SCREENING OF VOLATILE COMPOUNDS IN SHRIMP PASTE AS CANDIDATES FOR ALZHEIMER'S DRUGS THROUGH BIOINFORMATIC ANALYSIS

Isdaryanti*[1], Alexander Kurniawan Sariyanto Putera[1], Susi Indriani[2]
[1]Prodi Pendidikan Biologi Universitas Sulawesi Barat
Jl. Prof. Baharuddin Lopa, Majene, Sulawesi Barat
[2]Prodi Teknologi Produksi Tanaman Pangan ,Politeknik Negeri Pangkep
*email : Isdaryanti@unsulbar.ac.id

Abstrak

Penyakit Alzheimer (AD) merupakan salah satu penyakit yang hingga saat ini metode pengobatan yang tepat belum ditemukan. Paradigma pengobatan telah bergeser kearah pemanfaatan senyawa bioaktif dari berbagai produk makanan fermentasi. Beberapa riset telah membuktikkan bahwa beberapa jenis makanan fermentasi memiliki kandungan senyawa yang dapat mencegah dan mengobati penyakit AD. Akan tetapi, terasi sebagai produk khas Indonesia dan digemari masyarakat serta kaya akan kandungan senyawa bioaktif belum dianalisis potensinya dalam mencegah dan mengobati AD. Oleh karena itu, penelitian ini dilaksanakan untuk mencari jenis senyawa yang dapat menjadi kandidat dalam pengobatan AD. Adapun tahapan penelitian meliputi kajian literatur untuk koleksi data senyawa volatil pada terasi Indonesia. Kedua, kajian virtual untuk skrining senyawa potensial melalui 3 tahapan yaitu tahap analisis aktivitas biologis, prediksi protein target serta analisis toksisitas. Berdasarkan hasil analisis diperoleh sekitar 68 senyawa volatil, 9 senyawa potensial dan hanya 6 senyawa yang memiliki nilai probabiltas aktif >0,3, teruji secara in silico berkorelasi dengan penyakit AD berdasarkan pada situs way2drug. Protein target yang diperoleh yaitu 30 jenis serta berpotensi sebagai target dalam pengobatan AD. Akan tetapi, senyawa golongan pirazina yang diperoleh memiliki aktivitas karsinogenik. Oleh karena itu, diperlukan analisis lebih lanjut baik secara in vitro dan in vivo untuk potensi senyawa volatil terasi sebagai kandidat dalam pengobatan AD.

Kata kunci— *Alzheimer, fermentasi, terasi, bioaktif, in silico*

Abstract

Alzheimer's Disease (AD) currently has no definitive medication. The trend has shifted towards utilizing bioactive compounds found in fermented foods as potential medications for AD. Several studies have identified AD medication and prevention compounds in certain types of fermented foods. However, the potential of shrimp paste, a popular Indonesian food rich in bioactive compounds, in preventing and curing AD has not been studied yet. Therefore, this research aims to identify potential volatile

compounds in shrimp paste that could be candidates for AD medication. The first method involves a systematic review to collect volatile compound data from Indonesian shrimp paste. Subsequently, an in silico approach is used to screen potential compounds through three steps: analyzing biological activity, predicting target proteins, and analyzing toxicity. The results reveal 68 volatile compounds, 9 potential compounds, and only 6 compounds with a probability value greater than 0.3. These compounds are then tested in silico for correlation with AD based on the way2drug website. The analysis identifies 30 potential target proteins for AD medication. However, the pirazina compound is found to have carcinogenic activity, highlighting the need for further in vitro and in vivo analyses to identify potentially volatile compounds that could be candidates for AD medication.

Keywords— *Alzheimer, fermentation, shrimp paste, bioactive compounds, in silico approach.*

1. INTRODUCTION

Alzheimer's Disease (AD) is highly correlated with dementia because it is a major cause of the disease. AD is a degenerative disease that affects the brain and is caused by amyloid proteins that form plaques in neurons. These plaques are toxic and contribute to misfolding in the protein of the neuron cells, leading to damage or even death of the cells [1]. This damage to the neuron cells interferes with cognitive abilities, such as recalling memory, language, and intellectual disorders. Patients may find it difficult to remember names, locations, and activities they did just a short while ago, and they may struggle to communicate with family and others. Patients may also exhibit changes in their behavior, such as being unmanageable and emotional. These disorders can gradually worsen until the patient loses their memory or develops dementia [2].

