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Measuring the potential antioxidant activity of methyl gallate: Molecular docking study

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ABSTRACT

Objectives: This study aims to obtain predictions of the interactions that occur between methyl gallate and various antioxidant receptors while comparing it with gallic acid as its parent compound. **Materials and Methods:** The protein crystal structures of several antioxidant receptors from several classes such as an oxidoreductase (PDB ID 1HD2, 3MNG, and 3NRZ), transferase (PDB ID 2HCK), and transport proteins (PDB ID 4JK4) were selected as receptors. Molecular docking was performed using methyl gallate and gallic acid as test ligands with AutoDock 4.2.6. The main parameters used were the free energy of binding as an affinity marker and amino acid residues to see the level of similarity of interactions. **Results:** Of the five receptors used, methyl gallate showed the highest potential antioxidant activity at the 3NRZ receptor (xanthine oxidase) with the free energy of binding value of −7.45 kcal/mol, with the whole receptor showing a negative value, indicating a spontaneous reaction occurs. Both the affinity and the interaction between methyl gallate and gallic acid are not too much different, meaning the potential for comparable antioxidant activity. **Conclusion:** Overall, methyl gallate shows the potential for the antioxidant activity that is not too different from that indicated by gallic acid.

Keywords: Antioxidant, docking, gallic acid, methyl gallate, xanthine oxidase

INTRODUCTION

Our previous research has found that *Mangifera casturi*, a plant typical of Kalimantan Island, contains various compounds that have antioxidant activity in the fruit, such as 2,3-dihydroxybenzoic acid, dihydroxyquercetin, glucogallin, β-sitosterol, lupeol, gallic acid, and methyl gallate.[1-3] One that is already well known but not too much studied is methyl gallate, a derivative of gallic acid with lower polarity and better permeation ability.[4] Even though it is absorbed better in the body, it is known that the antioxidant activity of methyl gallate is lower than gallic acid as its parent compound.[5] However, the content of methyl gallate in medicinal plants is known to still play a significant role in antioxidant activity from the extracts of medicinal plants, including *M. casturi*.[6]

The antioxidant activity of a secondary metabolite of medicinal plants is often associated with free radical scavenging, wherein these compounds interact directly with a free radical without involving specific proteins in the body.[7,8] That matter is especially true of compounds with reducing groups such as hydroxyl and thiol which are commonly found in phenolic compounds, as shown in methyl gallate.[9] However, it does not rule out the possibility that antioxidant activity is also related to the interaction of these compounds with proteins that act as antioxidants in the body. As is known, the body has a defense mechanism against the oxidation process that involves various proteins and enzymes such as catalase, superoxide dismutase, peroxidase, and various small molecules such as glutathione.[10] Besides, direct interactions with several proteins such as myeloperoxidase, peroxiredoxin, Src family tyrosine kinase, xanthine oxidase, and serum albumin are known to have a significant impact on the antioxidant activity of a compound. For example, a group of oxidoreductase enzymes such as peroxiredoxin that regulates the amount of peroxide in mammalian cells is one of the critical targets in the mechanism of internal antioxidants. Another example is various kinase enzymes and transferases that involve the mechanism of reactive oxygen species (ROS).[11-14]

This study aims to obtain predictions of the interactions that occur between methyl gallate and various antioxidant

receptors while comparing it with gallic acid as its parent compound as well as determining the receptor with the highest potential antioxidant activity. The study was conducted *in silico* by molecular docking method. The results obtained are expected to provide a complete picture of the different types of interactions that occur between methyl gallate and gallic acid. Besides, the results obtained will reinforce how the potential of methyl gallate as an antioxidant compared to gallic acid, especially when compared through the mechanism of action involves several antioxidant receptors.

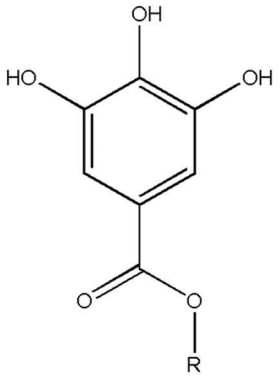
MATERIALS AND METHODS

Ligands Preparation

The test ligands used consisted of methyl gallate and gallic acid, as shown in Figure 1. The two-dimensional structure was sketched using HyperChem 8.0.8 from Hypercube, Inc. with geometry optimization ab initio basis set 6-31G*. Optimization was done by Polak-Ribiere algorithm and RMS Gradient 0.1 kcal/mol. Optimization with large basis sets was carried out to obtain the ideal molecular conformation which approves conformation of these compounds in nature.[15] The optimized structure then changes the format from.hin to.pdb using Open Babel 2.4.1 software.[16] The use of Open Babel makes it very easy to change ligands from one format to another without losing their ideal conformation.[17] Docking software used was AutoDock 4.2.6 from The Scripps Research Institute. One of the advantages of AutoDock 4 is that it can provide predictive value for the dissociation constant (K_i) so that it can provide predictions for the *in vitro* analysis process later. Both ligands then are given the charge and set torque using AutoDockTools 1.5.6.[18]

