performed both for publication and new compounds, which allow the filtering of unpromising compounds at the earliest stage of the investigation. Therefore, the

utilization of bioinformatics science-based computing technology in massive data management has become a big business [10].

Previous research has evaluated the potential of bioactive peptides contained in tilapia viscera hydrolyzate extract as antiviral. The PASS analysis showed that the bioactive peptides of tilapia viscera hydrolyzate extract had simian immunodeficiency virus proteinase inhibitor activity, 3C-like protease (Human corona-virus) inhibitor, Viral entry inhibitor, antiviral for adenovirus, influenza, and rhinovirus. [11]. This article aims to obtain a potential profile of hydrolysate sewage of tilapia viscera (*Oreochromis niloticus*) as an anti-inflammatory with a bioinformatics analysis approach.

2. Materials and Methods

2.1. Materials

The material used in this study was the hydrolysate of tilapia viscera waste (*Oreochromis niloticus*) [12, 13]. The tools used in this study are Liquid Chromatography-High Resolution Accurate Mass Spectrometry (LC-HRMS) and a set of computers.

2.2. Hydrolisate analysis using LC-HRMS

Compound screening was performed with LC-HRMS Shimadzu (Shimadzu Corp, Kyoto, Japan). The capillary column was injected with 1 µL of the sample. Chromatography separation was achieved using Hyperil Column Gold (1.9 µm x 1 mm x 50 mm). The mobile process consisted of a mixture of A (0.1 % formic acid in water, v / v) and B (0.1 % formic acid in acetonitrile, v / v). The liner gradient ranged from 4 to 20 percent B (v / v) at 40 min, to 35 percent B at 60 min, to 100 percent B at 61 min and head at 100 percent B at 65 min. The ThermoFisher Scientific Q Exactive with 70000 resolution for MS1 plus 17500 resolution for MS2 is the LC-HRMS used. The polarity was optimistic. The program used to read the results was mzCloud MS / MS Library with the most recent updates (May 2019) [14].

2.3. Bioinformatics Analysis (PASS)

The first step is to access the PubChem server (https://pubchem.ncbi.nlm.nih.gov/) to get canonical SMILE information. The second stage is to predict the biological activity of hydrolysate compounds using PASS (http://www.way2drug.com/PASSOnline/index.php) by including canonical SMILE [15, 16].

