ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

Jurnal Kefarmasian Indonesia
Available online at https://jkefarind.com/index.php/jki
Original Research Article
JKI

Potential of Snakehead Fish and Sea Cucumber Extracts on Increase Anticancer Drugs Potency through Inhibition of Glutathione S-Transferase

Andi Suhendi[1], Ega Annisa Putri[2], Thalia Fiandra Tasyaningtyas[2], Safira Mafaza Abdillah[2], Inandrohatul Aisy[2], Wahyu Utami[1]*

[1]Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Surakarta, Central Java, Indonesia
[2]Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Surakarta, Central Java, Indonesia

ARTICLE INFO

Article history:
Received 15 May 2024
Revised 21 June 2024
Accepted 03 July 2024
Published online 31 August 2024

*Corresponding author.
E-mail: *wahyu.utami@ums.ac.id*

DOI: https://doi.org/10.22435/jki.v14i2.6653

Citation: Suhendi A, Putri EA, Tasyanintyas TF, Abdillah SM, Aisy I, Utami W. Potential of Snakehead Fish and Sea Cucumber Extracts on Increase Anticancer Drugs Potency through Inhibition of Glutathione S-Transferase. Jurnal Kefarmasian Indonesia. 2024;14(2):194-203

Copyright: © 2024 Suhendi *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

In certain conditions of cancer, isoenzymes of glutathione-S-Transferase (GST) showed increased activity. Consequently, anticancer drugs would be inactive due to increase of phase II metabolism through conjugation with glutathione. In order to increase the effectiveness of anti-cancer drugs, a GST inhibitor is required. One of the natural sources have antioxidant activity is snakehead fish. The study aims to determine GST inhibition activity of the combination of extract of snakehead fish (SF) and sea cucumber (SC). This activity was determined using rat liver and lung microsomal-cytosolic fractions obtained through the Lundgren method. GST activity was measured spectrophotometrically using a simple kinetic program at a wavelength of 345 nm with a DCNB (1.2-dichloro-4-nitrobenzene) as a substrate. The results showed an increase in the percentage of inhibition GST enzymes of the liver and lungs of rats by extracts and their combinations. The data of IC_{50} values showed that the smallest value for liver GST was found in SC with the value of 117.7 µg/mL and for lung GST enzymes, it was a combination of SC-SF (1:2) with a value of 45.6 µg/mL. This showed that these two samples had the greatest activity in inhibiting lung and liver GST enzymes and the potential as a complementary therapy for cancer patients.

***Keywords*:** Glutathione s-transferase; Snakehead fish; Sea cucumber; Simple kinetics; 1,2 Dichloro-4-nitrobenze

INTRODUCTION

Cancer cells can become resistant to anti-cancer drugs and one of its resistance mechanisms is the inactivation of anti-cancer drugs by detoxifying enzymes, particularly glutathione S-transferase (GST). In xenobiotic metabolism, GST is known as a phase II enzyme whose main role is to catalyze the reaction of hydrophobic and electrophilic compounds. The anti-cancer drugs of the group of alkylating substances are electrophilic compounds that will be metabolized through a reaction of conjugation with GSH. In general, GST experiences an excessive expression as a characteristic in many tumors, including transitional cell carcinoma of the bladder, renal cell cancer, ovarian cancer, breast cancer, and colorectal cancer.[1–4] An increase in GST in cancer cells will decrease the drug's ability to kill cancerous cells. To increase the effectiveness of anti-cancer medication therapy, a GST inhibitor is required. In addition, the combination of indomethacin

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic

(as an inhibitor of GST) with chlorambucil will increase effectiveness of chlorambucil in the treatment of certain types of cancer.[5-6] The involvement of GST in resistance to anticancer drugs and an inverse correlation between expression and prognosis in many tumors provided a rationale for the design of inhibitors and prodrugs to enhance the therapeutic index.[7]

GST was reported to be inhibited by saturated fatty acids, stearate acid, palmitic acid, fatty acid esters, ascorbate stearate, and palmitate ascorbate.[8] In addition, flavonoids and polyphenols are compounds that can inhibit the activity of GST [8]. Research by Özaslan and Demir (2017) showed that Cd^{2+}, Cu^{2+}, Zn^{2+}, and Ag+ could inhibit GST activity.[9] Snakehead fish is one of a good source of important nutrients for containing albumin, Zn, and Cu. It was also stated to have an antioxidant activity.[10,11] Other pharmacological effects are anti-inflammatory effects on both acute and chronic inflammation.[12–14] Meanwhile, fatty acids contained in snakehead fish are palmate acid, stearate acid, oleic acid, linoleic acid, EPA, and DHA. [15,16]

Based on chemical content such as Zn, Cu, and fatty acids of snakehead fish extract and sea cucumber extract it is expected to have high GST inhibitory activity. If such activity is proven, it is very promising to be used as a complementary therapy in cancer patients. This study therefore aims to find out the inhibitory effect of snakehead fish and sea cucumber extracts on GST enzymes from liver and pulmonary GST rats with 1.2-dichloro-4-nitrobenzene (DCNB) as a substrate.