Receptors Preparation

The molecular structure of all receptors was obtained from the website of RCSB Protein Data Bank http://www.rscb.org in the.pdb format. The unused portion of the receptors was removed, added the non-polar hydrogen group, given the charge, and set the grid box size and coordinate using the AutoDockTools 1.5.6.[19] The size and coordinates of the grid box are adjusted automatically with the ligand cocrystal position of each receptor by making the ligand position the center of the grid box.[20] Five protein structures are used as receptors consisting of oxidoreductase (PDB ID 1HD2, 3MNG, and 3NRZ), transferase (PDB ID 2HCK), and transport proteins (PDB ID 4JK4). All of these receptors are known to play a role in the process of oxidative regulation in the body and have been previously investigated as target receptors for antioxidant compounds.[11-14] The receptor part used is an active site that has a cocrystal ligand, both in the form of natural ligand and known antioxidant compounds.



R	Compound
-H	Gallic acid
-CH3	Methyl gallate

Figure 1: Two-dimensional structure of methyl gallate and gallic acid

Molecular Docking

The docking process is preceded by a validation process, with the redocking method using cocrystal ligands as reference which have been extracted from receptors as test ligands and cocrystal ligand location as the binding site.[21] The parameters observed in the validation process are root mean square (RMSD) of each cocrystal ligand at the selected binding site. The RMSD score illustrates the average difference in ligand atom position redocking with crystallographic results.[22] Docking software is preferred to predict results from experimental positions with RMSD no more than 2 Å. Smaller RMSD shows that the position of the redocking result is closer to the cocrystal ligand.[23,24]

Docking for both test ligands performed in the same way as validation process with similar size and position of grid box form each cocrystal ligands. Docking search parameter used is Lamarckian genetic algorithm with the number of genetic algorithm 100 runs, population size 150, the maximum number of energy evaluation is medium with 2,500,000, and the maximum number of generations 27,000, with the default docking parameter used for run options. The primary parameter used in the docking process was the free energy of binding (ΔG), the dissociation constant (K_i), amino acid residues, and the number of hydrogen bonds.[19] ΔG and K_i scores determine ligand affinity to the receptor in the docking method. The more negative ΔG and lower K_i indicated higher ligand affinity toward the active site of the used receptor. All test ligands then compared with the validation result of cocrystal ligand to determine the potency of both test ligands as each receptor inhibitor.[25] The amino acid residues of both test ligands for each receptor then compared with amino acid residues of cocrystal ligand to assess the similarity of interaction between test and cocrystal ligand. The more similar amino acid residues are indicating a higher probability that the test ligand will have similar activity with the cocrystal ligand.[26]

Furthermore, to confirm the potential uses and benefits of both methyl gallate and gallic acid, identification of absorption, distribution, metabolism, and excretion (ADME) properties and a study of Lipinski's rule of five of the two compounds were carried out. The method is carried out using SwissADME web server (http://www.swissadme.ch/) with the.smiles file format. The results obtained will show some important ADME properties such as water solubility, blood–brain barrier (BBB) permeability, P450 cytochromes inhibitors, as well as whether or not there is a violation of Lipinski's rule of five.[27]

RESULTS AND DISCUSSION

Validation is carried out at the active site of each receptor using the cocrystal ligand as a reference to determine the size

and coordinates of each box.[28] Redocking results are shown in Figure 2 until 6, where it can be seen that the position of each redocking signal is almost overlapped with the crystallographic ligand position of receptors 2HCK, 3NRZ, and 4JK4 with RMSD values smaller than 2 Å. The ligand position which is slightly different is shown in 1HD2 and 3MNG, with a considerable RMSD value of more than 2 Å but still below 3 Å. However, RMSD values resulting from docking validation below 3 Å still show results that are biologically meaningful and can be used in docking with lower accuracy.[29] Overall, the redocking process still shows results that can be used for the docking process. Other parameters observed in the validation process are ΔG, K_i, amino acid residues, and the number of hydrogen bonds, including size and grid coordinates, as shown in Table 1.

Docking was performed using AutoDock 4.2.6 at the active site of each receptor with 100 genetic algorithms.[3] For both test ligands, one poses with the most negative ΔG and lowest K_i is selected as representatives of test ligand.[30] The docking results of all test ligands to each receptor are shown in Table 2.

