STABILIZATION OF THE INTERACTION OF THE COMPUNDS OF *Mallotus paniculatus Müll.Arg*. ON GLUTHATIONS S-TRANSFERASE (GSTs)

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***Abstract**: **Gluthathione s-transferases (GSTs) play an important role against carcinogens, therapeutic drugs, and various types of cellular oxidative damage.** Judging from its role in overcoming cellular oxidative damage, the activity of GSTs is one of the antioxidant enzymes. One of the plants that has been tested for its antioxidant ability is M. paniculatus. The content of M. paniculatus has been found so that it can be used to test the stability of the interaction between ligands and receptors. The aim of this research is to find compounds from M. paniculatus that have the potential to stabilize the interaction with the receptors. This docking method begins with ligand preparation and protein preparation followed by docking, visualization of interactions and analysis of the results. The research resulted in various docking scores with the various proteins involved. The stability of the interaction is obtained when the ligand binds to Glutathione S-transferase type A3 is hesperetin, sitosterol and sitosterol, to Glutathione S-transferase type theta-2 is β-daucostero, to Glutathione S-transferase type P is quercetin.*

***Keywords:** GSTs, M. paniculatus, docking*

INTRODUCTION

Since the 1970s much attention has been focused on the properties of glutathione S -tranferases (GSTs) which facilitate the catalysis of the thioether bond between glutathione (GSH) and the electrophilic centers of small molecules. Microsomal GSTs contain three isoforms designated as mGST 1, 2, and 3 which are encoded by a single gene located on chromosome 12 (MGST1) and are involved in the endogenous metabolism of leukotrienes and prostaglandins. All cytosolic GSTs have genetic polymorphisms in human populations. The promoter region varies between classes and may contain one or more response elements: antioxidant response elements; xenobiotic response elements; GSTP adder 1; glucocorticoid response elements; Barbie-box elements. The promoter can also contain binding sites for transcription factors such as, AP-1, MAF, Nrf1, Jun, Fos, and NF-kappa B, which are species specific.[1]

Cytosolic GSTs have catalytic activity as homo- or hetero-dimer proteins, allowing the formation of a large number of enzymes from a number of genes; However, dimerization is usually limited to subunits within the same class. Subunit sizes range from 24 to 29 kDa. Each subunit contains an active site with two sub-sites: a highly conserved G site for GSH binding and an H site for hydrophobic substrates. Although <10% protein is crystallized, all GSTs isozymes have two domains and a similar topology.[1]

GSTs play an important role against carcinogens, therapeutic drugs, and various types of cellular oxidative damage.[2] GSTs also regulate the biosynthesis and transport of intracellular hormones. Based on their similarity in order and substrate specificity, GSTs are divided into at least ten subfamilies: alpha, delta, epsilon, kappa, mu, P, sigma, theta, zeta, and omega. In this study only focused on the subfamily GSTP_human (PDB ID code: 18GS), GSTA3_human (PDB ID code: 1TDI) and GSTT2_human (PDB ID code: 3LJR) this is because the three gluthation families are expressed in large numbers in response to the existence of a radical . The antioxidant activity test of plants is often carried out to look for potential compounds in overcoming a radical. One of the plants that has antioxidant activity is M. paniculatus.

The content of the M. paniculatus plant with the local name balik angin is 3'-O-dimethylluteolin, 4-methoxybenzoic acid, acetylaleuritolic acid, apigenin, erythordiol, hesperetin,[3] isoscopoletin, kaempherol, quercetin, scopoletin, sitosterol, syringaresinol, β-daucostero[4] Leaf ethanol extract has the antioxidant ability of IC50 DPPH method of 30 µg / ml[5], from the bark of methanol extract IC50 71.94 µg / ml, n-hexane fraction = 59.92 µg / ml, ethyl fraction acetate = 49.92 µg / ml,[6] Dayak people from East Kalimantan use it as a medicine for diarrhea, fever, and itching on the skin,[7] so that the content in this plant can be examined more on the mechanism of antioxidants in interacting with gluthathione S-transferase.