METHODS

Materials: Snakehead fish extract and sea cucumber (CV. Herbal Nusantara, Karanganyar, Central Java, Indonesia), bovine serum albumin (analytical grade; Sigma-Aldrich), glutathione (analytical grade; Sigma-Aldrich), 1,2-dikloro-4-nitrobenzen (analytical grade; Sigma-Aldrich), Wistar Rats (5 male rats, 150-200 g, 2 months old, from Pharmacology Laboratory of Faculty of Pharmacy Universitas Muhammadiyah Surakarta), Spectrophotometer UV Mini 1280 (Shimadzu), analytical balance (Ohaus), Ultra-Turrax® (Ika), Ultrasonic (Branson), Micro-centrifuge (Mikro 200R Hettich Zentrifugen). The protocol study was evaluated and approved by the Ethics Committee of Health Research of the Faculty of Medicine Universitas Muhammadiyah Surakarta (No.3046/A.1/KEPK-FKUMS/XI/2020). The Wistar-Albino rats used in our study weighed 150-200g and were kept in an environment with established standard environmental conditions (temperature 23 ± 2 °C and 12 h light/dark cycle).

Preparation of fraction of microsomal-cytosolic liver and lung rats

The rats were injected using ketamine injection followed by neck dislocation before being dissected. Immediately, the liver and lungs were removed and washed in refrigerated saline solution (0.9 % NaCl). Liver and lung were mixed with phosphate buffer 0.1 M pH 7.5, and sliced by blender and ultra turrax. The homogenate obtained was then gradually centrifuged, followed by the modification of Lundgren. [17,18] Modification performed was at the initial centrifugation of homogenate at 600x g for 10 minutes. Supernatants were then centrifuged at 10,000 g for 10 minutes. The second supernatants were called microsomal-cytosol fraction containing GST enzymes that were stored at a temperature of -80°C.

Protein determination

The protein concentration was determined by the Lowry method [19,20] and bovine serum albumin (BSA) was used as the protein standard. A series of BSA were 0.10; 0.15; 0.20; 0.25 and 0.30 mg/mL. The operating time and maximal wavelength obtained were within 18 minutes and at 748 nm, respectively. Protein determination is conducted in triplicate. Glutathione s-transferase activity using DCNB as substrate.[21] GST enzyme activities were determined by monitoring the thioether

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

bond formed between GST and DCNB spectrophotometrically at 340 nm wavelength for five minutes. Table 1 presents the reaction system. Measurements were performed in triplicate.

Table 1. Reaction system of GST activity

Reagents	S_0	S_1
0.1 M phosphate buffer pH 7.4 (µL)	647.5	632.5
microsomal and cytosol fraction (µL)	17.5	17.5
Extracts (µL)	0	15
Incubation (minutes)	0	4
50 mM GSH in phosphate buffer (µL)	75	75
DCNB 50 mM (µL)	10	10
Incubation for 4 minutes at room temperature		
Abs were measured at 345 nm using a simple kinetics program		

The rate of conjugate product form (V) calculated by equation (1)

$$V = \frac{rate}{\Delta\varepsilon_{\text{GS-CNB}} \times \text{d} \times \text{C}} \ldots \text{(equation 1)}$$

V = GST activity/ conjugate product GS-CNB (µmol min-1 mg-1)
= molar extinction of conjugate product GS-CNB = 8.5 mM-1 cm-1
d = path length (cm)
C = protein concentration (mg/mL)

Inhibition activity (%) was calculated by Equation (2)

$$\%\ \text{inhibition} = \frac{S_0 - S_1}{S_0} \times 100\% \quad \text{(equation 2)}^{22}$$

S0 = rate of GST without inhibitor; S1 = rate of GST with inhibitor

IC_{50} was calculated based on linear regression of extracts vs %Inhibition.

Data analysis

Linear regression of BSA and %inhibition calculated using Excel® software. Evaluation of the linearity of the graph based on R2 value, which the value of higher than 0.9. The results are given by means and standard deviation (means ± S). Data obtained were homogenous and they were analyzed Excel®, and SPSS 24 for one-way ANOVA followed by post hoc Tukey. The signification value used is 0.05.