Both test ligands in all receptors show negative ΔG scores, indicating that the interaction between all receptors with both methyl gallate and gallic acid will occur spontaneously. Surprisingly, of the five receptors used, two of them showed methyl gallate to have a more negative ΔG value and K_i which was smaller than gallic acid. These results are exciting because as mentioned earlier, methyl gallate is known to have antioxidant activity through a direct scavenging mechanism that is weaker than gallic acid.[5,31] At a glance, the results obtained show that the potential of methyl gallate in some antioxidant receptors is higher than gallic acid, even some of them are higher than comparable ligands.

In general, from all receptors, both methyl gallate and gallic acid show affinities that are not much different from cocrystal ligands, except for the 4JK4 receptor which shows a relatively lower affinity for test ligands. These results can be understood because the size of the two test ligands is relatively not much different from the cocrystal ligand so that the receptor part that interacts tends to be similar. These results are seen as shown in various oxidoreductase receptors such as 1HD2, 3MNG, and 4JK4. In Figures 2 and 3-5 for each

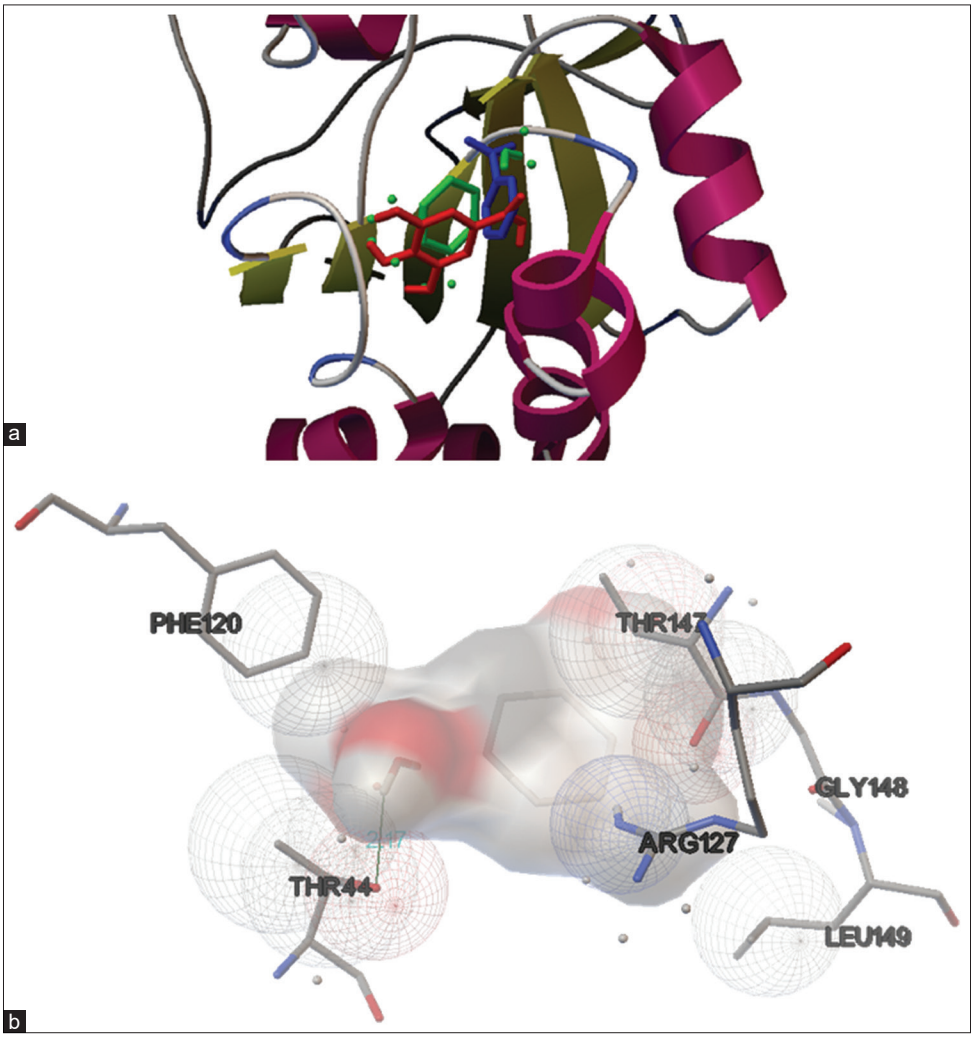


Figure 2: Comparison of the ligand position between cocrystal ligand (blue), gallic acid (red), and methyl gallate (green) on 1HD2 receptor (a) and interaction between receptor protein and methyl gallate (b)

