Antioxidant Analysis of Kawa Daun (*Coffea canephora*) Beverage by *In Vitro* and *In Silico* Approaches

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Abstract: *In Tanah Datar Regency, West Sumatra, Indonesia, the waste of pruning coffee leaves (*Coffea canephora*) is utilized as a traditional beverage called "Kawa Daun". For a consistent quality of Kawa Daun functional beverage, we evaluated the effect of different smoking times (0, 2, 4, and 6 h) on its* in vitro *DPPH antioxidant activity. Estimation of antioxidant components from the coffee leaf was conducted* in silico *using Peroxiredoxin V (PrxV) with 3MNG code as a receptor, and 33 phytochemicals were reported to be present in the coffee leaves as ligands. As a result, Kawa Daun, with a 2-h smoking time, had the highest antioxidant activity. Molecular docking between PrxV and the 33 compounds resulted in the ten most potential compounds based on the affinity energy. They were xanthone (-4.9 kcal/mol), uric acid (-4.8 kcal/mol), xanthosine (-4.8 kcal/mol), caffeine (-4.6 kcal/mol), 3-methylxanthine (-4.6 kcal/mol), 7-methylxanthosine (-4.6 kcal/mol), theobromine (-4.5 kcal/mol), theophylline (-4.5 kcal/mol), caffeic acid (-4.5 kcal/mol), and xanthine (-4.4 kcal/mol). These ten ligands had stronger interactions than the control ligand 1,2-dithiane-4,5-diol (-3.6 kcal/mol). This research showed the potential of Kawa Daun as a functional beverage with antioxidant activity. Further confirmation on the antioxidant potential of this beverage using an* in vivo *method is recommended.*

Keywords: *functional beverage; peroxiredoxin V; coffee leaves; Kawa Daun; smoking time*

■ INTRODUCTION

Pruning the leaves of coffee plants is a common practice in coffee cultivation to stimulate the development of coffee beans, which leads to a shorter harvesting period. Coffee leaves from pruning are typically discarded as waste. The farmers in Batipuh Ateh, Tanah Datar Regency, West Sumatra Province, Indonesia, traditionally utilize the wasted coffee leaves, particularly the Robusta variety (*Coffea canephora*), in a traditional beverage known as "*Kawa Daun*." The term "*Kawa*" is derived from an Arabic term defined as brewed water from the coffee leaves. The distinctiveness of this beverage is in the drying methods of coffee leaves. There are two drying methods commonly used by the local *Kawa Daun* industries: (1) using a *cabinet dryer* with a mechanically controlled temperature and (2) the traditional way by smoking the leaves in a fireplace of cinnamon wood for 2–4 h [1]. Empirically, Minang society believes that *Kawa Daun* beverage positively impacts their health. They feel that their bodies become fitter, stronger, and warmer after consuming the *Kawa Daun* beverage. Several studies reported the potency of functional characteristics of *Kawa Daun* since it contains bioactive components for antioxidants, such as 3-methylxanthine, xanthine, and theophylline [2], 7-methylxanthosine, xanthosine [3], caffeic acid [4], uric acid [5], caffeine, xanthone, and theobromine [6].

Most traditional *Kawa Daun* producers in Tanah Datar Regency produce the *Kawa Daun* beverage with inconsistent quality. Thus, the effect of different smoking times (0, 2, 4, and 6 h) on antioxidant activity must be evaluated. It is difficult to predict and identify the bioactive components of coffee leaves that are most beneficial as antioxidants. *In silico* is a computational method that can be implemented for such a purpose. *In silico* can provide preliminary information that can be utilized as the foundation for future strategy in developing *Kawa Daun* as a functional beverage. Target proteins (receptors) of Peroxiredoxin V (PrxV) with 3MNG code are used to investigate the antioxidant potential of the Kawa Daun beverage. Peroxiredoxin (Prxs) is an essential peroxidase involved in antioxidant and redox signaling protection in humans. It has a very high catalytic efficiency which dominates peroxide reduction in all body cells. At 1.45 A° resolution, the new structure of human PrxV has a dithiothreitol bond in the active site, with its diol moiety emulating the two oxygens of the peroxide substrate. This structure classifies the diol and related dioxygen as a new competitive inhibitor for Prxs. The PrxV protein developed into a suitable and efficient receptor to be used as the target receptor of antioxidant activity in the human body [7].