RESULTS AND DISCUSSION

Microsomal-cytosolic fraction and protein content in liver and lung GST

Microsomal-cytosolic fractions of the liver and lungs of mice were obtained by step-by-step centrifugation methods such as in Lundgren.[17] The determination of protein content by the Lowry method (Figure 1) was based on the reaction between phosphomolybdic acid and phosphotungstic acid with Cu ions and side chains of tyrosine, tryptophan, and cysteine producing a greenish-blue color[19,20] in which absorbance could be measured at wavelengths between 650 nm and 750 nm.[23]

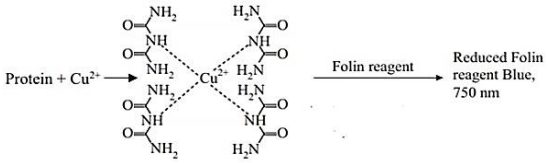

Figure 1. Reaction of Lowry method with protein

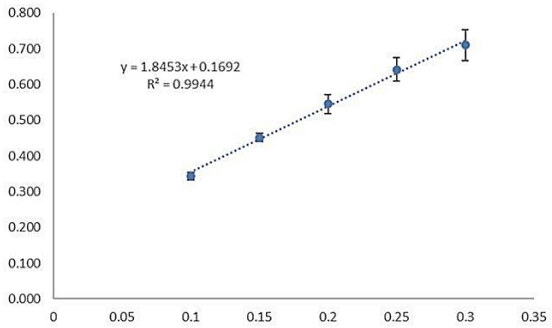

Figure 2. Graphic of linear regression of Bovine Serum Albumin (mg/mL) vs. absorbance using Lowry Method at 748 nm and operating time at 18 minutes

The maximum wavelength for determining protein levels was within 18 minutes and at 748 nm. Protein levels were calculated based on the regression equation of plotting the absorption vs. albumin levels. The regression equation was y =

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic

1.844x + 0.1692, R^2 0.9944 (Figure 2) and protein contents in the microsomal fraction of liver and lung rats were 0.22 ± 0.06 and 0.32 ± 0.13 mg/mL

Determination activity of GST of liver and lung rats

The determination of the activity of GST was measured by spectrophotometry using a simple kinetic method. As GST obtained from fractionations was crude, then GST activity was stated as specific activity, the activity of several enzymes in catalyzing the formation of conjugate products of 1µmol per minute per mg of protein. The substrate used in this study was 1,2-dichloro-4-nitrobenzene (DCNB) due to a specific substrate for mu class GST.[21] The conjugation reaction occurred between DCNB and GSH compounds catalyzed by GST, namely the C1 atom in the para position of DCNB would be substituted by a thiolate anion (GS-) from GSH.[24] This reaction produced the GS-CNB conjugate product.

The measurement of the conjugate product was carried out at λ 345 nm as the maximum wavelength for the GS-CNB conjugate product. The results of measurements with simple kinetic obtained the value of the rate with the units of absorbance/minute. The rate value was then used to calculate the GST activity with and without the addition of the extract at various concentrations.

Based on data (Table 2 and 3), GST activity of liver was higher than that of lung. Differences in the ability to inhibit GST activity might be caused by the type of organ used. The liver is the main site for metabolizing drugs[25], and class mu GST isoenzymes are mostly found in the liver. [24,25] The rate of GST activity with the addition of the sample showed a decreasing trend. The greater the concentration of the extract given, the lower the rate of formation of conjugate products. Therefore, it could be stated that sea cucumber extract has an inhibiting activity of GST of liver and lung. In line with sea cucumber activity, snakehead fish extract showed the inhibiting activity of GST in the liver and lungs. The decreasing value of GST activity of snakehead fish extract was higher than sea cucumber extract.

Table 2. Rate activity of GST with and without sea cucumber extract

Conc. (µg/ mL)	Rate/ V (nmol/min/mg protein)	
	Liver GST	**Lung GST**
0	2021.9 ± 67.97*	146.84 ± 8.56*
50	2021.9 ± 67.97*	95.45 ± 11. 77*
75	1464.9 ± 65.68*	66.08 ± 18.43*
100	1251.8 ± 37.81*	31.82 ± 21. 29*
125	967.6 ± 7.39*	

GST = Glutathione-S Transferase; * ($p < 0.05$); Conc.= concentration

Table 3. Rate activity of GST of snakehead fish extract

	Rate/ V (nmol/min/mg protein)		
Conc. (µg/ mL)	**Liver GST activity**	**Conc. (µg/ mL)**	**Lung GST activity**
0	5336.7 ± 592.0*	0	274.5 ± 18.9*
50	4996.7 ± 618.8*	25	190.9 ± 15.8*
100	4773.8 ± 856.6*	50	166.0 ± 9.1*
150	3897.8 ± 721.2*	75	99.3 ± 26.1*
200	2784.1 ± 446.8*	100	34.1 ± 20.3*
250	1854.6 ± 267.3*		

GST = Glutathione-S Transferase; * ($p < 0.05$); Conc.= concentration

The combination of snakehead fish extract and sea cucumber was expected to have higher GST activity on GST than the alone extract. Data (Table 4) showed that the trend of GST activity with the addition of extract combination declined. The combination of 1:1 of those extracts had a lower activity than the extract alone of snakehead fish and sea cucumber. In addition, the activity of extract combination (1:1) was almost similar to snakehead fish and sea cucumber extract.