Table 1: Validation results of all test receptors

Receptor		1HD2	2HCK	3MNG	3NRZ	4JK4
RMSD (Å)		2.548	1.968	2.665	0.190	1.677
ΔG (kcal/mol)		−3.47	−8.25	−3.62	−6.23	−7.49
K_i (μM)		2860.00	0.8984	2240.00	26.93	3.26
Amino acid residues		40-Pro	273-Leu	40-Pro	802-Glu	149-Tyr
		44-Thr	293-Ala	44-Thr	880-Arg	217-Arg
		45-Pro	295-Lys	45-Pro	914-Phe	218-Leu
		46-Gly	323-Val	120-Phe	1009-Phe	221-Lys
		120-Phe	338-Thr	127-Arg	1010-Thr	237-Leu
		127-Arg	339-Glu	147-Thr	1078-Ala	241-His
			340-Phe		1079-Ala	256-Arg
			341-Met			286-Ser
			344-Gly			289-Ile
			393-Leu			
			403-Ala			
			404-Asp			
Number of hydrogen bonds		0	4	3	2	3
Grid box coordinate	x	7.089	30.556	7.283	37.618	95.873
	y	41.659	45.903	41.723	20.226	16.048
	z	34.385	99.090	34.112	17.926	13.494
Grid box size (Å)		30×30×30	40×40×40	30×30×30	40×40×40	40×40×40

Table 2: Docking results of each test ligand in all receptors

Receptor	1HD2		2HCK		3MNG		3NRZ			4JK4	
Ligand	**GA**	**MG**	**GA**	**MG**	**GA**	**MG**	**GA**	**MG**	**F**	**GA**	**MG**
ΔG (kcal/mol)	−3.44	−3.35	−5.13	−4.93	−3.48	**−3.59**	−5.93	−7.45	**−10.2**	**−5.66**	−4.51
K_i (μM)	3010.00	3510.00	175.01	214.62	2840.00	**2330.00**	44.99	3.48	**0.03325**	**70.62**	491.22
Amino acid residues	-	44-Thr	276-Gly	-	40-Pro	-	-	-	648-Leu	149-Tyr	149-Tyr
	45-Pro	-	277-Gln	-	44-Thr	44-Thr	-	-	768-Asn	217-Arg	217-Arg
	46-Gly	-	-	281-Val	46-Gly	46-Gly	-	-	771-Lys	-	218-Leu
	-	120-Phe	-	293-Ala	47-Cys	-	-	799-Gly	-	221-Lys	221-Lys
	-	127-Arg	-	294-Val	116-Leu	-	802-Glu	802-Glu	802-Glu	-	222-Phe
	147-Thr	147-Thr	295-Lys	295-Lys	120-Phe	120-Phe	-	-	873-Leu	237-Leu	237-Leu
	148-Gly	148-Gly	-	323-Val	-	127-Arg	-	-	876-Ser	241-His	241-His
	149-Leu	149-Leu	-	338-Thr	147-Thr	147-Thr	880-Arg	880-Arg	880-Arg	256-Arg	256-Arg
			-	339-Glu	-	148-Gly	-	913-Gly	-	-	263-Ile
			-	340-Phe	-	149-Leu	914-Phe	914-Phe	914-Phe	286-Ser	286-Ser
			-	341-Met			-	1009-Phe	1009-Phe	289-Ile	289-Ile
			390-Ala	-			1010-Thr	1010-Thr	1010-Thr	290-Ala	-
			391-Asn	-			-	-	1011-Val		
			-	393-Leu			-	-	1013-Phe		
			-	403-Ala			-	-	1014-Leu		
			404-Asp	404-Asp			-	-	1076-Pro		
							1078-Ala	1078-Ala	-		
							1079-Ala	1079-Ala	-		
							1261-Glu	1261-Glu	-		
Number of hydrogen bonds	2	1	4	1	2	0	4	1	3	6	2
Similar amino acid residues (%)	37.5		12.5		40		21.1			66.7	

GA: Gallic acid, MG: Methyl gallate, F: Febuxostat. Bold number indicates higher affinity. Similar amino acid residues calculated based on the percentage of the number of similar amino acids to the combined overall amino acids that interact with all ligands