Protein function targeting is accomplished by substituting the native ligand of PrxV with 33 bioactive components previously reported to be identified in the coffee leaves as tested ligands. The binding mode of the ligand-receptor complex is interpreted by comparing the tested ligand to the original ligand as a control, specifically the competitive antioxidant inhibitor 1,2-dithiane-4,5-diol (D1D). The molecular docking method can predict the proper ligand by obtaining affinity energy and bond stability between the receptor and the ligand [7]. Molecular docking is a tool for studying the interactions within a molecular complex. Hydrophobic and hydrogen interactions classify interactive positions that will be potential ligand-binding sites at each possible position. *In silico* research on coffee leaves, particularly associated with antioxidant compounds, has never been conveyed. The interaction between methyl gallate with various antioxidant receptors was predicted while comparing it with gallate acid as the main compound. The results showed that the affinity energy for the 3MNG receptor was 3.6 kcal/mol, which indicates that the methyl gallate had the potential as an antioxidant [8]. Another experiment showed that molecular docking on the meglumine catalyst exhibited the best docking results on the compound of 3,3'-((3,5-dimethoxyphenyl) methylene)bis(1*H*-indole), which had the strongest binding to 3MNG with an affinity energy of -7.6 kcal/mol. The binding energy value correlates with the highest value of the antioxidant activity, which was 78.48 µg/mL [9]. Meanwhile, *in vitro* antioxidant power of Robusta coffee leaves with water fractions at several concentrations, i.e., 100, 80, 60, 40, and 20 ppm, was reported. The IC_{50} value was 73.62 ppm [10-11].

The combination of *in silico* and *in vitro* antioxidants is crucial to obtain more accurate data results regarding the potential of *Kawa Daun* beverage as a functional beverage with the best antioxidant potential, as well as the bioactive components of coffee leaves that play the most crucial role as antioxidants. This research aimed to find the smoking time which gave *Kawa Daun* the highest antioxidant activity and to identify the possible antioxidant compounds. The fresh coffee leaves were smoked using a cinnamon fireplace at different smoking times. The *in vitro* antioxidant activity of *Kawa Daun* methanolic extracts was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In parallel, a literature study was conducted to list phytochemicals previously identified in the coffee leaves. *In silico* study was then conducted to analyze the interaction and the affinity energy of those bioactive components in coffee leaves as ligands to the target protein of PrxV (receptor) using molecular docking.

■ EXPERIMENTAL SECTION

Materials

Fresh coffee leaves of Robusta variety (*C. canephora*) were harvested from Mus's coffee plantation, Tanah Datar, West Sumatra, Indonesia, from August to January 2021. Only broad, green, and young and old aged leaves in pseudostem parts were selected. The coffee leaves were washed before being subjected to the

subsequent treatment. Cinnamon wood for the fireplace was freshly harvested from the Batipuh Ateh forest, Tanah Datar, West Sumatera, Indonesia. The chemicals used were DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, dimethyl sulfoxide (DMSO), and ascorbic acid. They were all analytical grade and acquired from Sigma-Aldrich (Darmstadt, Germany).

For *in silico* study, protein target 3D structure (receptor) of Peroxiredoxin V (PrxV) utilizing PDB code (3MNG) was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB), Protein Data Bank (PDB) (https://www.rcsb.org/) sites, which is in the .pdb format. Table 2 displays the 33 tested ligands. The D1D was used as a control ligand. All ligands acted as oxidation inhibitors and were obtained in the form of SDF from the PubChem database of chemical molecules (https://pubchem.ncbi.nlm.nih. gov/). The personal computer specification for *in silico* study was as follows: Intel 2Core N3350 2.4 Ghz, Intel HD Graphics, Endless OS, Windows Operation System 10 64-bit. The software used in this research comprised USCF Chimera 1.13.1, VMD version 1.9.3, Avogadro, Open Babel version 2.3.2, Autodock Tools (ADT) version 1.5.6, LigPlot+ version 2.1, and PyMOLMolecular Graphics System Version 2.4.0.

Procedure

Kawa Daun preparation [12]

Coffee leaves were smoked on a cinnamon wood fireplace with different smoking times (0, 2, 4, and 6 h at 70 °C). The 0-h sample (control sample) was the coffee leaves dried in a cabinet drier at 70 °C for 8 h. In a waring blender, all the dried coffee leaves were ground into very fine powder. The powder was then stored in airtight plastic containers. To prepare the *Kawa Daun* beverage, the powdered leaves were mixed with freshwater (1:100 b/v), boiled (100 °C, 15 min), and filtered. The resulting beverage was stored in a dark bottle and kept cold in the refrigerator (8 °C). For *in vitro* antioxidant activity measurement, the *Kawa Daun* beverage was dried in a freeze dryer for 72 h and stored in the freezer (-10 °C) before the measurement.