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

Table 4. Rate activity of GST with and without sea cucumber-snakehead fish extract (1:1)

Rate/ V (nmol/min/mg protein)			
Conc. (µg/ mL)	**Liver GST**	**Conc. (µg/ mL)**	**Lung GST**
0	1243.2 ± 102.4*	0	134.6 ± 24.6*
50	1009.7 ± 9.7*	50	95.4 ± 6.5*
100	901.7 ± 36.9*	75	71.0 ± 5.1*
150	737,3 ± 21.4*	100	58.7 ± 8.2*
200	606.8 ± 59.7*	125	36.7 ± 6.1*

GST = Glutathione-S Transferase; * ($p < 0.05$); Conc. =Concentration

The combination of sea cucumber and snakehead fish extract at a ratio of 1:2 (Table 5) exhibited similar activity to previous combination on decreasing GST activity of liver and lung. This indicated that the combination of 1:1 and 1:2 of sea cucumber-snakehead fish extract was the evident of antagonistic activity of compounds in the extract.

Table 5. Rate activity of GST with and without combination sea cucumber-snakehead fish extract (1:2)

Rate/ V (nmol/min/mg protein)			
Conc. (µg/ mL)	**Liver GST**	**Conc. (µg/ mL)**	**Lung GST**
0	1243.2 ± 102.4*	0	33.7 ± 3.5*
90	1009.7 ± 9.7*	30	24.8 ± 2.0*
120	901.7 ± 36.9*	60	16.0 ± 0.9*
150	737,3 ± 21.4*	90	10.6 ± 0.9*
180	606.8 ± 59.7*		

GST = Glutathione-S Transferase; * ($p < 0.05$); Conc.=concentration

The data of combination 1:2 of sea cucumber-snakehead fish extract (Table 6) showed a similar profile in GST activity with two previous combinations. The data confirmed that the combination of sea cucumber-snakehead fish extracts evidence of the antagonist activity of the chemical compound on it. Based on one-way ANOVA with the degree of freedom 95% obtained, all groups of snakehead fish extract, sea cucumber extract, and their combination (1:1; 1:2; 2:1) showed a significant difference. It described that all groups had inhibition activity on liver and lung GST. The difference of GST activity of the liver and lung was due to the different levels of production.

Table 6. Rate activity of GST with and without combination sea cucumber-snakehead fish extract (2:1)

Rate/ V (nmol/min/mg protein)			
Conc. (µg/ mL)	**Liver GST activity**	**Conc. (µg/ mL)**	**Lung GST activity**
0	924.5 ± 32.3*	0	64.7± 8.3*
90	842.7 ± 12.3*	30	45.4 ± 2.1*
120	728.3 ± 12.7*	45	34.4 ± 1.6*
150	660.0 ± 21.4*	60	18.9 ± 7.2*
180	577.5 ± 10.7*		
210	458.5 ± 9.8*		

GST = Glutathione-S Transferase; * significance ($p < 0.05$)

As shown in Figure 3, all linear regression equations had a good correlation between concentration and percentage of inhibition as marked with the value of $R^2 > 0.9$. Therefore, in the determination of IC_{50} value, the results were found valid.

The results showed an increase in the percentage of inhibition GST enzymes in the liver and lungs of rats (Figures 3 and 4) from extracts and their combinations. This showed that sea cucumber extract can inhibit GST enzyme activity in the liver and lungs of rats. Based on the calculation of linear regression (Figure 3 and Figure 4), the IC_{50} values were obtained. The data of IC_{50} (Table 7) showed the smallest IC_{50} value for liver GST, namely sea cucumbers. Meanwhile, for lung GST enzymes, it was a combination of sea cucumbers and snakehead fish extract (1:2). This showed that these two samples had the greatest activity in inhibiting lung and liver GST enzymes.

Table 7. IC_{50} value of extracts and their combinations on GST liver and lung

Sample extracts	IC_{50}	
	GST of liver	GST of Lung
Sea cucumber (SC)	117.7	93.0
Snakehead fish (SF)	209.7	55.7
SF:SC(1:1)	195.1	85.1
SF:SC(1:2)	155.8	81.9
SF:SC(2:1)	212.4	45.6