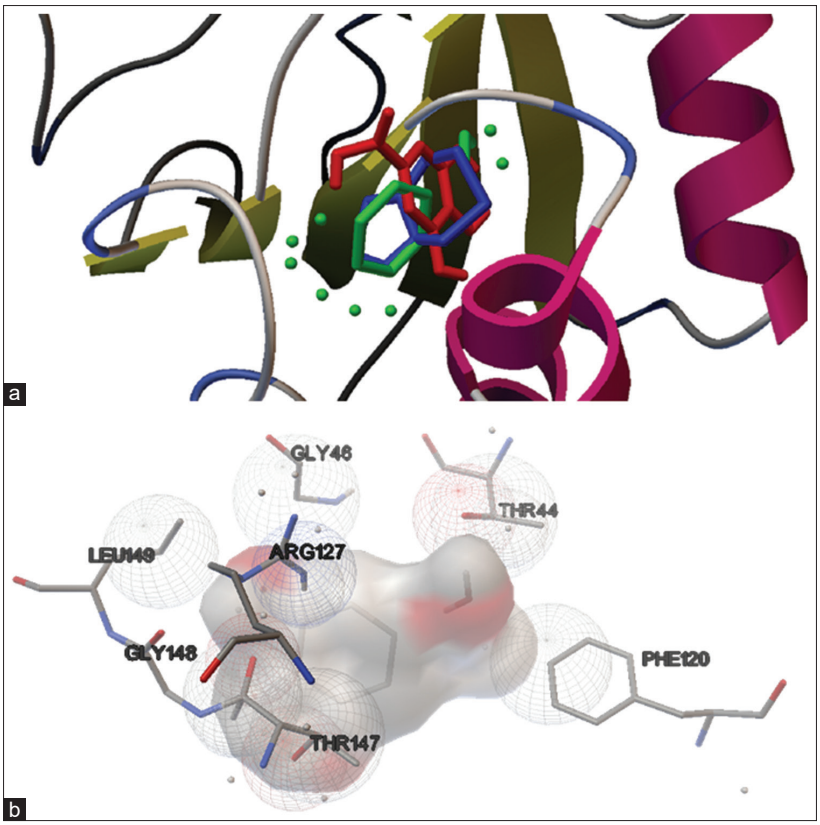


Figure 3: Comparison of the ligand position between cocrystal ligand (blue), gallic acid (red), and methyl gallate (green) on 3MNG receptor (a); and interaction between receptor protein and methyl gallate (b)

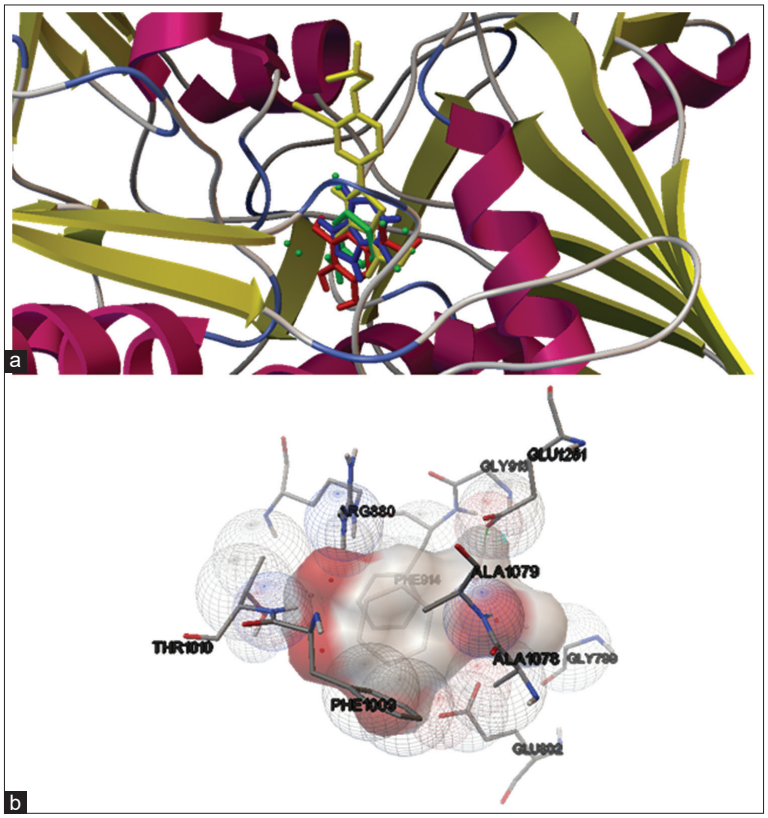


Figure 4: Comparison of the ligand position between cocrystal ligand (blue), febuxostat (yellow), gallic acid (red), and methyl gallate (green) on 3NRZ receptor (a); and interaction between receptor protein and methyl gallate (b)

