The Compounds of Tuna-Shredded (*Thunnus* sp.) Fortified Banana Blossom Extracts' Antioxidant Activity and Xanthine Oxidase Enzyme Inhibition Capacity: An *In Vitro-In Silico* Study

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Abstract: *Tuna is one of the fish source of nutrition for humans because it contains high-quality protein and omega-3 fatty acids, which are beneficial for health. Tuna can be processed into various products, such as tuna-shredded. But it still has a drawback, i.e., the lower-fiber content. To enrich the fiber of tuna-shredded, fortification with banana blossoms can be developed as functional food such as preventing gout arthritis. The aims of this study were to develop a diversified product of tuna-shredded fortified banana blossoms and to determine the antioxidant activity* in vitro *and anti-arthritis gout through inhibition of the xanthine oxidase (XO) enzyme* in silico. *The method used was a simple, completely randomized design. The formulation of tuna-shredded used fortification and active compounds analyzed by LC-HRMS. The antioxidant activity was analyzed by the DPPH. Inhibition of the XO enzyme was analyzed by molecular docking* in silico. *The results showed that tuna-shredded extract contained 32 compounds, which had total phenolic was 0.00134 mg GAE/g, total flavonoid was 0.0006670 mg QE/g, and* IC_{50} *was 4.38 ppm. Ferulic acid had the potential to inhibit the XO enzyme with binding affinity was -9.70 kcal/mol through hydrogen bonds and hydrophobic interactions.*

Keywords: *anti-arthritis gout; antioxidant;* in silico; in vitro; *tuna-shredded*

■ INTRODUCTION

Gout is a degenerative disease with a prevalence that increases with age. Gout is caused by the absence of uric acid in the joints, causing inflammation (causing pain). The presence and formation of monosodium urate (MSU) crystals in the joints cause severe pain. Hyperuricemia can be caused by impaired renal excretion or excess production of uric acid due to excessive production of foods high in purine content [1]. Patients with gout are at risk for chronic disease, cardiovascular disease, metabolic disorders, and psychosis [2].

During purine metabolism, hypoxanthine and xanthine are produced and then metabolized in the liver to uric acid. The reaction is catalyzed by the xanthine oxidase (XO) enzyme [3]. Humans lack uricase, an enzyme that degrades uric acid to soluble allantoin; therefore, uric acid is not degraded, leading to the accumulation of insoluble uric acid crystals in joints, bones, and many other organs such as the kidneys [4]. According to Kostalova et al. [3], XO is a key enzyme in the pathogenesis of arthritis gout, and its inhibition is very important in the management of this pathological condition.

Nonsteroidal anti-inflammatory drugs (NSAIDs), as well as systemic and intra-articular corticosteroids, are used to treat acute gout. In patients with gout who could not tolerate NSAIDs or systemic corticosteroids, oral colchicine is generally the treatment of choice. However, these various treatments have side effects, such as the occurrence of gastrointestinal and liver toxicity. Various risks of degenerative diseases require increased knowledge for each individual so that they can recognize early and be aware of the health problems they are experiencing, especially the diet and nutritional content of the food consumed [5].

Tuna (*Thunnus* sp.) meat contains a high nutritional value, i.e., protein and fat of 28.34% and 0.51%, respectively [6]. Tuna also contains omega-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid [7], minerals and vitamins such as calcium, phosphorus, iron, sodium, vitamin A (retinol), and vitamin B (thiamin, riboflavin, and niacin) [8]. For the availability of food made from tuna, it can be processed into various processed products, one of which is tuna-shredded. Tuna-shredded is tuna meat that is chopped and dried with the addition of certain spices. This type of fish processing is one of the businesses of processing fish products. When compared with other traditional forms of processing, tuna-shredded has a relatively long shelf-life, which is still acceptable in storage for 50 d at room temperature [9]. As a processed fish product, tuna-shredded can be used as a side dish for consumers with degenerative disease conditions, including arthritis and gout. Polyunsaturated fatty acids and omega-3 contained in tuna meat can reduce the risk of arthritis gout. However, shredded fish still has a drawback, i.e., low fiber content. Where fiber can help fill the stomach and prevent obesity, obesity is one of the triggers for arthritis gout and other joint diseases [10].

Currently, dietary fiber is being studied comprehensively to understand its role in the prevention of degenerative diseases, such as arthritis gout, heart disease, obesity, diabetes, cancer, and others. The consumption of fiber-rich foods is rich in antioxidants and other nutrients that protect against cell damage and reduce inflammation throughout the body, including joints [11]. Dietary fiber addition by fortification has several usages both from technological and nutraceutical perspectives. Dietary fiber is a necessary component of the human diet [12]. Fortification, especially dietary fiber fortification, may increase the proximate as well as the nutritional content of food products [13-14]. One of the ingredients that can be used as a source of fiber is banana (*Musa paradisiaca*) blossoms. The banana blossoms have great nutritional value and health benefits. Many *in vitro* studies have used parts of the banana plant, including banana blossoms, as other drugs in surgical dressings, pain relief, food and medicine, nanotechnology, induction of apoptosis, and cell cycles. The banana blossoms are also a good source of calories because it is rich in fiber and a good source of vitamin C [15]. Thus, the aims of this study were to develop a diversified product of tuna (*Thunnus* sp.) shredded fortified banana blossoms as a functional food and to determine the antioxidant activity *in vitro* and also anti-arthritis gout through inhibition of XO enzyme through molecular docking *in silico*.