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic

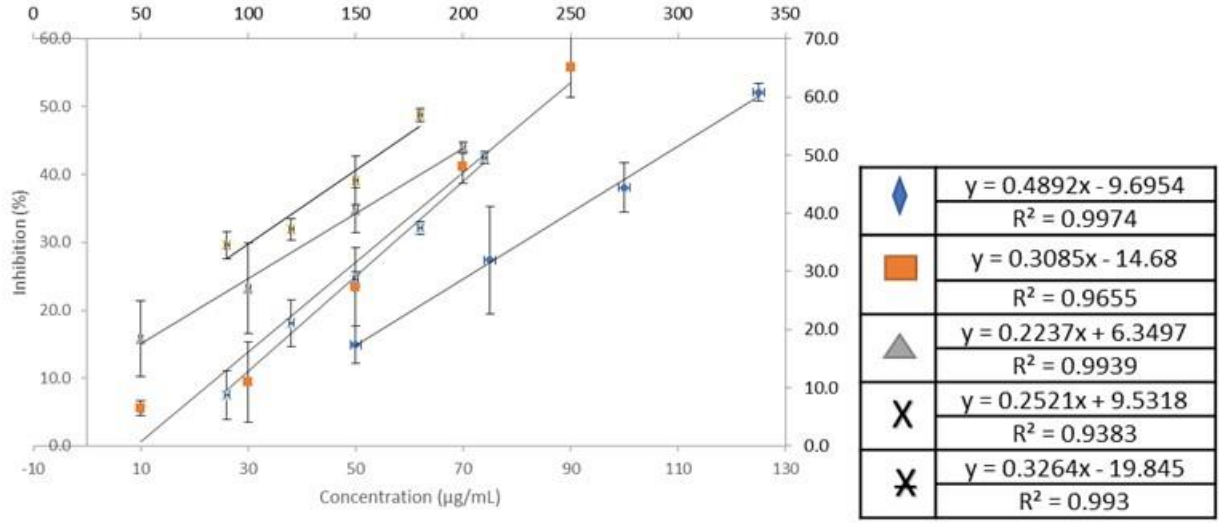

Figure 3. Linear regression of liver GST activity extract vs %inhibition of extract of sea cucumber (◊), snakehead fish extract (□); combination sea cucumber: snakehead fish extract 1:1 (▲), 1:2 (x), and 2:1 (✶)

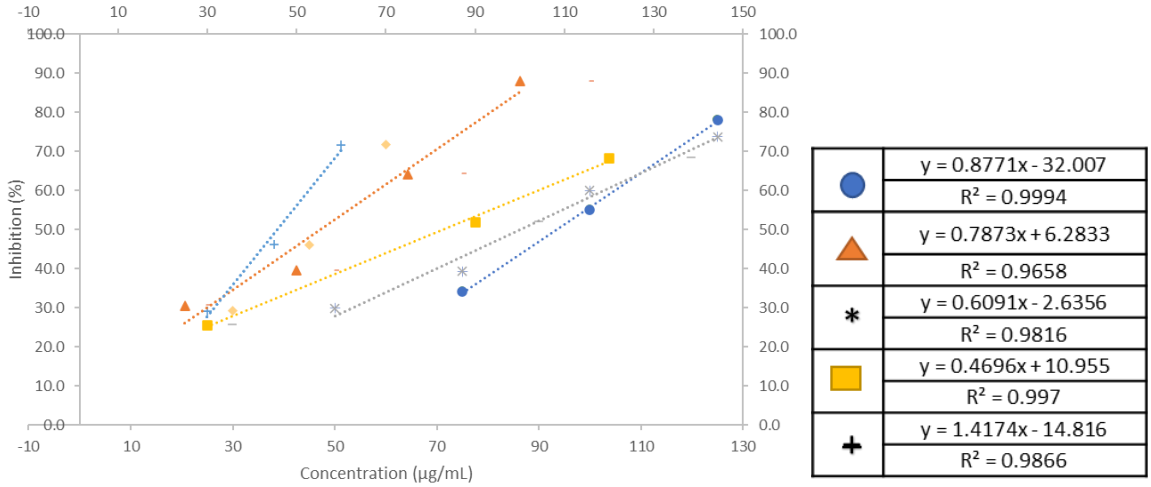

Figure 4. Linear regression of lung GST activity extract vs %inhibition of extract of sea cucumber (○), snakehead fish extract (Δ); combination sea cucumber: snakehead fish extract 1:1 (*), 1:2 (□), and 2:1 (+)

The inhibition of GST by Cu was by accelerating the disulfide binding so there was a decrease in the levels of thiol groups bound to GST.[26] In addition, the presence of Zn could be an inhibitor of GST with a competitive inhibition mechanism.[9] Fatty acids, also have been reported to inhibit glutathione-S-transferase (GST) activity. The highest inhibition was ascorbic ester, ascorbyl palmitate, or stearate, whereas the IC_{50} value of palmitic acid was 423 µM and stearic acid was 349 µM.[7] Several fatty acids were also found to inhibit GST activity, one of which was oleic acid.[27] In addition, flavonoids and polyphenols are compounds that can inhibit GST activity.[28] Several minerals have been reported to inhibit GST, such as Cd2+, Cu2+, Zn2+, and Ag+.[9] GST has two active sites, namely a GSH binding site (hydrophilic G-site), which is specific for GSH, and an electrophilic binding site (hydrophobic H-site), which allows GST to react with a variety of xenobiotics.[29] The ability to inhibit GST activity by fatty acids occurs because fatty acids are lipophilic, which will bind to the hydrophobic side of the active site of GST, which results in no conjugate product being formed.[7] While, the inhibition of GST activity by flavonoids