3. Results and Discussion

3.1. Results

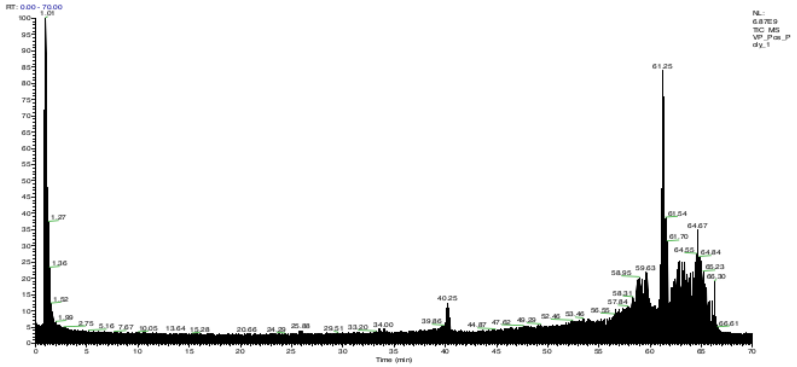

Figure 1. Chromatogram LC-HRMS from The Hydrolysate of Tilapia Viscera Waste

Table 1. Compounds with Pa value higher than 0.7

No	Compounds	Relative amount (%)	Pa	Pi
1	Eicosapentaenoic acid (EPA)	14.57	0.804	0.006
2	Anacardic acid	3.00	0.706	0.003
3	9-Oxo-10(E),12(E)-octadecadienoic acid	2.72	0.770	0.009
4	1-Linoleoyl glycerol	2.71	0.746	0.011
5	(+/-)12(13)-DiHOME	2.23	0.710	0.003
6	Andrographolide	1.08	0.845	0.005
7	Medrysone	1.02	0.921	0.001
8	α-Eleostearic acid	0.96	0.720	0.002
9	Arachidonic acid	0.83	0.730	0.012
10	14(S)-HDHA	0.40	0.871	0.005
11	Pinolenic acid	0.37	0.730	0.012
12	γ-Linolenic acid ethyl ester	0.30	0.827	0.005
13	9(Z),11(E),13(E)-Octadecatrienoic Acid methyl ester	0.22	0.751	0.002
14	Sedanolide	0.08	0.717	0.014
15	Adrenic acid	0.03	0.730	0.012
	Total (%)	**30.52**		

Table 2. Compounds with 0.5 < Pa < 0.7

No	Compounds	Relative amount (%)	Pa	Pi
1	Ethyl palmitoleate	3.95	0.672	0.019
2	Acetophenone	1.51	0.552	0.005
3	(+/-)12-HpETE	1.49	0.521	0.006
4	(3E)-3-(Hydroxymethyl)-2-oxo-5-[(1S,8aS)-5,5,8a-trimethyl-2-methylenedecahydro-1-naphthalenyl]-3-pentenoic acid	1.32	0.647	0.023
5	L-Norleucine	1.29	0.521	0.006
6	Monoolein	1.02	0.689	0.117
7	Stearoylbenzoylmethane	0.58	0.592	0.004
8	Docosahexaenoic acid ethyl ester	0.47	0.683	0.018
9	D-Sphingosine	0.44	0.535	0.047
10	(5ξ,9ξ,16ξ)-17-Hydroxykauran-19-oic acid	0.41	0.500	0.057
11	Cannabidiolic acid	0.33	0.512	0.053
12	3-Methyl-5-[(1S,2R,4aR)-1,2,4a,5-tetramethyl-7-oxo-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenyl]pentanoic acid	0.27	0.529	0.049
13	3-Methyl-5-(5,5,8a-trimethyl-2-methylene-7-oxodecahydro-1-naphthalenyl)pentyl acetate	0.27	0.679	0.019
14	6-Gingerol	0.26	0.566	0.004
15	5-Aminovaleric acid	0.26	0.526	0.005
16	11-Deoxy prostaglandin F1β	0.18	0.533	0.005
17	2-Aminooctadec-4-yne-1,3-diol	0.17	0.516	0.006
18	Palmitoleic acid	0.16	0.685	0.003
19	Bis(2-ethylhexyl) phthalate	0.14	0.537	0.046
20	4-Phenylbutyric acid	0.13	0.546	0.005
21	Butyl benzoate	0.08	0.510	0.006
22	1-Stearoyl glycerol	0.07	0.644	0.024
23	5-[(Z)-Pentadec-8-enyl]benzene-1,3-diol	0.03	0.514	0.053
	Total (%)	**14.83**		