■ EXPERIMENTAL SECTION

Materials

The raw materials used for producing tuna-shredded fortified with banana blossom, such as tuna (*Thunnus* sp.), banana blossom, and spices. The tuna used was obtained from the Probolinggo Regency, East Java Province and had the characteristics of clear eyes, bright red gills, a little slimy, the texture of the flesh is dense and elastic when touched with a finger, and measuring less than 50 cm long and about 2 kg. The banana blossom used is the type of kepok banana obtained from Bunul Market and Muharto Market, Malang City, East Java Province. The seasonings used include garlic, shallots, coriander powder, sugar, table salt, turmeric, galangal, bay leaf, lemongrass, lime leaves, coconut milk, and cooking oil. The materials for parameter testing include quercetin (Sigma-Aldrich), gallic acid (Sigma-Aldrich), Folin-Ciocalteu (Sigma-Aldrich), sodium carbonate (Sigma-Aldrich), sodium nitrite (Sigma-Aldrich), aluminum chloride (Sigma-Aldrich), acetic acid (Sigma-Aldrich), chloroform

(Sigma-Aldrich), potassium Iodide (Sigma-Aldrich), aquades, sodium thiosulfate (Sigma-Aldrich), tapioca flour (Cap Swan), filter paper Whatman No. 1 (Sigma-Aldrich), tissue, label paper, aluminum foil (Klin Pak 8 m × 30 cm) and plastic wrap (Cling Wrap 30 m × 30 cm). Materials for *in silico* analysis were XO P80457 (XDH_BOVIN, code: 1FIQ) and its resolution 2.5 Å obtained from Protein Data Bank https://www.rcsb.org/, allopurinol (ligand control) and active compounds of tuna-shredded fortified banana blossoms extract obtained from PubChem https://pubchem.ncbi.nlm.nih.gov/).

Instrumentation

The instrumentation used for LC-HRMS analysis consists of LC Alliance brand LC equipment 2996 (waters) with photodiode-array detector (PDA) 2996 (Waters) and MS type XEVOG2QTOF (Waters) equipment. LC-HRMS analysis using HPLC Thermo Scientific Dionex Ultimate 3000 RSLCnano with microflow meter. UV–Visible Spectrophotometer (Shimadzu UV-1601PC, Japan). The instrumentation used for *in silico* process is Acer TravelMate P633-M Laptop, Intel(R) Core™ i5-3230M CPU@2.60 GHz (4 CPUs), 4096 MB RAM Memory, Open Babel GUI, PyMOL, Discovery Studio Visualizer, and PyRx software.

Procedure

The process of making tuna-shredded fortified banana blossom

The process of making tuna-shredded refers to the method of Hardoko et al. [16]. Making tuna-shredded consists of 3 steps, i.e., making crushed banana blossom, preparing fish meat, and making seasonings. The banana blossom mash is made by washing and cleaning the banana blossom, removing the outer skin to leave a yellowish white inside, steaming for 15 min, and then blending it. The crushed banana blossom is squeezed using a filter cloth to reduce the water content so that the crushed banana blossom juice is ready to be used as an additional ingredient in the manufacture of shredded tuna. While the process of making banana blossom mash, tuna fish are weeded by removing the contents of the stomach, fins, head, and gills, and washed with running water. Clean tuna is steamed for 30 min, cooled, and separated from the meat. The tuna meat is then shredded using a grater. While the shredding process is carried out, the seasoning is also prepared by weighing the required spices according to the tuna-shredded formulation (Table 1), mixing and mashing the spices with a blender so that a smooth seasoning is obtained. The spices are then mixed with coconut milk, grated fish meat, and crushed banana heart, then boiled until boiling or cooked. Furthermore, the stew of the shredded ingredients is filtered with a filter cloth and squeezed to obtain the residue of the shredded material. The remaining shredded juice is fried for about 5 min or until light brown. Abon, which has been light brown in color, is removed, and the oil is drained using a spinner in order to obtain a dry floss that is ready to be packaged and analyzed for its compounds.

Extraction of tuna-shredded and active compound analysis

The extraction process for tuna-shredded is carried out using the stratified maceration method. The maceration process begins with weighing the shredded 50 g and adding *n*-hexane solvent in a ratio of 1:5, and macerating at room temperature for 24 h. The maceration results were filtered with filter paper, and the residue was macerated at room temperature for 24 h with ethyl acetate solvent in a ratio of 1:5. The result of

Table 1. Formulation of tuna-shredded fortified banana blossom

Ingredients	Number
Tuna (g)	250
Banana Blossom (g)	175
Garlic (g)	11
Onion (g)	21
Coriander powder (g)	4
Sugar (g)	21
Salt (g)	16
Turmeric (g)	10
Galangal (g)	25
Bay leaves (g)	3
Lemongrass (g)	19
Lime leaves (g)	3
Coconut milk (mL)	25

maceration with ethyl acetate was filtered and the residue was macerated at room temperature for 24 h with ethanol solvent in a ratio of 1:5. Furthermore, the results of maceration with methanol were filtered through Whatman filter paper No.1 and the filtrate was taken to be evaporated at a temperature of 50 °C at a speed of 100 rpm until the solvent evaporated completely and a methanol extract of tuna-shredded was obtained for compound analysis using LC-HRMS. The mobile phase used was A: Water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid. The column used is Hypersil GOLD aQ 50 × 1 mm × 1.9 particle size withflow rate 40 L/min. The mass spectrometry used was Thermo Scientific Q Exactive with a full scan at a resolution of 70,000, an analysis time of 30 min with positive and negative ion modes.

Analysis of total phenolic content

Total phenolic contents (TP) of the tuna-shredded fortified banana blossoms extract were determined using Folin-Ciocalteu (FC) assay. A 40 µL of properly diluted tuna-shredded fortified banana blossoms extract solution was mixed with 1.8 mL of FC reagent. The reagent was pre-diluted 10 times with distilled water. After standing for 5 min at room temperature, 1.2 mL of (7.5% w/v) sodium carbonate solutions were added. The solutions were mixed and incubated for 1 h at room temperature. Then, the absorbance was measured at 765 nm using a UV-Vis. A calibration curve was prepared using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L, $R^2 = 0.997$). The total phenolic content was indicated as mg gallic acid equivalent per 1 g of extract weight (mg GAE/g) [17].

Analysis of total flavonoid content

The total flavonoid content (TF) of the tuna-shredded fortified banana blossoms was determined according to the colorimetric assay. A 1 mL of properly diluted tuna-shredded fortified banana blossoms extract was mixed with 4 mL of distilled water. At zero-time, 0.3 mL of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 mL of (10% w/v) $AlCl_3$ was added. At 6 min, 2 mL of 1 M solution of sodium hydroxide was added. After that, the volume was made up to 10 mL immediately by the addition of 2.4 mL of distilled water. The mixture was shaken vigorously, and the absorbance of the mixture was read at 510 nm. A calibration curve was prepared using a standard solution of quercetin (20, 40, 60, 80, and 100 mg/L, $R^2 = 0.996$). The total flavonoid content was as mg equivalent of quercetin per 1 g of extract weight (mg QE/g) [17].