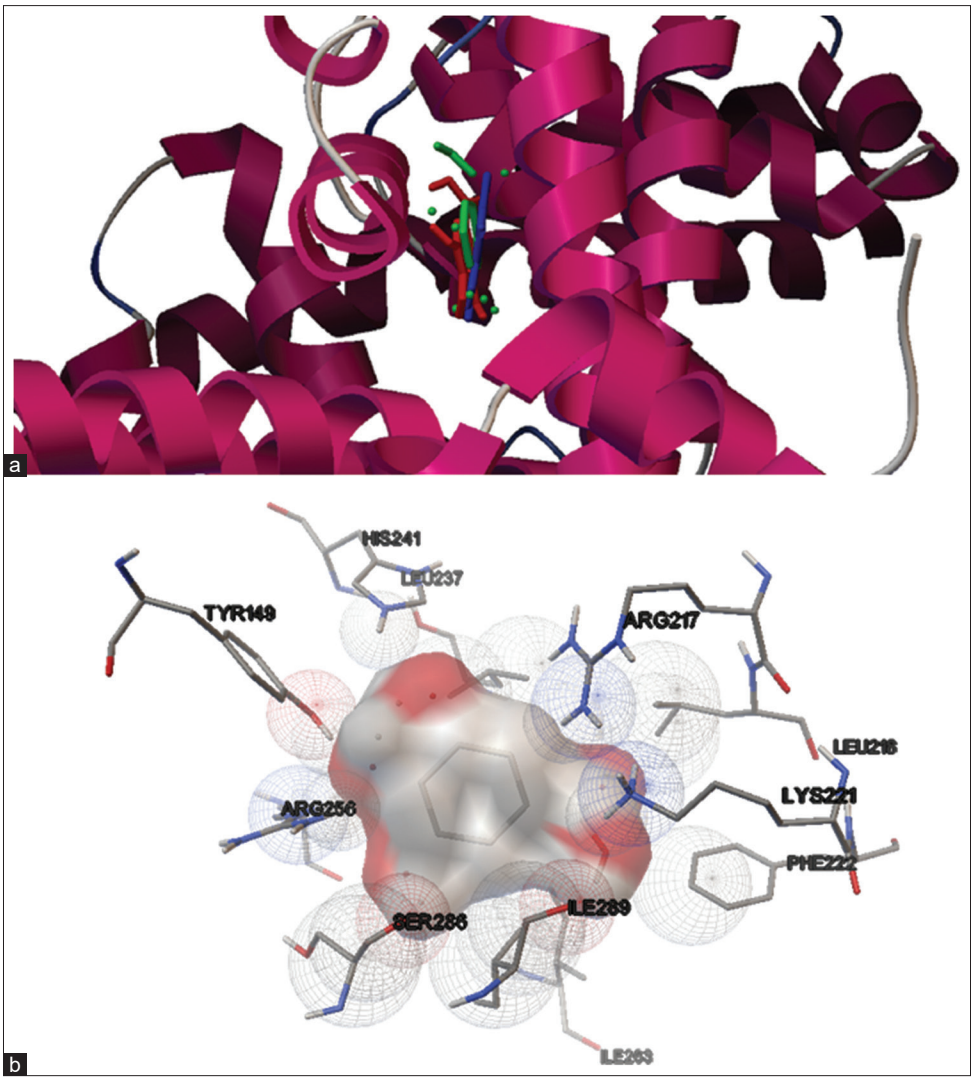


Figure 5: Comparison of the ligand position between cocrystal ligand (blue), gallic acid (red), and methyl gallate (green) on 4JK4 receptor (a); and interaction between receptor protein and methyl gallate (b)

receptor, it can be seen that the position of the two test ligands and the cocrystal ligands overlaps, indicating a similar bond position. In addition, some groups of oxidoreductase inhibitors themselves are known to tend to have polar groups that can form hydrogen bonds in some parts.[32] This resembles the profile shown by the two test ligands.

At the transferase receptor, which is 2HCK, there are quite distinct differences in affinity, which clearly shows that the cocrystal ligand affinity is higher than the two test ligands. The size of the cocrystal ligand which is much larger than the test ligand is suspected to be one of the causes of the difference in affinity. However, the visual observation in Figure 6 shows that in contrast to gallic acid whose bond position is very different from the cocrystal ligand, methyl gallate binds to a position similar to the comparable ligand. Transferase inhibitors themselves are generally large-sized compounds because at the active site, they will compete with large-sized natural ligands such as ATP.[33]

The receptor that shows the superiority of methyl gallate versus gallic acid is 3NRZ, the xanthine oxidase receptor. Besides being a receptor for hyperuricemia disorders, xanthine oxidase itself is often associated with antioxidant activity, where often a compound is known to have acted as a xanthine oxidase inhibitor also has antioxidant activity and vice versa.[12] As is known, the inhibition of xanthine oxidase is a strategy to treat and prevent the accumulation of uric acid and consequently the ROS. The formation of ROS itself is closely related to the increase in pro-oxidant compounds associated with various health problems.[34]

The 3NRZ receptor itself has a cocrystal compound hypoxanthine, a natural ligand of xanthine oxidase and very well-filled xanthine oxidase binding pocket.[35] Unexpectedly, it turned out that methyl gallate had a very high affinity on the active side, with K_i values almost 9 times lower than hypoxanthine. When compared with gallic acid, the value of K_i methyl gallate is almost 13 times smaller, indicating a much higher affinity than the parent compound. Whereas compared to the interacting amino acid residues, as shown in Table 2, there are only three of the 10 different amino acid residues from the two compounds which is glycine at 799 and 913 as