Currently, there is no known cure for Alzheimer's disease. Only five medications, including rivastigmine, donepezil, galantamine, and memantine, have been approved for use [3]. However, these drugs only reduce the marginal effects and do not address the underlying cause of the disease. Additionally, they can have negative side effects such as brain bleed, swollen brain, and hepatotoxicity [4]. Therefore, there is a shift towards using diet and fermented food as alternative interventions to prevent the formation of abnormal proteins that cause AD. Several studies have shown that consuming fermented food can improve memory [5] and reduce the number of plaques in the neuron cells of AD patients [6].

Given the great potential of locally fermented food as an alternative AD prevention, the exploration of many products made in Indonesia is needed. One such product is shrimp paste, a fermented fish or shrimp-based product with a unique strong scent. Shrimp, the base component, contains a variety of bioactive compounds, including antioxidants, antibacterials, anticancer, anti-inflammation, and anti-hypertension compounds [7]. While many of the functions of these compounds have been proven, the potential of shrimp paste as an alternative food to prevent AD is still unclear. Therefore, this research aims to gain information about the potential compounds in shrimp paste to prevent AD through a virtual approach: in silico. Medication will be tested through computation on various valid and relevant websites. This research will be the first step in early screening to obtain potential compounds, identify protein targets, and assess the safety of the compounds. Hopefully, the data

from this research can be used as a baseline for further in vivo and in vitro approaches. If the three-step process is successful, the volatile compounds inside the shrimp paste could be defined as an alternative medication for AD

2. RESEARCH METHODOLOGY

2.1 Time and Location of Research

This research will be conducted from January to March 2023 and carried out in silico/dry lab .

2. 1.1 Steps of Research

Briefly, the steps of research can be seen in the flow diagram below:

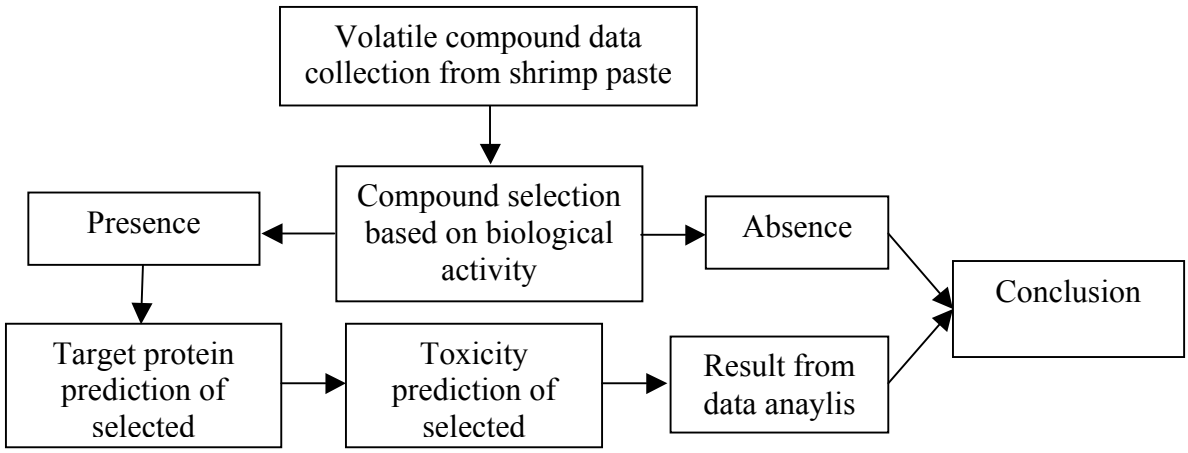

Figure 1 Research Process Scheme

2. 2 Research Procedure

2. 2.1 Volatile Compound Data Collection from Many Shrimp Pastes around Indonesia

The character of research article we use is that article analyzing the compound by utilizing a new and exact identification method, which is Gas Chromatography-Mass Spectrometry (GC-MS). The research articles are available at the following links:
1. http://www.bioflux.com.ro/docs/2020.938-950.pdf [8],
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8874951/[9],
3. https://europepmc.org/article/med/33189071[10],
4. https://pubmed.ncbi.nlm.nih.gov/35804754/[11].