RESEARCH METHOD

Equipments that were used in this research were Laptop with 2GB ram specifications, Intel ®Pentium Quad core Processor N3700, PLANTS 1.2 software, Discovery studio, MarvinBean.[8] The materials were GSTP_human (PDB ID code: 18GS),[9] GSTA3_human (PDB ID code: 1TDI)[10] and GSTT2_human (PDB ID code: 3LJR).[11]

The docking process using PLANTS 1.2[12] involves the chemical content of the M. paniculatus a plant against the GSTP_human receptor (PDB ID code: 18GS), GSTA3_human (PDB ID code: 1TDI) and GSTT2_human (PDB ID code: 3LJR).

Visualization of docking results using Discovery studio.[13]

The data seen were the docking score and the bond residue equation from the chemical content of M. paniculatus to natural ligands.

RESULTS AND DISCUSSION

M. paniculatus is a plant that is often used by the public, especially as an antioxidant. The enzymes involved in antioxidants are SOD, catalase and Gluthation transferase.[14] Therefore, the researchers chose one of the enzymes involved as an antioxidant, namely Gluthation transferase. The docking process of the chemical components involved as antioxidants begins with the release of the ligands from the receptionist in the database and begins the redocking process. After the redocking was complete, it was followed by docking the compounds from the M. paniculatus plant.

Glutathione S-transferase type A3 with ID code: 1TDI interacts with the original gluthathione ligand, the amino acid residue interacting with the ligand is used as a benchmark for analysis, because the amino acid residue that interacts with the ligand is a binding site and may be a catalytic site, while determining The catalytic site required laboratory testing, so the researchers were only able to predict the binding site and the binding site equation between the ligand from M. paniculatus and the original ligand. Regarding the bond equation with amino acid residues, namely 3'-O-dimethylluteolin (0%), 4-methoxybenzoic acid (0%), acetylaleuritolic acid (0%), apigenin (0%), erythordiol (66%), hesperetin (33 %), isoscopoletin (33%), kaempherol (0%), quercetin (0%), scopoletin (33%), sitosterol (33%), syringaresinol (66%), β-daucostero (33%). What is interesting about the Glutathione S-transferase type A3 interaction is the presence of a water bridge, the water bridge contributes to the docking score, this can be seen from syringaresinol and erythordiol, although they have the same 66% but have a poor level of stability. The type of interaction of each ligand from M. paniculatus to gluthathione S-transferase A3 can be seen in Table 1.

Glutathione S-transferase theta-2 has an original ligand, namely 1-Menaphthyl Glutathione Conjugate. The binding site equation between the ligand M. paniculatus and the original ligand is 3'-O-dimethylluteolin (50%), 4-methoxybenzoic acid (40%), acetylaleuritolic acid (50%), apigenin (50%), erythordiol (40%) , hesperetin (50%), isoscopoletin (6%), kaempherol (40%), quercetin (50%), scopoletin (50%), sitosterol (30%), syringaresinol (70%), β-daucostero (70%) . What is interesting about this type of interaction is that the best docking score is only 76% (this is not good enough), this is because the original ligand is able to interact with SO4245 while the ligands from M. paniculatus are not able to interact with SO4. The type of interaction of each ligand from M. paniculatus to gluthathione S-transferase theta-2 can be seen in Table 2.

Table 1.Types of interaction between ligand and Glutathione S-transferase type A3

Compound	hydrogen bond	hydrophobic interaction	Another interaction (electrostatic, PI-lone Pair, PI-Sulfur, water bridge)
ligand original	MET16, ARG13,	-	GLU162, HOH336, HOH479
3'-O-dimethylluteolin	-	TYR166, TRP21, PRO18, LEU163, ILE19, LEU72	SER159, HOH479, TRP21, HOH435
4-methoxybenzoic acid	SER159,	TRP21	HOH507, GLU162
acetylaleuritolic acid	ASN103	PRO18,LEU72,ILE19,TRP21,TYR166	HOH370, HOH479, TRP21
apigenin	-	TYR166, TRP21, LEU163, ILE19, LEU72	TRP21
erythordiol	VAL161, MET16, ARG15	ARG15, PRO18, ILE19, LEU72, LEU163, VAL161, PRO56, TRP21, TYR166	-
hesperetin	ILE19, ILE158, ARG15	TRP21, PRO18, LEU72	-
isoscopoletin	-	PRO18	GLU162
kaempherol	ILE19, PRO18, GLY14, EU160	TRP21	-
kuersetin	PRO18	TRP21	-
scopoletin	GLU162	PRO18	GLU162
sitosterol	ARG15	ARG15, PRO18, ILE19, LEU72, TRP21	-
syringaresinol	ASN103, GLU162, VAL161, MET16, LEU160	ARG13, PRO18, LEU72, TYR166, LEU163, ILE19	-
β-daucostero	THR68, GLN67, ARG15	TRP21, ARG15, PRO18, LEU72, VAL161, TRP21	HOH566