Table 3. Compounds with 0.3 < Pa < 0.5

No	Compounds	Relative amount (%)	Pa	Pi
1	4-Piperidone	4.86	0.367	0.019
2	L-Phenylalanine	3.24	0.429	0.016
3	Promethazine sulfoxide	1.78	0.294	0.165
4	4-tert-Butylcyclohexyl acetate	1.27	0.376	0.108
5	3-Methoxy prostaglandin F1α	1.23	0.490	0.008
6	Valine	1.21	0.403	0.022
7	Palmitoyl ethanolamide	1.01	0.324	0.054
8	5(Z),8(Z),11(Z)-Eicosatrienoic acid ethanolamide	0.74	0.467	0.067
9	Prolinamide	0.63	0.302	0.075
10	Ornithine	0.56	0.351	0.040
11	R-Palmitoyl-(2-methyl) ethanolamide	0.55	0.387	0.026
12	Betahistine	0.49	0.255	0.166
13	(3S)-5-[(4aR,8aS)-2,5,5,8a-Tetramethyl-3-oxo-4a,6,7,8-tetrahydro-4H-naphthalen-1-yl]-3-methylpentanoic acid	0.46	0.389	0.101
14	Hexadecanamide	0.45	0.472	0.101
15	Oleamide	0.42	0.444	0.014
16	Diethyl phthalate	0.41	0.422	0.007
17	Stearamide	0.41	0.472	0.010
18	DL-Leucineamide	0.40	0.341	0.034
19	Indole-3-acrylic acid	0.40	0.358	0.037
20	Anandamide (AEA)	0.38	0.467	0.067
21	N,N-Dimethylsphingosine	0.35	0.370	0.030
22	16,16-Dimethyl prostaglandin A1	0.34	0.431	0.016
23	Glycyl-L-leucine	0.30	0.307	0.068
24	Oleoyl ethanolamide	0.30	0.353	0.120
25	2-Amino-1,3,4-octadecanetriol	0.26	0,470	0,010
26	Guanidinosuccinic acid	0.21	0.389	0.026
27	N-Acetyltyramine	0.18	0.309	0.064
28	Triphenyl phosphate	0.16	0.372	0.031
29	Kahweol	0.15	0.345	0.126
30	4-Piperidinecarboxamide	0.15	0.386	0.013
31	Tyramine	0.14	0.413	0.019
32	L-Iditol	0.14	0.446	0.013
33	9S,13R-12-Oxophytodienoic acid	0.14	0.379	0.029
34	4-(Dimethylamino)benzophenone	0.11	0.473	0.004
35	L-Glutamic acid	0.11	0.493	0.008
36	β-Hydroxyfentanyl	0.09	0.345	0.043
37	6-Methylquinoline	0.09	0.323	0.050
38	Muscone	0.09	0.379	0.015
39	19-Nortestosterone	0.08	0.479	0.004
40	4-Hydroxybenzaldehyde	0.06	0.451	0.013
41	Isoproturon	0.06	0.375	0.016
42	Z-Leu-OH	0.06	0.344	0.032
43	3-Aminopyrrolidine	0.04	0.346	0.030
44	1-Dodecyl-2-pyrrolidinone	0.03	0.315	0.060
45	Erucamide	0.02	0.444	0.014
46	Leu-Leu	0.02	0.327	0.045
	Total (%)	**21.47**		

Table 4. Compounds with Pa less than 0.3

No	Compounds	Relative amount (%)	Pa	Pi
1	Promethazine sulfoxide	1.78	0.294	0.165
2	Cafestol	1.09	0.274	0.118
3	Betahistine	0.49	0.255	0.166
4	Glycerophospho-N-palmitoyl ethanolamine	0.42	0.240	0.224
5	Histamine	0.38	0.242	0.196
6	N-Acetylputrescine	0.26	0.255	0.166
7	Lys-Pro	0.15	0.204	0.183
8	L-(+)-Arginine	0.14	0.283	0.085
9	N-Butylbenzenesulfonamide	0.14	0.280	0.087
10	4-Indolecarbaldehyde	0.12	0.220	0.157
11	Sulfadiazine	0.12	0.220	0.159
12	DAUDA	0.10	0.208	0.177
13	Thymine	0.09	0.276	0.120
14	Val-Tyr	0.09	0.296	0.084
15	Viru-merz	0.09	0.295	0.086
16	Gly-l-Pro	0.06	0.274	0.093
17	α-Phenylpiperidine-2-acetamide	0.04	0.258	0.158
18	Leucyl-leucyl-norleucine	0.04	0.291	0.092
	Total (%)	**5.60**		

Table 5. Compounds with undata Pa

No	Nama	Relative amount (%)	Pa	Pi	References
1	Choline	24,76	-	-	Rowley et al., 2010
2	XLR11 N-(4-hydroxypentyl) metabolite	1,54	-	-	-
3	Buprenorphine	1,04	-	-	-
4	Citalopram	0,11	-	-	-
5	Asp-Trp	0,07	-	-	-
6	Cytisine	0,06	-	-	-
	Total (%)	**27.58**			

3.2. Discussions

The anti-inflammatory property was the living organisms cellular response due to infection or injury to cells or tissues [17]. Inflammation is closely related to the development of chronic human diseases [18], including atherosclerosis, arthritis [19], diabetes [20], cancer [21], inflammatory bowel disease [22], and Alzheimer's [23]. Therefore, it is necessary to develop a therapeutic agent candidate, which can be used as a nutraceutical and pharmaceutical material.