The collected data from the research articles will be saved as a Microsoft Excel format file to be managed and analyzed.

2.2.2 Compound Selection Based on AD Biological Activity

Each compound data collected from the research articles is categorized and prepared to find out the 3D structure and to identify every Canonical SMILES

(Simplified Molecular-Input Line-Entry System) through the PubChem website [12]. The collected data is the part of best match or top rates from all provided data based on the result of the search. The next step is to choose structure column and click on 3D, then click the download icon and choose "save as SDF mode". Information on canonical smiles can be obtained by clicking "Names and Identifiers". Then, choose canonical smiles, copy the displayed data, and move it into Notepad for the next step. Canonical SMILES data can be inputted into the Way2Drugs website [13]. The compound showing activity that is correlated to AD can be saved for the next step of analysis.

2.2.3 Target Protein Prediction in Human for selected compound

Target protein prediction can be conducted through the SuperPred website [14] with menu option of target prediction. Data of target protein obtained by inputted Canonical SMILES in the website and the attribute was set to 'homo sapiens' to predict the targets of compounds based on calculating the Tanimoto similarity. The target of protein data then analyzed manually based on the percentage of similarity.

2.2.4 Analysis of the Toxicity of Collected Compound

Toxicity compound data analysis from the selected volatile compund can be conducted on the ProTox-II-Prediction of Toxicity of Chemicals website [15].

3. RESULTS AND DISCUSSION

3.1 Biological Activity Analysis

According to the literature review, shrimp paste contains essential and non-essential amino acids, unsaturated fatty acids, single-chain and multiple-chain unsaturated fatty acids, and volatile compounds. The volatile compounds in shrimp paste include alcohols, carbonyls, organic acids, esters, hydrocarbons, nitrogen-containing compounds, sulfur-containing compounds, and phenols. Different regions produce different compound profiles, and controlled fermentation using Lactobacillus as a starter has also been studied. Overall, 68 volatile compounds were identified from the literature review using GC-MS and HP-LC. The biological activity analysis identified 9 compounds that correlate with Alzheimer's disease. The details can be seen in Table 1.

Table 1: Potentially volatile compounds from shrimp paste for AD medication.

Compounds Names	Isomeric SMILES	Functional	Pa (Probability activity)	Pi (Probability in activity)
1-Methyl-2-Pyrrolidinone	CN1CCCC1=O	*Alzheimer's disease treatment*	0,293	0,068
Methionol	CSCCCO	*Alzheimer's disease treatment*	0,203	0,146
N-Methyl-2-Pyridone	CN1C=CC=CC1=O	*Alzheimer's disease treatment*	0,325	0,054
		Antiamyloidogenic	0,328	0,066
2-Methylpyrazine	CC1=NC=CN=C1	*Alzheimer's disease treatment*	0,662	0,005

		Antiamyloidogenic	0,532	0,011
2.5-dimethylpyrazine	CC1=CN=C(C=N1)C	*Alzheimer's disease treatment*	0,688	0,005
		Antiamyloidogenic	0,518	0,012
2.6-dimethylpyrazine	CC1=CN=CC(=N1)C	*Alzheimer's disease treatment*	0,635	0,005
		Antiamyloidogenic	0,432	0,023
2.3-dimethylpyrazine	CC1=NC=CN=C1C	*Alzheimer's disease treatment*	0,332	0,051
2.3.5-trymethylpyrazine	CC1=CN=C(C(=N1)C)C	*Alzheimer's disease treatment*	0,612	0,005
Trimethylamine	CN(C)C	*Alzheimer's disease treatment*	0,229	0,117
		Antiamyloidogenic	0,367	0,044

Based on the value of pa and pi, the compounds have not been tested for Alzheimer's drug using either clinical or in vivo approaches. However, 6 compounds, including N-methyl-2-pyridone, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and 2,3,5-trimethylpyrazine, have been tested in silico. These compounds have a positive impact on Alzheimer's patients because pa values greater than 0.7 indicate clinically tested compounds, while pa values greater than 0.3 indicate compounds tested only in silico [16].