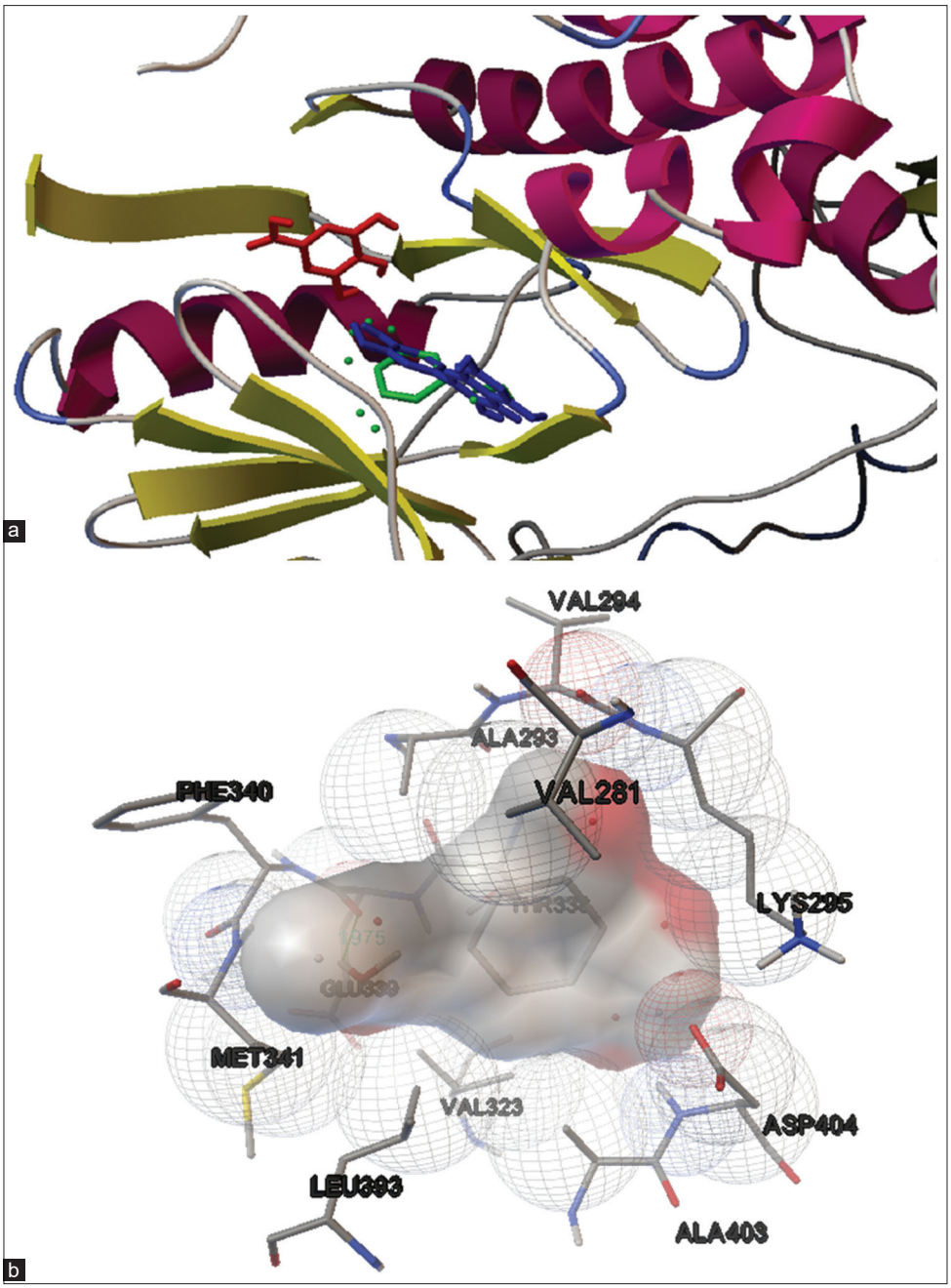


Figure 6: Comparison of the ligand position between cocrystal ligand (blue), gallic acid (red), and methyl gallate (green) on 2HCK receptor (a); and interaction between receptor protein and methyl gallate (b)

well as phenylalanine at 1009. The big difference caused from three amino acids is interesting to observe, considering that the structure between gallic acid and methyl gallate is not much different.[36]

In addition, we also compared the affinity of methyl gallate with compounds known to have affinity as xanthine oxidase inhibitors, namely, febuxostat which can block the binding site of xanthine oxidase. The comparison of febuxostat is mainly done to measure the comparison of the affinity of methyl gallate with xanthine oxidase inhibitors that are already available in the market. As shown in Table 2, the affinity of febuxostat is much higher than that of all other ligands, with a comparison value of K_i which is 100 times smaller than K_i of methyl gallate. This is not surprising considering that febuxostat has gone through various stages of drug development and is known to be one of the options for hyperuricemia therapy other than allopurinol.[37]

To obtain more information regarding the differences in interactions between these compounds, visual observations were carried out, as shown in Figure 4. The observations turned out to provide some interesting information, including the position of both test ligands and the corresponding ligands. As observed in Tables 1 and 2, all the ligands are in the same plane and even overlap with each other. This result