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

can occur because flavonoids are electrophilic enabling them to compete with the substrate on the active site of GST, which causes no conjugate products to form.[30,31]

The mechanism for inhibiting GST activity by Zn minerals is by occupying the active site of GST so that DCNB cannot bind to GSH and no conjugate products are formed.[9] Whereas, Cu was reported to be capable of inhibiting GST activity for having a high affinity for the thiol group (-SH) causing a change in the conformation of the enzyme, which causes the enzyme to lose activity.[26]

The reports informed that snakehead fish extract contains amino acids, fatty acids, and minerals.[32,33] Based on research by Che Ku Daud[34], snakehead fish extract extracted with water contained various kinds of fatty acids such as palmitic acid (18.39%) and stearic acid (10.88%). In addition, snakehead fish contain several minerals such as Zn and Cu.[35] Meanwhile, sea cucumbers contain phenolics and flavonoids.[36] Damaiyanti research[37] stated that one of the active compounds found in sea cucumber water extract was flavonoids with a percentage of 0.16%.

Therefore, to overcome the resistance of anticancer drugs in patients' therapy, one of the strategies is how to inhibit GST enzyme activity, so the sensitivity of cancer cells to anticancer drugs increases.[38,39] Currently, an example of an inhibitor used with chemotherapy drugs is piperlongumine combined with cisplatin or doxorubicin.[15,40] Piperlongumine inhibits GSTP1-1 by directly binding to the enzyme mechanism.[41] The combination of cancer chemotherapy drugs with GST inhibitors can reverse resistance by suppressing GST activity and increasing the sensitivity of chemotherapy drugs.[42] Therefore, snakehead fish extract is expected to have the potential to become an inhibitor of GST enzymes used together with anti-cancer drugs.

CONCLUSION

The data of IC_{50} values showed that the smallest value for liver GST, namely sea cucumbers with a value of 117.7 µg/mL, and lung GST enzymes, was a combination of SC-SF (1:2) with a value of 45.6 µg/mL. This showed that these two samples have the greatest activity in inhibiting lung and liver GST enzymes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

Thank you to the Faculty of Pharmacy UMS and CV. Herbal Nusantara, Karanganyar, Central Java.

REFERENCES

1. Pljesa-Ercegovac M, Savic-Radojevic A, Matic M, Coric V, Djukic T, Radic T, *et al.* Glutathione Transferases: Potential Targets to Overcome Chemoresistance in Solid Tumors. International Journal of Molecular Sciences. 2018;19(2):3785. doi: 10.3390/ijms19123785.
2 Abbas Z, Rehman S, Abbas Z, Rehman S. An Overview of Cancer Treatment Modalities. In: *Neoplasm*. IntechOpen. 2018;1:138-57. doi:10.5772/intechopen.76558.
3 Singh RR, Reindl KM. Glutathione S-Transferases in Cancer. Antioxidants. 2021;10:701. doi.org/10.3390/antiox10050701.
4 Kennedy L, Sandhu JK, Harper M-E, Cuperlovic-Culf M. Role of Glutathione in Cancer: From Mechanisms to Therapies. Biomolecules. 2020;10(10):1429. doi.org/10.3390/biom10101429.

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic

5 Jo G, Kim EJ, Hyun H. Tumor Targeting by Conjugation of Chlorambucil with Zwitterionic Near-Infrared Fluorophore for Cancer Phototherapy. International Journal of Molecular Sciences. 2022;23(22):14093. doi.org/10.3390/ijms232214093

6 Gaté L, Tew KD. Glutathione S-transferases as emerging therapeutic targets. Expert Opinion on Therapeutic Targets. 2021;5(4): 477–89. doi: 10.1517/14728222.5.4.477.

7 Mitra A, Govindwar S, Joseph P, Kulkarni A. Inhibition of human term placental and fetal liver glutathione-*S*-transferases by fatty acids and fatty acid esters. Toxicology Letters. 2022;60(3): 281–8. doi.org/10.1016/0378-4274(92)90286-S.

8 Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as Potential Anti-Inflammatory Molecules: A Review. Molecules. 2022;27(9):2901. doi.org/10.3390/molecules27092901.