The biological activity contains antiamyloidogenic properties that are present in the 5 compounds identified above. These compounds play an important role in inhibiting the formation of amyloid-β fibrils [17]. Inhibiting the formation of beta-amyloid activity can reduce the number of plaques in Alzheimer's patients. Restricting beta-amyloid activity can prevent the formation of plaques in the neurons and can serve as a preventive step in preventing Alzheimer's disease [17]. An et al. [18] found that the antiamyloidogenic compound SPA1413 exhibited strong inhibitor activity against the fibrillation of amyloid-β and oligomerization. Additionally, this compound can inhibit neural inflammation and prevent cytotoxicity. An et al. [18] also revealed that the SPA1413 compound can repair cognitive disorders and activate microglia in the mouse brain. Therefore, in vitro tests of these compounds, including N-methyl-2-pyridone, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and 2,3,5-trimethylpyrazine, from shrimp paste are necessary.

The pyrazine group of compounds has the highest pa value for the treatment of Alzheimer's disease and is also antiamyloidogenic. Pyrazine is an organic compound in the heterocyclic aromatic group that produces a unique aroma in fermented products such as shrimp paste. Additionally, this compound is found in coffee, nuts, tempe, and other products that undergo the Maillard reaction. The functional spectrum of pyrazine group compounds is very wide, such as antibacterial and antioxidant properties [19]. Antioxidants play an important role in neuroprotection by preventing Alzheimer's disease through the binding of free-radical compounds. Pyrazine has the activity to inhibit protein kinase, which can be implemented as an antiproliferative agent and become an inhibitor of β-secretase to cure AD [20]. In vitro, tetramethylpyrazine is known to have the activity to fight free radicals and has the potential to become a neuroprotective agent [21]. However, there is no evidence of in vitro and in vivo tests on the pyrazine compound from shrimp paste to prevent and cure AD.

3.2 Target Protein Analysis

Potential compounds such as N-methyl-2-pyridone, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, and 2,3,5-trimethylpyrazine in the AD medication must be provided with information about the target protein data from humans. This is because the development and utilization of bioactive compounds as medication require knowledge of the target protein. Through the SuperPred website, data on target protein prediction has been collected from six compounds. Here is the elaboration:

Table 2. The Result of Target Protein Prediction from 6 Compounds inside Shrimp Paste by SuperPred

Prediction protein target of N-Methyl-2-Pyridone by SuperPred :	**Prediction protein target of 2-Methylpyrazine by SuperPred :**	**Prediction protein target of 2.5-dimethylpyrazine by SuperPred :**
-Cathepsin D -intermediary factor 1-alpha -Kruppel-like factor 5 -Nuclear factor NF-kappa-B p105subunit	-G-protein coupled bile acid receptor 1 -C-X-C chemokine receptor type 4 -G-protein coupled receptor 6 -Dual specificty protein kinase CLK1 -Vascular endothelial growth factor receptor 1 -CDK2/Cyclin A -Pregnane X receptor	-G-protein coupled bile acid receptor 1 -C-X-C chemokine receptor type 4 -Pregnane X receptor
Prediction Protein target of 2.6-dimethylpyrazine by SuperPred :	**Prediction Protein target of 2.3-dimethylpyrazine by SuperPred :**	**Prediction protein target of 2.3.5-trymethylpyrazine by SuperPred :**
-Dual specificty protein kinase CLK1 -G-Protein coupled bile acid receptor 1 -Monoamine oxidase B -Cyclooxygenase-2	-Pregnane X receptor -G-protein coupled bile acid receptor 1 -Nuclear factor NF-kappa-B p105 subunit -C-X-C chemokine receptor type 4	-G-protein coupled bile acid receptor 1 -Cyclooxygenase-2 -Anandamide amidohydrolase -Monoamine oxidase B -Pregnane X receptor -Dual specificity protein phosphatase 3 -Histone deacetylase 2 -C-X-C chemokine receptor type 4