Analysis of antioxidant activity

The antioxidant activity of the tuna-shredded fortified banana blossoms extract was also studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. An aliquot (10 µL) of tuna-shredded fortified banana blossoms extract was mixed with 90 µL of distilled water and 3.9 mL of 25 mM DPPH methanolic solution. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was measured later, at 515 nm, against a blank of methanol without DPPH. Results were expressed as a percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the Eq. (1):

$$\%\ \text{Inhibition of DPPH} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \qquad (1)$$

$\text{Abs}_{\text{control}}$ is the absorbance of DPPH solution without extracts [18].

Analysis of pharmacokinetic (drug-likeness)

The drug-likeness analysis of the test compound/ligand is aimed at finding out whether the test compound/ligand complies with Lipinski's rule. The analysis was carried out online through the page http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp [19-21]. This stage is carried out before molecular docking *in silico* analysis.

Molecular docking

Molecular docking of the active compound obtained from the LC-MS results of tuna-shredded fortified banana blossoms with XO P80457 (XDH_BOVIN, code: 1FIQ) and its resolution 2.5 Å as a receptor using PyRx software. The XO enzyme as a receptor was obtained from https://www.rcsb.org. The test ligands used were obtained from the active compound content of tuna-shredded fortified banana

blossom and control ligands in the form of allopurinol. The 3D structure of the ligand was obtained from PubChem https://pubchem.ncbi.nlm.nih.go in sdf format. Then, it is converted into pdb format using the Discovery Studio software. Receptor preparation was carried out by separating the C chain structure from the intact structure, then saving it in *.pdbqt format. After being stored, the water molecule was removed, and the natural ligand was separated from the enzyme chain C structure. The file was then saved in *.pdbqt format. The molecular docking process is carried out in the PyRx software, and the type of docking is blinded docking. After docking is complete, the results of several docking modes along with the value of binding affinity (kcal/mol), are obtained. Next, visualize the docking results in 2D and 3D using Discovery Studio software.

Analysis of toxicity and bioavailability

The test ligands with the best binding affinity were tested for toxicity predictions one by one using an online toxicity test program accessed http://tox.charite.de/protox_II/ [22]. Ligands that showed non-toxic results were tested for bioavailability (ADME) using a program that can be accessed at http://www.swissadme.ch/ [23].

Analysis of statistical

Data were analyzed using SPSS software. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences. The difference was considered significant at a p-value < 0.05.

■ RESULTS AND DISCUSSION

The results of compound analysis using LC-HRMS from ethanol extract of tuna-shredded fortified with banana blossom identified 32 compounds divided into groups of phytochemical compounds (steroids, alkaloids, flavonoids, terpenoids, and phenolics), organic compounds, fatty acids, and amino acids. The detail of the compounds identified can be seen in Table 2.

Table 2. Identified compounds in tuna-shredded fortified banana blossom extracts

No.	Retention time (min.)	Molecule weight (g/mol)	Formula	Compound	Compound Group
1	2.409	436.22734	$C_{24}H_{33}FO_6$	Flurandrenolide	Steroids
2	2.415	143.09410	$C_7H_{13}NO_2$	DL-Stachydrine	Alkaloids
3	8.702	464.09450	$C_{21}H_{20}O_{12}$	Quercetin-3β-D-glucoside	Flavonoids
4	13.51	286.08324	$C_{16}H_{14}O_5$	Sakuranetin	Flavonoids
5	14.692	368.12517	$C_{21}H_{20}O_6$	Curcumin	Flavonoids
6	17.016	218.16640	$C_{15}H_{22}O$	Nootkatone	Sesquiterpenoid
7	17.372	216.15096	$C_{15}H_{20}O$	(+)-ar-Turmerone	Sesquiterpenoid
8	20.483	120.05732	C_8H_8O	Acetophenone	Organic compound
9	7614	194.05744	$C_{10}H_{10}O_4$	Ferulic acid	Phenolic
10	6.949	132.05712	C_9H_8O	trans-Cinnamaldehyde	Organic compound
11	2.422	145.10976	$C_7H_{15}NO_2$	Acetylcholine	Organic compound
12	2.826	109.06384	$C_5H_7N_3$	2-Amino-4-methyl pyrimidine	Organic compound
13	3.391	129.07855	$C_6H_{11}NO_2$	L-Pipecolinic acid	Organic compound
14	3.554	136.03800	$C_5H_4N_4O$	Hypoxanthine	Organic compound
15	3.559	109.05264	C_6H_7NO	4-Aminophenol	Phenolic
16	3.561	267.09593	$C_{10}H_{13}N_5O_4$	Adenosine	Organic compound
17	8.54	195.19820	$C_{13}H_{25}N$	*N*-Cyclohexyl-*N*-methylcyclohexanamine	Organic compound
18	22.612	390.27559	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl)-phthalate	Organic compound
19	11.019	255.16155	$C_{17}H_{21}NO$	Diphenhydramine	Antihistamine
20	15.057	250.15611	$C_{15}H_{22}O_3$	3,5-di-tert-Butyl-4-hydroxybenzoic acid	Phenolic
21	2.44	228.14672	$C_{11}H_{20}N_2O_3$	Prolylleucine	Amino acid
22	2.248	203.11516	$C_9H_{17}NO_4$	Acetyl-L-carnitine	Amino acid

23	2.743	117.07870	$C_5H_{11}NO_2$	Valine	Amino acid
24	2.787	113.05866	$C_4H_7N_3O$	Creatinine	Amino acid
25	2.835	155.06894	$C_6H_9N_3O_2$	L-Histidine	Amino acid
26	2.898	174.11104	$C_6H_{14}N_4O_2$	DL-Arginine	Amino acid
27	3.56	204.08933	$C_{11}H_{12}N_2O_2$	DL-Tryptophan	Amino acid
28	8.469	197.11992	$C_{14}H_{15}N$	Dibenzylamine	Aromatic amine
29	21.613	281.27083	$C_{18}H_{35}NO$	Oleamide	Fatty acid
30	21.826	255.25545	$C_{16}H_{33}NO$	Hexadecanamide	Fatty acid
31	22.386	283.28658	$C_{18}H_{37}NO$	Stearamide	Fatty acid
32	25.762	337.33315	$C_{22}H_{43}NO$	Erucamide	Fatty acid

Total Phenolic Content

Determination of the total phenolic content of extract tuna-shredded was performed using FC assay and analyzed by UV-Vis spectrophotometer at 765 nm. Phenolic contents react with the FC reagent and form complex blue compounds. The standard solution used was gallic acid, a phenolic compound derived from hydroxybenzoic acid. The total phenolic contents of tuna shredded extract are shown in Table 3.