Table 3: Absorption, distribution, metabolism, and excretion properties of methyl gallate, gallic acid, and febuxostat

Ligand	ESOL class solubility	Ali class solubility	Silicos-IT class solubility	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Lipinski's rule of five #violations
MG	Very soluble	Soluble	Soluble	No	No	No	No	No	No	0
GA	Very soluble	Soluble	Soluble	No	No	No	No	No	Yes	0
F	Moderately soluble	Moderately soluble	Moderately soluble	No	Yes	Yes	Yes	No	No	0

MG: Methyl gallate, GA: Gallic acid, F: Febuxostat, ESOL: Estimated solubility, BBB: Blood–brain barrier, CYP: P450 cytochromes

implies that although some ligands have the same position in a binding pocket, amino acid residues that will interact with each other may not necessarily be the same.[38]

Amino acid residues that have a significant role in the activity of a receptor or often called a "hot spot" are often identified as being in a specific binding pocket, where all the amino acids contained in it are generalized to have an equivalent role.[39,40] The observations confirm that even though they are in the same pocket binding, each amino acid residue has different roles and influences. The visual observation in Figure 4 shows that the presence of the methyl group on methyl gallate determines the interaction at that position, where the interaction did not occur in gallic acid because of the absence of the methyl. The key amino acid that binds to the methyl ester group in the methyl gallate is glycine at 799, where the interaction does not occur with the gallic acid or the reference ligand. Interactions that occur involve van der Waals interactions between methyl groups and side chains of glycine. Interestingly, the interaction at that position also does not occur on hypoxanthine or febuxostat. In other words, if the methyl group is replaced by another larger group like ethyl in ethyl gallate, it is more likely that the affinity for the binding pocket of the xanthine oxidase will be even higher. In the end, gallic acid may have higher antioxidant activity than methyl gallate on most pathways of action mechanisms, but not on the xanthine oxidase pathway. This result also opens the opportunity that methyl gallate might also be developed in the treatment of hyperuricemia, especially those related to the inhibition of xanthine oxidase.

The results of the identification of ADME properties show interesting results, where although most of them show similarities, there are several different parameters of ADME properties between methyl gallate and gallic acid, as well as febuxostat as a comparison for 3NRZ receptors. Both methyl gallate and gallic acid are more easily soluble in water than febuxostat, while the three compounds are also predicted to not pass through the BBB. The most striking difference is shown in the metabolism properties, where febuxostat can inhibit CYP1A2, CYP2C19, and CYP2C9; gallic acid can inhibit CYP3A4; while methyl gallate does not inhibit all. These results imply that the metabolic profile of methyl gallate is slightly better than gallic acid.[27] The three compounds also fulfill the element of druglikeness, in the absence of a violation of Lipinski's rule of five. The complete results of identifying the ADME properties of methyl gallate, gallic acid, and febuxostat are presented in Table 3.

Computational studies of gallic acid and its derivatives are quite rare, at least what has been done is to link them with anticancer receptors as Bcl-xL inhibitors. Even then the results obtained indicate that both gallic acid and methyl gallate have the poor potential to be developed as Bcl-xL inhibitors.[41] With this research, it is hoped that it can pave the way for other studies aimed at developing the potential of methyl gallate as a potent antioxidant. This study also provides an explanation of the antioxidant mechanism of methyl gallate as reported by Ekaprasada which proves the antioxidant activity of methyl gallate.[31]

CONCLUSION

This study has succeeded in showing some similarities in the types of interactions that occur in methyl gallate and gallic acid in some antioxidant receptors, with the highest affinity shown on the xanthine oxidase receptor. Although, in general, the antioxidant activity of methyl gallate is still lower than gallic acid, in the xanthine oxidase pathway, methyl gallate shows better potential as an antioxidant. Furthermore, isolation and derivatization of methyl gallate from plant extracts such as from *M. casturi* can be done to optimize the desired antioxidant activity. The results obtained also reveal the fact regarding the relationship between amino acid residues and binding pocket hot spots. Further research with the method of derivatization can be done to find methyl gallate derivatives that have better antioxidant potential on the xanthine oxidase pathway. In addition, the potential shown by methyl gallate does not only act as an antioxidant but also can be developed as one of the alternative compounds for the treatment of hyperuricemia. However, since the interaction between the ligand and the receptor is not only caused by a functional group but also by several other factors, further testing of other compounds with a profile that resembles gallic acid and its derivatives needs to be done. One of them is by setting criteria for each of the target receptors then comparing it with antioxidant compounds that have been tested *in vitro* to have activity on each receptor. These results can later sharpen the predictions produced, especially for gallic acid derivatives which have the potential activity of each antioxidant receptor.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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