The target protein prediction data has an accuracy level of 85.3% to 98.88%, which indicates a high level of validation [22]. These proteins are target candidates based on the compounds having biological activity in AD (Table 1), and they contribute both directly and indirectly to the presence of abnormal protein, plaque formation, and cognitive disorder due to neurological damage in other parts of the neural system. These

proteins can be used as targets for the docking process to test compound activity as a medicure candidate through in silico. However, the selection process of the best target protein can be conducted by analyzing the pathway from the disease process. For example, Kruppel-like factor 5 protein is a relevant protein to cognitive function and accelerates AD. This protein can cause apoptosis and inflammation to the neuron and is thus chosen as a target to cure AD [23].

Analyzing the target protein is conducted to investigate the new type of target protein to cure AD. Currently, the AD medication only concerns a protein that inhibits the formation of amyloid beta in the brain [24-25]. However, until now, this method has not been effectively tested to cure AD. Cathepsin D (CatD) is one of the proteins tested clinically as a target in AD therapy. CatD maintains the balance inside neurons in the brain and degrades amyloid beta, which causes AD. This candidate can be explored to understand its role in AD and can be tested in silico, in vitro, and in vivo.

3.3 Toxicity Analysis

Toxicity analysis is necessary to understand the impact of the compound on the human body. The compound can be toxic or drug-affected based on the consumption rate. Based on the results of the analysis on the Swiss Adme website, the collected data are shown below:

Table 3. The Result of Toxicity Analysis based on the Six Compound on the Human

Compunds Name	Prediction accuracy	Predicted LD50	Predicted Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
N-Methyl-2-Pyridone	68,07%	1200mg/kg	4	Inaction	inactive	inactive	inactive	inactive
2-Methylpyrazine	100%	1800mg/kg	4	Inaction	active	inactive	inactive	Inactive
2.5-dimethylpyrazine	100%	1020mg/kg	4	Inactive	active	inactive	inactive	inactive
2.6-dimethylpyrazine	100%	880mg/kg	4	Inactive	active	inactive	inactive	inactive
2.3-dimethylpyrazine	100%	613mg/kg	4	Inactive	active	inactive	inactive	inactive
2.3.5-trymethylpyrazine	100%	806mg/kg	4	Inaction	active	inactive	inactive	inactive

The table above shows that 2-Methylpyrazine, 2.5-dimethylpyrazine, 2.6-dimethylpyrazine, 2.3-dimethylpyrazine, and 2.3.5-trimethylpyrazine display carcinogenic activity with 100% accuracy. Pyrazine compound is a natural product from several products and compounds caused by heating activity. This material is used as a seasoning in various products. Based on the safety review, this compound will not

display any health problems to the consumer if consumed at the lowest suggested dose [26]. As a pure compound, the six compounds are irritants and are categorized into four classes based on the results above. The compounds categorized into four classes show that the compound is dangerous if swallowed. However, the impact of the compound in different conditions can give different results. Therefore, it is important to analyze the potential shrimp paste compounds containing high pyrazine for the possibility of carcinogenic effects and other impacts on the body.

4. CONCLUSION

Based on the results above, there are six volatile compounds in shrimp paste that can potentially be used in the process of AD medication, according to biological activity analysis and target protein prediction. This potential is still low enough because the pa value displays >0.3, so tests are urgently needed in the future through compound interaction analysis and target protein, followed by tests in vitro and in vivo. Additionally, the results of the toxicity analysis show the presence of compound capability in displaying cancer. Therefore, it is necessary to analyze the pure result from the shrimp paste and solid formation as the shrimp paste.

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