Table 2.Types of interactions between ligand and glutathione S-transferase type theta-2

Compund	hydrogen bond	hydrophobic interaction	Another interaction (electrostatic, PI-lone Pair, PI-Sulfur, water bridge)
ligand original	ARG15, MET168, GLN12	PRO13, ARG15, ALA16, ILE213	SO4245, ARG107, GLU172, GLN175,
3'-O-dimethylluteolin	LEU9, GLU172	ARG15, PRO13,ALA16,MET168	SER11
4-methoxybenzoic acid	GLU172, LEU167, MET168	ARG15,ALA16	GLU172
acetylaleuritolic acid	MET168	PRO13, ARG15, ALA16, PRO55,MET174, ILE213, HIS210	ARG107
apigenin	SER67, GLU171, SER67	PRO13, ALA16, PRO13, ALA16, ARG15	GLU172, MET168
erythordiol	LEU167	ARG15, ALA16, ARG107, MET174, PRO13, MET174, HIS210	-
hesperetin	ARG107	ARG15, MET174, HIS210, PRO13, ARG15, MET168	-
isoscopoletin	GLN12, GLU171, MET168, GLU172	PRO13, ARG15, ALA16	GLU172
kaempherol	GLN175, GLU171, SER67	ARG15	GLU171, GLU172, GLN12
kuersetin	GLN175, SER11	ARG15, PRO13, ALA16	SER11, MET168
scopoletin	GLU171, MET168	ALA16, PRO13, ARG15	GLU172
sitosterol	-	PRO13, ARG15, ALA16, ILE213, LEU71, LEU99, HIS102	-
syringaresinol	HIS102, MET168, LEU164, SO4245, GLU172, GLN175	ALA16, LEU71, LEU167, PRO13, ARG107, VAL10, ILE213, HIS102, PRO13, ARG15	-
β-daucostero	ARG15, HIS210, GLU171, LEU170	ARG15,ALA16, ARG107, MET168, PRO13, ARG107, PRO13, LEU54, HIS 102	-

The 18GS (Glutathione S-transferase P) has an original ligand, namely Gluthatione-2,4 dinitrobenzene. The binding site equation between the ligand from M. paniculatus and the original ligand is 3'-O-dimethylluteolin (40%), 4-methoxybenzoic acid (40%), acetylaleuritolic acid (30%), apigenin (20%), erythordiol (40%), hesperetin (20%), isoscopoletin (40%), kaempherol (20%), quercetin (30%), scopoletin (50%), sitosterol (20%), syringaresinol (30%), β-daucostero (20%). The docking score of the M. paniculatus ligand is not good because the equation of the amino acid residue that interacts with the M. paniculatus ligand is below 80%, besides that the original ligand can form a large number of hydrogen bonds. The type of interaction of each ligand from M. paniculatus to Glutathione S-transferase P can be seen in table 3.

The best docking scores for gluthathione S-transferase A3 belonged to hesperitin, sitosterol and β-daucostero with a score of 99% each; 109% and 114%. The stability of the interaction between the compound from M. paniculatus and gluthathione S-transferase A3 is influenced by the equalization of the amino acid residues that interact with the original ligand (gluthathione). Hisperitin has an amino acid residue equation of 33% when compared to the original ligand, namely ARG15, sitosterol has a 33% bond residue equation, namely ARG15, while β-daucostero has a 33% bond residue equation, namely ARG15. The best docking score for Glutathione S-transferase theta-2 is owned by β-daucostero this is due to the equation of the amino acid residue bonding to the original ligand (1-Menaphthyl Glutathione Conjugate). This docking score when compared to the original ligand is 76%, the equation of the interacting residue is ARG15; ALA16; ARG107; MET168, PRO13. Quercetin has the best docking score for Glutathione S-transferases P with a score of 67% against the original ligand. The residual equation that interacts with the original ligand (Gluthatione-2,4 dinitrobenzene) is 30%, the interaction of this ligand with the amino acid residue ASN93; ARG100; ASP157. The docking scores of various types of Glutathione S-transferases can be seen in table 4.