The total phenolic content of tuna-shredded fortified banana blossoms extract was 0.00286 mg GAE/g. Each g of the extract was equivalent to 0.00286 mg of gallic acid—the presence of spices used in the processing of tuna-shredded act as a source of phenolic compounds. Sukisman et al. [24] reported that the total phenolic content in the spices for making shredded meat was 82.49 ± 0.19 mg GAE/g. Phenolic compounds contained in plants act as provide antioxidant activity to chelate redox-active metal ions and lipid-free radical chains and block the conversion of hydroperoxides to reactive oxyradicals [25]. The presence of total phenolic contributes to antioxidant activity. As their free radical scavenging ability is induced by the hydroxyl (OH) group, thus the total phenolic content could be used as a basis for rapid screening of antioxidant activity.

Total Flavonoid Content

The tuna-shredded extract's total flavonoid content was analyzed by the complex formation between $AlCl_3$ with the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom from the flavone and flavanol groups. Quercetin was used as a standard for analyzing flavonoids' content since quercetin was a flavanol group with a keto group on the C-4 atom and the OH group on the C-3 and C-5 atoms. The results of total phenolic contents are shown in Table 4.

The total flavonoid content of tuna-shredded extract as mg equivalent per g of dry tuna-shredded was 0.0006670 mg QE/g (Table 4). This results in sync with the total phenolic content obtained. Another research has shown that phenolic content also contains amounts of flavonoid content [25]. Herbs, as spices ingredients in this research, were a major source of minerals, vitamins,

Table 3. Total phenolic content of extracts banana blossoms and tuna-shredded fortified banana blossoms extracts

No.	Sample	Total phenolic content (mg GAE/g)
1	Extract Banana Blossoms	0.00286
2	Extract Tuna-Shredded Fortified Banana Blossoms	0.00134

Table 4. Total flavonoid content of extracts banana blossoms and tuna-shredded fortified banana blossoms extracts

No.	Sample	Total flavonoid content (mg QE/g)
1	Extract Banana Blossoms	0.0006672
2	Extract Tuna-Shredded Fortified Banana Blossoms	0.0006670

and other essential nutrients. Most medicinal plants, including herbs, contain phenolic acids, flavonoids, and other phytochemicals. Flavonoids such as flavone, flavanol and condensed tannins are plant secondary metabolite compounds, the antioxidant activity of which depends on the presence of free OH group, especially 3-OH [26].

Antioxidant Activity

The antioxidant compound in a food product can inhibit or prevent the oxidation of lipids. Compounds that can be easily oxidized will cause food damage, causing rancidity. One of the parameters that can describe the percentage of a food ingredient's ability to inhibit free radical is by determining the antioxidant activity. The antioxidant activity of tuna-shredded fortified banana blossoms was carried out using the DPPH method. Determination of antioxidant activity using the DPPH method shows the ability of antioxidant substances to neutralize free radicals *in vitro*.

The antioxidant activity of tuna-shredded fortified banana blossoms extract shown in Table 5. The antioxidant activity of tuna-shredded fortified banana blossoms extract was 4.38 ppm, while the banana blossoms extract 3.06 ppm, and also ascorbic acid as standard was 0.61 ppm. Based on the results of the IC_{50} value showed the ability of the tuna-shredded extract to have a very strong antioxidant category. Compounds that have biological activity as antioxidants also have the potential for other biological activities [27]. The difference in antioxidant activity in the three samples is thought to be caused by a decrease in the antioxidant compound content in shredded. The decreasing antioxidant activity after processing tuna-shredded fortified banana blossoms is caused by a deep-fried or heating process. During the processing process, the ingredients for making tuna-shredded go through two heating processes. The first heating is the process of boiling the banana blossoms-tuna meat, and the second heating is the process of frying the tuna-shredded dough using hot oil until cooked.

Cooking tuna-shredded using hot oil is done using the deep-frying method [13]. During the heating process, the antioxidant compounds in the banana blossoms and herbs as spices, i.e., phenol and flavonoid compounds, will be destroyed. Research shows that the antioxidant activity of raw vegetables decreases when the vegetables are sautéed [28].

Pharmacokinetic Drug-Likeness Lipinski's Rule

Before docking the ligand to the receptor/enzyme XO, each ligand of the active compound of tuna-shredded fortified banana blossom was analyzed according to Lipinski's rule. The Lipinski rule states that a ligand can be continued for the docking process if (1) the mass is less than 500 g/mol, (2) the Log P value is less than 5, (3) the number of hydrogen bond donors is less than 5, and (4) hydrogen bond acceptor is less than 10 [19]. The suitability of the test ligand characteristics with the parameters of Lipinski's rule is a requirement that must be met before molecular docking is carried out. Lipinski's rule can be used in the physicochemical properties of a ligand that can cross cell membranes in the body. The results of the analysis of the suitability of the test ligand characteristics with Lipinski's rule parameters are presented in Table 6.

Ligands with a molecular weight of less than 500 Da more easily penetrate cell membranes than ligands with a molecular weight is more than 500 Da. The log P value is related to the polarity of the ligand in fat, oil, and non-polar solvents. Ligands with a log P value is more than 5 will interact more quickly through

Table 5. Antioxidant activity of extracts banana blossoms and tuna-shredded fortified banana blossoms extracts

No.	Sample	Antioxidant activity (IC_{50}) (ppm)
1	Extract Banana Blossoms	3.06
2	Extract Tuna-Shredded Fortified Banana Blossoms	4.38
3	Ascorbic Acid (Vitamin C)	0.61