9 Özaslan MS, Demir Y, Küfrevioğlu OI, Çiftci M. Some metals inhibit the glutathione S-transferase from Van Lake fish gills. Journal of Biochemical and Molecular Toxicology. 2017; 31: e21967. doi.org/10.21597/jist.525154.

10 Suhendi A, Puspa FE, Pawarti H. Aktivitas Antioksidan Ekstrak Ikan Gabus (*Channa striata*) Pada Tikus yang Diinduksi dengan Rifampisin-Isoniazid. Jurnal Kesehatan. 2020;3(1): 69–77. doi: 10.23917/jk.v13i1.11103.

11 Ren M, Yin T, You J, Liu R, Huang Q, Xiong S. Comparative Study of the Nutritional Composition and Antioxidant Ability of Soups Made from Wild and Farmed Snakehead Fish (Channa Argus). Foods. 2022;11(20):3294.doi.org/10.3390/foods11203294.

12 Ramadhanti NA, Sandhika W, Widodo ADW. The Effect of Snakehead Fish (*Channa striata*) Extract on Inflammation Reaction of Skin Wound Tissue in Rattus novergicus Wistar Strain. Berkala Ilmu Kesehatan Kulit dan Kelamin. 2021;33(1):48–54. doi: 10.20473/bikk.V33.1.2021.48-54

13 Suhendi A, Muhtadi M, Sutrisna EM. Anti-inflammatory and antidiabetic of Channa striata powder and Nephelium lappaceum fruit peel ethanolic extracts on albino Wistar mice. Drug Invent. Today. 2019;12(11):2472-6.

14 Suhendi A, Rohman A, Nurrochmad A, Wahyono D. Effect of Snakehead Fish (*Channa striata*) Extracts on Liver Function in Rats Which Receiving Anti-tuberculosis. International Journal of Pharmaceutical Research 2020;12. doi:10.31838/ijpr/2020.SP1.147.

15 Kung F-P, Lim Y-P, Chao W-Y, Zhang Y-S, Yu H-I, Tai T-S *et al.* Piperlongumine, a Potent Anticancer Phytotherapeutic, Induces Cell Cycle Arrest and Apoptosis In Vitro and In Vivo through the ROS/Akt Pathway in Human Thyroid Cancer Cells. Cancers 2021; 13: 4266. doi: 10.3390/cancers13174266

16 Pangestuti R, Arifin Z. Medicinal and health benefit effects of functional sea cucumbers. Journal of Traditional and Complementary Medicine. 2018;8(3): 341–51. doi: 10.1016/j.jtcme.2017.06.007

17 Lundgren B, Meijer J, Birberg W, Pilotti A, Depierre JW. Induction of cytosolic and microsomal epoxide hydrolases in mouse liver by peroxisome proliferators, with special emphasis on structural analogues of 2-ethylhexanoic acid. Chemico-Biological Interactions. 2018;68(3): 219–40. doi: 10.1016/0009-2797(88)90018-x.

18 Scotcher D, Billington S, Brown J, Jones CR, Brown CD, Rostami-Hodjegan A, Galetin A. Microsomal and cytosolic scaling factors in dog and human kidney cortex and application for in vitro-in vivo extrapolation of renal metabolic clearance. Drug Metabolism and Disposition. 2017;45(5):556-68. doi: 10.1124/dmd.117.075242.