Figure 1 shows the chromatogram of LC-HRMS. Analysis with the LC-HRMS tool shows there is 160 peak with 99 compounds and eight peptides. Classification of compounds and peptides sorted from most percentages (area of LC-HRMS analysis) is unsaturated fat 34% (28 compounds), protein non-nitrogen 24% (4 compounds), metabolite 13% (27 compounds), 10% protein (16 compounds and eight peptides), other 10% (9 compounds), terpenoids and alkaloids 7% (7 compounds), unsaturated fats 2% (8 compounds).

Table 1 shows the value of Pa (Probability activity) above 0.7 on 15 compounds with a relative number of 30.52% of the total hydrolysate of the tilapia viscera. Pa value more than 0.7 indicates that the compounds are very likely to demonstrate activity in the experiment. However, the substance may

have similarities with other known drug agents. A categorical description of biological activity as "active " or "inactive" is used in the PASS program. The nature or character of a biological compound can be predicted using a PASS spectrum activity prediction for substances that can be accessed online. The PASS software can predict more than 300 pharmacological effects and biochemical mechanisms based on the structural formula of a material and can be used efficiently to find new targets or mechanisms for some ligands. Besides, it can also be used to uncover new ligands for some biological targets [24].

Table 2 shows the value of Pa between 0.5 up to 0.7 on 23 compounds with a relative amount of 14.83% of the total hydrolysate of the tilapia viscera. Pa values between 0.5 and 0.7 indicate that the substance tends to show activity, and may not be the same as other known medications. PASS provides predictions for over 4000 types of biological activity with a mean accuracy of 95%, which is much higher than for other web resources that also predict biological activity profiles using the structural formulas of organic compounds [25], in particular, ChemSpider [26], SuperPred [27], and DRAR-CPI [28].

Table 3 shows the value of Pa in between 0.3 to 0.5 in 46 compounds with a relative number of 21.47% of the total hydrolysate of the tilapia viscera. Table 4 shows Pa value less than 0.3 on 18 compounds with a comparable number of 5.6% of the total hydrolysate of the tilapia viscera. PASS allows us to estimate the possible profile of biological activity of organic compounds such as drugs (which the molecular masses range from 50 to 1250 dA) based on structural formulas [29]. These estimates are based on analysis of structure-activity relationships for a broad set of training involving medicinal substances, drugs – candidates in various stages of the clinical and preclinical investigation, pharmaceutical agents and chemical probes, and compounds, specific toxicity information known.

Table 5 shows no Pa value on six compounds with a relative amount of 27.58% of the overall total hydrolysate of tilapia viscera. Not that the mixture has no potential as an anti-inflammatory. It is evidenced in Choline compounds (24.76%) that have activities as an anti-inflammatory. Choline has activities as an anti-inflammatory and antinociceptive in a mouse model of postoperative pain [30]. Based on these results, the development potential of the hydrolysate tilapia viscera waste (*Oreochromis niloticus*) as an anti-inflammatory can be developed further. These results can be used as a basis for testing at a later stage, in vitro, and in vivo.

4. Conclusions

The hydrolysate of tilapia viscera waste (*Oreochromis niloticus*) has the potential of biological activity as an anti-inflammatory based on PASS Server analysis. This can be the basis of subsequent studies in vitro and in vivo.

Acknowledgment

The author thanked the LPPM of Diponegoro University, who had given the research fund. Research fund through RPP 2020 (No. 233-84/UN 7.6.1/PP/2020).

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