Table 6. Ligand parameters to comply with Lipinski's rules

No.	Ligand	Mass (g/mol)	Log P	H Bond Donor	H Bond Acceptor
1	Flurandrenolide	436.000000	2.498700	2	6
2	DL-Stachydrine	143.000000	1.024900	0	2
3	Quercetin-3β-D-glucoside	463.000000	1.143710	4	8
4	Sakuranetin	274.000000	0.318340	2	5
5	Curcumin	350.000000	0.439250	2	6
6	Nootkatone	218.000000	3.904199	0	1
7	(+)-ar-Turmerone	196.000000	0.682600	0	1
8	Acetophenone	120.000000	1.889200	0	1
9	Ferulic acid	194.000000	1.498600	2	4
10	trans-Cinnamaldehyde	132.000000	1.898700	0	1
11	Acetylcholine	146.000000	0.255700	0	2
12	2-Amino-4-methyl pyrimidine	109.000000	0.367220	2	3
13	L-Pipecolinic acid	129.000000	0.213100	2	3
14	Hypoxanthine	136.000000	0.187100	2	4
15	4-Aminophenol	109.000000	0.974400	3	2
16	Adenosine	267.000000	1.980000	4	8
17	*N*-Cyclohexyl-*N*-methylcyclohexanamine	195.000000	3.583599	0	1
18	Bis-(2-ethylhexyl) phthalate	388.000000	3.698699	0	4
19	Diphenhydramine	255.000000	3.354199	0	2
20	3,5-di-*tert*-Butyl-4-hydroxybenzoic acid	250.000000	3.685399	2	3
21	Prolylleucine	362.000000	2.403100	2	7
22	Acetyl-L-carnitine	203.000000	1.235701	0	4
23	Valine	117.000000	0.054300	3	3
24	Creatinine	113.000000	1.226900	2	4
25	L-Histidine	155.000000	0.635900	4	4
26	DL-Arginine	174.000000	1.548100	4	6
27	DL-Tryptophan	204.000000	1.122300	4	3
28	Dibenzylamine	197.000000	2.976400	1	1
29	Oleamide	281.000000	3.509200	2	2
30	Hexadecanamide	255.000000	3.952999	2	2
31	Stearamide	283.000000	3.062230	2	1
32	Erucamide	337.000000	4.083392	2	1

the lipid bilayer of cell membranes and are widely distributed in the body. It can affect the sensitivity of the ligand binding to the target molecule will decrease, and the ligand toxicity to increase. The smaller the log P value, the more ligands tend to be water soluble and hydrophobic [29]. The log P value of the ligand must not be a negative value because it cannot pass through the lipid bilayer membrane. Then, the number of hydrogen bonds in the donor and acceptor correlates with the biological activity of the ligand or drug [30].

Molecular Docking Active Compound of Tuna-Shredded Fortified Banana Blossom Extract

Various active compounds from natural ingredients have multifunction for health, one of which is as anti-arthritis gout. The initial method to determine the potential of the active compound as an anti-arthritis gout is by *in silico* molecular docking method, namely the binding of the active compound as a test ligand to the target anti-arthritis gout receptor XO enzyme. Prior to molecular docking, the docking method validation must

first be carried out. The validation of the molecular docking method was carried out with the aim of re-docking the ligand on the active site of the XO enzyme by selecting a conformation similar to the natural ligand conformation that was known through re-docking.

At this stage, grid box measurements are carried out to determine the area where the test and control ligands will attach. The grid box obtained shows that the two types of ligands will attach to the center position X 27.5292, Y 17.1729, and Z 107.1436 and the dimensions (Å) X 89.9546, Y 69.5777, and Z 70.1696. The docking process is carried out in 10 repetitions, and then the docking results are selected by looking at the conformation that is most similar to the native ligand. The overall ligand conformation that has been selected is then calculated as the Root Mean Square Deviation (RMSD) value using the Discovery Studio application. The results of molecular docking that show good performance have an RMSD value of less than 2 Å. The RMSD value resulting from the validation of re-tethering between the receptor and the natural ligand showed the lowest value of 0 Å and the highest value of 1.3521. This molecular docking validation is said to be valid or appropriate since the average RMSD value obtained is less than 2 Å [31].

The results of the molecular docking of the active compound of tuna-shredded fortified with banana blossom extract can be seen in Table 7. Based on the binding affinity score (Table 7), it can be seen that the *in silico* analysis of the activity of the tuna-shredded fortified with banana blossom extract to the XO enzyme showed that the more stable ligand to the XO enzyme is ferulic acid (a phenolic compound) with binding affinity score -9.70 kcal/mol and trans-cinnamaldehyde (an organic compound) with binding affinity score -9.20 kcal/mol. The results of method validation obtained an RMSD score of less than 2 Å. These results show that the calculation of the docking between protein and ligand gives results that are almost similar to the position of the native ligand since it has an RMSD score of less than 2 Å. In addition, the data presented in Table 7 only test ligands that have binding affinity scores lower than allopurinol as the control ligand (-5.90 kcal/mol). In contrast, test ligands with binding affinity scores greater than control ligands allopurinol are not shown. Since these ligands have a very low affinity for binding to the XO enzyme. Toppo et al. [32] explained that the lower the binding affinity score of the interaction of the ligand-receptor complex, the stronger the potential to bind and the higher the inhibitory ability of the ligand to the target enzyme.

Based on the binding affinity score of allopurinol as ligand control (-5.90 kcal/mol), the compound that can bind strongly to the receptor and have the potential as anti-arthritis gout is a compound that has a lower binding affinity score than allopurinol. The compound that has the lowest binding affinity score to the control and has the potential to bind to the XO enzyme is ferulic acid (-9.70 kcal/mol). This indicates that the ferulic acid compound has the potential to anti-arthritis gout. This is also supported by data that tuna-shredded fortified with banana blossoms has antioxidant capacity by DPPH assay. A compound that has antioxidant capacity also has the potential to have other biological activities [24]. This is confirmed by Nile et al. [33] reported that the ferulic acid compound had an inhibitory activity of more than 50% at 100 µg/mL against the XO enzyme and also against the cyclooxygenase-2 (COX-2) enzyme. Wang et al. [34] also reported that the ferulic acid compound had an IC_{50} value to the XO enzyme 1.35×10^{-5} M, and this IC_{50} value was higher than the IC_{50}

Table 7. Binding affinity score ligands of tuna-shredded fortified with banana blossom active compounds against xanthine oxidase enzyme