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

19 Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols. 2017;2(4):875-7.
20 Suhendi A, Rohman A, Cahyaningrum S. Validasi Metode Analisis Penetapan Kadar Protein Ekstrak Ikan Gabus dengan Metode Lowry dan Bromocresol Green. Jurnal Kefarmasian Indonesia. 2023; 13(1):50-8. doi: 10.22435/jki.v13i1.6219
21 Devi MC, Pirabaharan P, Rajendran L, Abukhaled M. An efficient method for finding analytical expressions of substrate concentrations for different particles in an immobilized enzyme system. Reaction Kinetics, Mechanisms and Catalysis. 2020;130:35-53. doi.org/10.1007/ s11144-020-01757-0
22 Yuniarti N, Martono S, Supardjan A M. Effect of aspirin on pi-class of rat kidney glutathione S-transferase activity. Indonesian Journal of Pharmacy 2005; 0: 87–93.
23 Noble JE, Bailey MJA. Chapter 8 Quantitation of Protein. In: Burgess RR, Deutscher MP (eds). Methods in Enzymology. Academic Press, 2009: 73–95. doi: 10.1016/S0076-6879(09)63008-1
24 Pljesa-Ercegovac M, Savic-Radojevic A, Matic M, Coric V, Djukic T, Radic T, Simic T. Glutathione transferases: potential targets to overcome chemoresistance in solid tumors. International journal of molecular sciences. 2018;19(12):3785. doi.org/10.3390/ijms19123785
25 Issa NT, Wathieu H, Ojo A, Byers SW, Dakshanamurthy S. Drug Metabolism in Preclinical Drug Development: A Survey of the Discovery Process, Toxicology, and Computational Tools. Current Drug Metabolism. 2017;18(6): 556–65. doi: 10.2174/ 1389200218666170316093301
26 Ahmed A, Malik A, Jagirdar H, Rabbani N, Khan MS, Al-Senaidy AM *et al.* Copper-Induced Inactivation of Camel Liver Glutathione S-Transferase. Biological Trace Element Research. 2016;169:69–76. doi: 10.1007/ s12011-015-0388-2
27 Aravindan V, Muthukumaravel S, Gunasekaran K. Interaction affinity of Delta and Epsilon class glutathione-s-transferases (GSTs) to bind with DDT for detoxification and conferring resistance in Anopheles gambiae, a malaria vector. Journal of vector borne diseases. 2014;51(1):8-15.
28 Boušová I, Hájek J, Dršata J, Skálová L. Naturally occurring flavonoids as inhibitors of purified cytosolic glutathione S-transferase. Xenobiotica. 2012;42(9):872879. doi: 10.3109/ 00498254.2012.670737.
29 Allocati N, Masulli M, Di Ilio C, Federici L. Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. Oncogenesis. 2018;7(1):8. doi: 10.1038/s41389-017-0025-3.
30 Martono S, Utami W. Daya Hambat Flavonoid Naringenin dan Naringin Terhadap Aktivitas GST Kelas P Hati Tikus (*Rattus norvegicus* L.) Secara In Vitro. Berkala Ilmiah Biologi. 2005; 4.
31 Utami W. Daya Hambat Ekstrak Daun Dewandaru (*Eugenia uniflora* L.) terhadap Aktivitas Gst Kelas Pi Ginjal Tikus secara In Vitro. 2007; 8: 110–118.
32 Suhendi A, Pawarti H, Rohman A, Wahyono D, Nurrochmad A. Snakehead fish extract (*Channa striata*): A review of pharmacological activity. Eurasian Journal of Biosciences. 2020; 14(2):4527.
33 Govender T, Brand M. Adverse gastrointestinal bleeding associated with over-the-counter nonsteroidal anti-inflammatory drug use: a cost study in two Gauteng public hospitals. SA. Pharmaceutical Journal. 2018;85(1):65–71. doi:10.10520/EJC-da075d25b
34 Dahlan-Daud CK, Jais AM, Ahmad Z, Abdah MD, Aishah AD. Amino and fatty acid compositions in Haruan traditional extract (HTE). Boletin latinoamericano y del Caribe de

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic

Plantas Medicinales Aromaticas. 2010;9(5):414-29.

35 Mustafa A, Widodo M, Kristianto Y. Albumin and Zinc Content of Snakehead Fish (*Channa striata*) Extract and Its Role In Health. International Journal of Scientific & Technology Research. 2012;1(2).

36 Xu C, Zhang R, Wen Z. Bioactive compounds and biological functions of sea cucumbers as potential functional foods. Journal of Functional Foods. 2018;49:73-84. doi.org/10.3390/ md9101761

37 Damaiyanti DW. Karakterisasi Esktrak Air Teripang Emas (*Stichopus hermanii*). DENTA 2015; 9: 74–81.

38 Oktaviyana ET, Utami W. Efektivitas Agen Pendeplesi GSH pada Sitotoksisitas Cisplatin terhadap Sel Kanker: Systematic Literature Review. Pharmacon: Jurnal Farmasi Indonesia 2021;18(2):177–186. doi: 10.23917/ pharmacon.v18i2.16254.

39 Liu Y, Zheng C, Huang Y, He M, Xu WW, Li B. Molecular mechanisms of chemo- and radiotherapy resistance and the potential implications for cancer treatment. MedComm *(2020)* 2021;2:315–340. doi: 10.1002/mco2.55.

40 Tripathi SK, Biswal BK. Piperlongumine, a potent anticancer phytotherapeutic: Perspectives on contemporary status and future possibilities as an anticancer agent. Pharmacological Research. 2020;156: 104772. doi: 10.1016/j.phrs.2020. 104772

41 Harshbarger W, Gondi S, Ficarro SB, Hunter J, Udayakumar D, Gurbani D, *et al.* Structural and Biochemical Analyses Reveal the Mechanism of Glutathione S-Transferase Pi 1 Inhibition by the Anti-cancer Compound Piperlongumine. Journal of Biological Chemistry. 2017; 292: 112–20. doi: 10.1074/jbc.M116.750299

42 Dong S-C, Sha H-H, Xu X-Y, Hu T-M, Lou R, Li H *et al.* Glutathione S-transferase π: a potential role in antitumor therapy. Drug Design, Development and Therapy. 2018;12: 3535–47. doi: 10.2147/DDDT.S169833