Table 3.Types of interaction between ligand and Glutathione S-transferase type P

compund	hydrogen bond	hydrophobic interaction	Another interaction (electrostatic, PI-lone Pair, PI-Sulfur, water bridge)
ligand original	CYS14, ARG18, TYR153, ASP157, ASN93, GLY12	ALA15	ARG13, ARG100, TYR103, HOH228, HOH230, HOH243
3'-O-dimethylluteolin	-	ALA15, MET19, ARG18, ALA16, ARG100, LEU158	HOH215, HOH220, HOH230, HOH217, HOH340, ASP157
4-methoxybenzoic acid	ASP157	ALA15, ARG13	ARG100, HOH215, HOH228, ASN154
acetylaleuritolic acid	ALA16, CYS14, LEU156	ALA15, ALA16, LEU69, VAL96, MET19, LEU160, MET19, LEU156, LEU160, LEU69, ARG13, LEU69	HOH283
apigenin	VAL96	LEU158, ALA15, MET19, ALA16, ARG100	HOH220, HOH230, ASP157
erythordiol	ASN154, VAL96	ALA15, ALA16, ARG18, ARG100, ALA151, LEU158, MET19, ARG11, CYS14, ARG18, VAL96, LEU155, LEU69	HOH217
hesperetin	ASN154, LEU17, VAL96	ARG100, LEU158, ALA16, VAL96, LEU158, ARG18, MET19	HOH215, HOH217, ARG100, ASP157
isoscopoletin	ARG18, MET19, GLY12, ASP157	ILE161, ARG100, ALA15, MET19	HOH230, HOH228, ASP157
kaempherol	ARG18, MET19, VAL96,	ALA15, MET19, ALA16, ARG100, LEU158	HOH230, ARG100, ASN154
kuersetin	ASN93	ALA16, ARG100, LEU158, MET19	HOH220, HOH217, ASP157, ASP154. HOH230
scopoletin	GLY12, ASP157, LEU20	ARG100, ILE161, ALA15, MET19, LEU69, ALA15	HOH228, ASP157, HOH230
sitosterol	-	ARG11, ALA15, ALA16, ARG18, MET19, LEU17, VAL96, ARG100, LEU158, ILE161, VAL96, LEU155	-
syringaresinol	ALA15, ARG13, LEU155, ASP152	ALA16, LEU155, LEU156, ARG13, LEU17, ARG100, ILE161, LEU20, LEU69, HIS162, ALA16, VAL96, ALA15, LEU17, LEU69	HOH283, HOH228
β-daucostero	ALA15	ALA15,ALA16, MET19, VAL96, ARG100, LEU158, VAL96, MET19, LEU156, LEU160, ARG13, LEU69	-

Tabel 4. Docking score

	Kode ID		
Compund	1TDI (Glutathione S-transferase A3)	3LJR (Glutathione S-transferase theta-2)	18GS (Glutathione S-transferase P)
ligand original	-81,727	-124,847	-111,687
3'-O-dimethylluteolin	-76,245	-78,817	-70,355
4-methoxybenzoic acid	-58,862	-61.548	-56,248
acetylaleuritolic acid	-70,877	-64.336	-51,851
apigenin	-75,414	-79.661	-70,491
erythordiol	-72,509	-61.991	-46,007
hesperetin	-81,077	-80,196	-68,859
isoscopoletin	-65,756	-69.338	-58,631
kaempherol	-71,206	-73.331	-64,461
kuersetin	-71,723	-74.184	-75,191
scopoletin	-68,864	-66.591	-59,151
sitosterol	-89,176	-88,031	-57,142
syringaresinol	-76,646	-79.367	-61,565
β-daucostero	-93,616	-95,818	-62,261

CONCLUSION

The docking score for M. paniculatus which results in a stable interaction with Glutathione S-transferase A3 is hesperetin, sitosterol and sitosterol, for Glutathione S-transferase theta-2 is β-daucostero, and for Glutathione S-transferase P is quercetin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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