No.	Ligand	Enzyme/Receptor	Binding affinity (kcal/mol)
1	Ferulic Acid	Xanthine Oxidase	-9.70
2	Trans-cinnamaldehyde		-9.20
3	Diphenhydramine		-7.00
4	Allopurinol (Positive Control)		-5.90

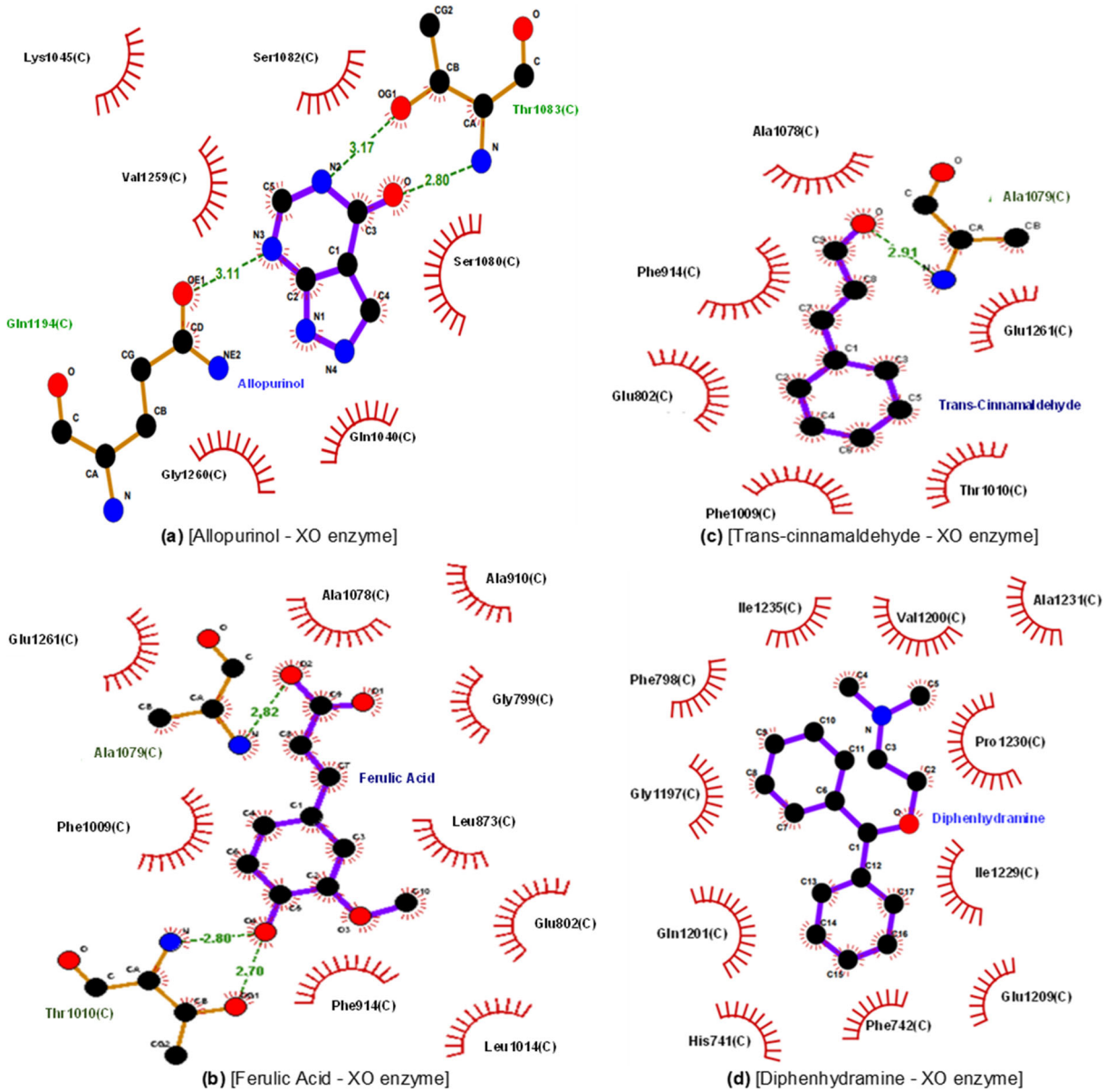

Fig 1. The 2D visualization of the interaction of the ligand allopurinol, ferulic-acid, Trans-cinnamaldehyde, and Diphenhydramine with XO enzyme as receptor

value of allopurinol as a positive control. The more OH group in the ferulic acid compound, the stronger its activity against XO enzyme inhibition.

The 2D Visualization

The lower binding affinity score of the control or higher inhibitory power of the drug to the XO enzyme becomes clearer through a 2D visualization. The visualization of the interaction between each ligand and with XO enzyme is shown in Fig. 1.

It can be seen that there are differences in the types and amino acid residues of the receptor that bind to the ligand, the type of bond formed between the amino acid residue of the receptor and the ligand and the distance between the amino acid residues and the ligand (Fig. 1). The interaction of these things determines the size of the binding affinity score of the ligand-receptor complex. According to Patrick [35], the strength of ionic bonds and hydrogen bonds, and hydrophobic interactions is determined by the difference in electronegativity of a

Table 8. The interaction type of each ligand complex of the active compound of tuna-shredded fortified banana blossom extract against the XO enzyme

[Complex ligand-receptor]	Type of interaction	Amino acids residues
[Ferulic Acid-XO enzyme]	Hydrogen bond	Ala1079, Thr1010
	Hydrophobic	Gly799, Ala910, Ala1078, Glu1261, Phe1009, Phe914, Leu1014, Glu802, Leu873
[Diphenhydramine-XO enzyme]	Hydrophobic	Phe798, Gly1197, Gln1201, His741, Phe742, Glu1209, Ile1229, Pro1230, Ala1231, Val1200, Ile1235
[Trans-Cinnamaldehyde-XO enzyme]	Hydrogen bond	Ala1079
	Hydrophobic	Ala1078, Phe914, Glu802, Phe1009. Thr1010, Glu1261
[Allopurinol-XO enzyme]	Hydrogen bond	Thr1083, Gln1194
	Hydrophobic	Lys1045, Ser1082, Val1259, Gln1040, Ser1080, Gly1260

compound. The greater the difference in electronegativity of a compound, the greater will be ionic bonds or hydrogen bonds or hydrophobic interactions. The shorter the bond distance, the stronger the bond [36].

The differences in the type and number of amino acid residues bound to allopurinol ligand as control positive ligand, as well as ligands from the active compound of tuna-shredded fortified banana blossoms, extract indicated differences in the mechanism of inhibition of each ligand on the XO enzyme as the target protein. The interactive form of each ligand complex of the active compound of tuna-shredded fortified banana blossoms extract against the XO enzyme can be seen in Table 8. *In silico* method used, the amino acid residues are amino acid residues on the active site of the XO enzyme, so the mechanism of inhibition of compounds from tuna shredded fortified banana blossoms extract is a competitive inhibition mechanism [37].

Toxicity and Bioavailability

The results of the pharmacokinetic and toxicity analysis of the potential ligands that have the lowest binding affinity score with the XO enzyme through the http://tox.charite.de/protox_II/ that the test ligand ferulic-acid showed the predicted toxicity class categorized as class 4 of 6 and predicted LD_{50} 1190 mg/kg. Thus, the ferulic acid as a test ligand compound is predicted not to be toxic to body organs.

After knowing the toxicity of the two potential compounds, the bioavailability analysis of the compound was then carried out. This analysis was carried out online through access at http://www.swissadme.ch/. The results of the analysis showed that ferulic acid had a high category of human gastrointestinal absorption. This analysis was carried out based on the fact that the material consumed orally should ideally be easily absorbed by the digestive system organs, be able to be distributed specifically to target sites, be able to be metabolized by the body and eliminated without causing any harm to the body's organs [38].

■ CONCLUSION

The results showed that the compound contents of the tuna-shredded fortified banana blossoms extract contained 32 active compounds, which had a total phenolic content was 0.00134 mg GAE/g, total flavonoid content was 0.0006670 mg QE/g, and the IC_{50} for the antioxidant activity was 4.38 ppm. The results of the *in silico* showed that the most potent compound, ferulic acid, had the potential to inhibit the XO enzyme with a binding affinity score was -9.70 kcal/mol through hydrogen bonds on Ala1079, Thr1010 amino acids residues and hydrophobic interactions on Gly799, Ala910, Ala1078, Glu1261, Phe1009, Phe914, Leu1014, Glu802, and Leu873 amino acids residues. As a potential compound, ferulic acid meets Lipinski's rules, is a high category of human gastrointestinal absorption, and is not potentially toxic to the body. The tuna-shredded fortified banana blossoms have the potency to be developed as a functional food, especially to prevent gout arthritis.

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■ REFERENCES

[1] Palani, T., Shobha, K., Thirunavukkarasu, P., and Hari, R., 2018, *In vitro* and *in silico* antigout arthritic activities of ethanolic and aqueous stem extracts of *Cissus quadrangularis* – A TLR2 and TLR4 Receptor approach, *J. Appl. Pharm. Sci.*, 8 (09), 015–022.

[2] Soltani, Z., Rasheed, K., Kapusta, D.R., and Reisin, E., 2013, Potential role of uric acid in metabolic syndrome, hypertension, kidney injury, and cardiovascular diseases: Is it time for reappraisal, *Curr. Hypertens. Rep.*, 15 (3), 175–181.

[3] Kostalova, E., Pavelka, K., Vlaskova, H., and Stiburkova, B., 2015, Hyperuricemia and gout due to deficiency of hypoxanthine-guanine phosphoribosyl transferase in female carriers: New insight to differential diagnosis, *Clin. Chim. Acta*, 440, 214–217.

[4] Raucci, F., Iqbal, A.J., Saviano, A., Minosi, P., Piccolo, M., Irace, C., Caso, F., Scarpa, R., Pieretti, S., Mascolo, N., and Maione, F., 2019, IL-17A neutralizing antibody regulates monosodium urate crystal-induced gouty inflammation, *Pharmacol. Res.*, 147, 104351.

[5] Billy, C.A., Lim, R.T., Ruospo, M., Palmer, S.C., and Strippoli, G.F.M., 2018, Corticosteroid or nonsteroidal antiinflammatory drugs for the treatment of acute gout: A systematic review of randomized controlled trials, *J. Rheumatol.*, 45 (1), 128–136.

[6] Hadinoto, S., and Idrus, S., 2018, Proporsi dan kadar proksimat bagian tubuh ikan tuna ekor kuning (*Thunnus albacares*) dari perairan Maluku, *Majalah BIAM*, 14 (2), 51–57.

[7] Hardoko, H., Suprayitno, E., Sulistiyati, T.D., Sasmito, B.B., Chamidah, A., Panjaitan, M.A.P., Tambunan, J.E., and Djamaludin, H., 2022, Banana blossom addition to increase food fiber in tuna (*Thunnus* sp.) floss product as functional food for degenerative disease's patient, *IOP Conf. Ser.: Earth Environ. Sci.*, 1036, 012095.

[8] Gang, M., 2013, *Changes in the quality and yield of fish fillets due to temperature fluctuations during processing*, Final Project, United Nations University Fisheries Training Programme, Iceland.

[9] Dara, W., and Arlinda, A., 2017, Mutu organoleptik dan kimia abon ikan gabus (*Channa striata*) yang disubstitusi sukun (*Artocarpus altilis*), *Jurnal Katalisator*, 2, 61–66.

[10] Parvez, G.M.M., and Akanda, M.K.M., 2019, "Foods and Arthritis: An Overview" in *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, 2nd Ed., Eds. Watson, R.R., and Preedy, V.R., Academic Press, Cambridge, MA, 3–22.

[11] Kesuma, C.P., Adi, A.C., and Muniroh, L., 2015, Pengaruh substitusi rumput laut (*Eucheuma cottonii*) dan jamur tiram (*Pleurotus ostreatus*) terhadap daya terima dan kandungan serat pada biskuit, *Media Gizi Indonesia*, 10 (2), 146–150.

[12] Asare, E.O., Bhujel, N.K., Čížková, H., and Rajchl, A., 2022, Fortification of fruit products – A review, *Czech J. Food Sci.*, 40 (4), 259–272.

[13] Djamaludin, H., Hardoko, H., Dailami, M., Nurhadianty, V., Ananta, D.R., and Prayoga, D.R., 2022, The peroxide, organoleptic and proximate content of *Thunnus albacares* shredded fortified with banana blossoms, *Jurnal Sumberdaya Akuatik Indopasifik*, 6 (4), 319–330.

[14] Sulistiyati, T.D., Suprayitno, E., Djamaludin, H., Tambunan, J.E., and Muchayaroh, U., 2022, The effect of fortification *Moringa oleifera* leaves powder on calcium content in otak-otak products of *Clarias* sp., *IOP Conf. Ser.: Earth Environ. Sci.*, 1036, 012071.

[15] Sharma, V., Shukla, K.V., and Golani, P., 2019, Traditional and medicinal effect of banana blossom, *Int. J. Sci. Dev. Res.*, 4 (5), 377–381.

[16] Hardoko, H., Sari, P.Y., and Puspitasari, Y.E., 2015, Substitusi jantung pisang dalam pembuatan abon dari pindang ikan tongkol, *JPK*, 20 (1), 1–10.

[17] Mohammed, E.T., Khalil, R.R., and Mustafa, Y.F., 2022, Phytochemical analysis and antimicrobial evaluation of quince seeds' extracts, *J. Med. Chem. Sci*., 5 (6), 968–979.

[18] Khalil, R.R., Mohammed, E.T., and Mustafa, Y.F., 2022, Evaluation of *in vitro* antioxidant and antidiabetic properties of *Cydonia oblonga* seeds' extracts, *J. Med. Chem. Sci*., 5 (6), 1048–1058.

[19] Lipinski, C.A., 2004, Lead- and drug-like compounds: The rule-of-five revolution, *Drug Discovery Today: Technol.*, 1 (4), 337–341.

[20] Jayaram, B., Singh, T., Mukherjee, G., Mathur, A., Shekhar, S., and Shekhar, V., 2012, Sanjeevini: A freely accessible web-server for target directed lead molecule discovery, *BMC Bioinf.*, 13 (Suppl. 17), S7.

[21] Jasim, S.F., Mustafa, Y.F., 2022, Synthesis, ADME study, and antimicrobial evaluation of novel naphthalene-based derivatives, *J. Med. Chem. Sci*., 5 (5), 793–807.

[22] Banerjee, P., Eckert, A.O., Schrey, A.K., and Preissner, R., 2018, ProTox-II: A webserver for the prediction of toxicity of chemicals, *Nucleic Acids Res.*, 46 (W1), W257–W263.

[23] Daina, A., Michielin, O., and Zoete, V., 2017, SwissADME: A free web tool to evaluate pharmacokinetics, drug- likeness and medicinal chemistry friendliness of small molecules, *Sci. Rep.*, 7 (1), 42717.

[24] Sukisman, S., Purnomo, H., Rosyidi, D., and Radiati, L.E., 2014, Quality properties, antioxidant capacity and total phenolic content of traditional deep-fried shredded meat (abon) of Palu, Central Sulawesi, *Am. J. Food Technol.*, 9 (2), 80–88.

[25] Esmaeili, A.K., Mat Taha, R., Mohajer, S., and Banisalam, B., 2015, Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (red clover), *BioMed Res. Int.*, 2015, 643285.

[26] Baba, S.A., and Malik, S.A., 2015, Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume, *J. Taibah Univ. Sci.*, 9 (4), 449–454.

[27] Djamaludin, H., Bintang, M., and Priosoeryanto, B.P., 2019, Cytotoxicity and antiproliferative effects of ethyl acetate fraction of *Padina australis* against MCM-B2 and K562 cell lines, *J. Appl. Biol. Biotechnol.*, 7 (2), 25–29.

[28] Aisyah, Y., Rasdiansyah, R., and Muhaimin, M., 2014, Pengaruh pemanasan terhadap aktivitas antioksidan pada beberapa jenis sayuran, *JTIPI*, 6 (2), 28–32.

[29] Yadav, D.K., and Khan, F., 2013, QSAR, docking and ADMET studies of camptothecin derivatives as inhibitors of DNA topoisomerase-I, *J. Chemom.*, 27 (1-2), 21–33.

[30] Giménez, B.G., Santos, M.S., Ferrarini, M., and Fernandes, J.P.S., 2010, Evaluation of blockbuster drugs under the rule-of-five, *Pharmazie*, 65 (2), 148–152.

[31] Hernández-Santoyo, A., Tenorio-Barajas, A.Y., Altuzar, V., Vivanco-Cid, H., and Mendoza-Barrera, C., 2013, "Protein-Protein and Protein-Ligand Docking" in *Protein Engineering – Technology and Application*, Eds. Ogawa, T., IntechOpen, Rijeka, 63–81.

[32] Toppo, A.L., Yadav, M., Dhagat, S., Ayothiraman, S., and Eswari, J.S., 2021, Molecular docking and ADMET analysis of synthetic statins for HMG-CoA reductase inhibition activity, *Indian J. Biochem. Biophys.*, 58 (2), 127–134.

[33] Nile, S.H., Ko, E.Y., Kim, D.H, and Keum, Y.S., 2016, Screening of ferulic acid related compounds as inhibitors of xanthine oxidase and cyclooxygenase-2 with anti-inflammatory activity, *Rev. Bras. Farmacogn.*, 26 (1), 50–55.

[34] Wang, F., Yang, L., Huang, K., Li, X., Hao, X., Stöckigt, J., and Zhao, Y., 2007, Preparation of ferulic acid derivatives and evaluation of their xanthine oxidase inhibition activity, *Nat. Prod. Res.*, 21 (3), 196–202.

[35] Patrick, G., 2001, *Instant Notes in Medicinal Chemistry*, BIOS Scientific Publishers, Oxford.

[36] Wardaniati, I., and Herli, M.A., 2018, Studi molecular docking senyawa golongan flavonol sebagai antibakteri, *JOPS*, 1 (2), 20–27.

[37] Ouertani, A., Neifar, M., Ouertani, R., Masmoudi, A.S., Mosbah, A., and Cherif, A., 2019, Effectiveness of enzyme inhibitors in biomedicine and pharmacotherapy, *Adv. Tissue Eng. Regener. Med.*, 5 (2), 85–90.

[38] Drwal, M.N., Banerjee, P., Dunkel, M., Wettig, M.R., and Preissner, R., 2014, ProTox: A web server for the *in silico* prediction of rodent oral toxicity, *Nucleic Acids Res.*, 42 (W1), W53–W58.