Antioxidant activities [13]

One hundred microliter of Kawa Daun solution at different concentrations (20, 40, 60, 80, and 100 ppm) were pipetted into 96 wells microplate, added by 100 µL DPPH (125 µM in methanol) incubated at room temperature in the dark condition (30 min). The absorbance reduction was measured using a microplate reader at 517 nm. The measurement was repeated two times.

$$\text{Percentage of antioxidant activity} = \frac{\left[\left(AB-AKB\right)-\left(AS-AKS\right)\right]}{\left(AB-AKB\right)}\times 100\%$$

where AB is blank absorbance, AKB is absorbance control blank, AS is sample absorbance, and AKS is sample control absorbance.

In silico *computation [14]*

The first step was gathering data on the bioactive components found in coffee leaves (*Coffea canephora*) based on the literature review. The obtained data were used to create ligand compounds, then examined via molecular docking. The molecular docking was carried out on the ligand test and the native ligand of D1D (from the 3D structure of 3MNG) was employed as a control. The second step involved preparing ligand tests using a Pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The database contained 33 molecular structures of tested ligands in SDF files. The tested ligand molecules were geometrically adjusted to resemble their natural condition using OCRA2 software [15]. Peroxiredoxin V (PrxV) ID: 3MNG was acquired as the target protein molecules (receptor), which was taken from Protein Data Bank (https://www.rcsb.org/). The receptor docking was repeated using a native ligand of D1D to recognize the coordinate of the receptor's active site. The system was prepared using Chimera 1.14.0 version (https://www.rbvi.ucsf.edu/chimera) [16]. The target protein was reduced, then segregation was performed as a ligand and receptor using USCF-Chimera software version 1.14.0; furthermore, it was planned to get rid of the free ion and water included in the protein's initial structure. The magnetic field and water molecules were

also eliminated after the native ligands were combined with hydrogen atoms to create an electric charge. The third step consisted of molecular docking. Ligand and receptor whose initial form was .pdb were converted to .pdbqt using AutoDock Tools 1.5.6. The grid box dimensions were set at x = 15, y = 15, and z = 15. The x, y, and z docking centers of 91.788, 91.838, and 104.806, respectively, were used so that the active site where the ligand binding took place was appropriately positioned. The database then used Autodock Vina to perform molecular docking. The results of the top 10 ligands-receptor that had the most potential as antioxidants were seen based on the interactions and the resulting affinity energy. The top 10 tested ligands were finally visualized in the fourth stage using PyMOL Molecular Graphics System Version 2.4.0 (https://pymol.org/) [17] and LigPlot [18]. The pharmacokinetic properties of the ligand with the lowest values (most negative) would be evaluated using SwissADME (http://www.swissadme.ch/) based on Lipinski's rules. This website allows us to compute physicochemical descriptors and predict ADME parameters (absorption, distribution, metabolism, elimination), pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery. The complex bond of a receptor with the native ligand as the comparison (control) and the complex bond of a receptor with the ten most robust tested ligands were simulated.

■ RESULTS AND DISCUSSION

Antioxidant Activity

The antioxidant activity of *Kawa Daun* methanolic extract obtained from four different smoking times ranged from 22.58 to 81.40% (Fig. 1). Sample B (2 h smoking) had the highest antioxidant activity among other fractions at all concentrations tested, followed by sample A (the unsmoked control). In contrast, sample D (6 h smoking) had the lowest antioxidant activity. Samples A and B had a shorter high-temperature exposure, resulting in minimal damage to the bioactive components in the coffee leaves.

The IC_{50} results of the *Kawa Daun* beverage from each treatment are presented in Table 1. Samples A and B had the lowest IC_{50} value (< 20 ppm). The compound is said to have very strong antioxidant activity if the IC_{50} value is < 50 ppm, strong if it is 50 to 100 ppm, moderate if it is 100 to 150 ppm, and weak if it is 150 to 200 ppm [19]. Thus, samples A and B are classified as having very

Table 1. IC_{50} values of *Kawa Daun* beverage

Smoking duration	IC_{50} (ppm)
A (0 h)*	< 20
B (2 h)	< 20
C (4 h)	53.56 ± 0.04
D (6 h)	64.37 ± 0.74
Vitamin C (control)	4.48 ± 0.01

(*)Drying in cabinet dryer (70 °C, 8 h)

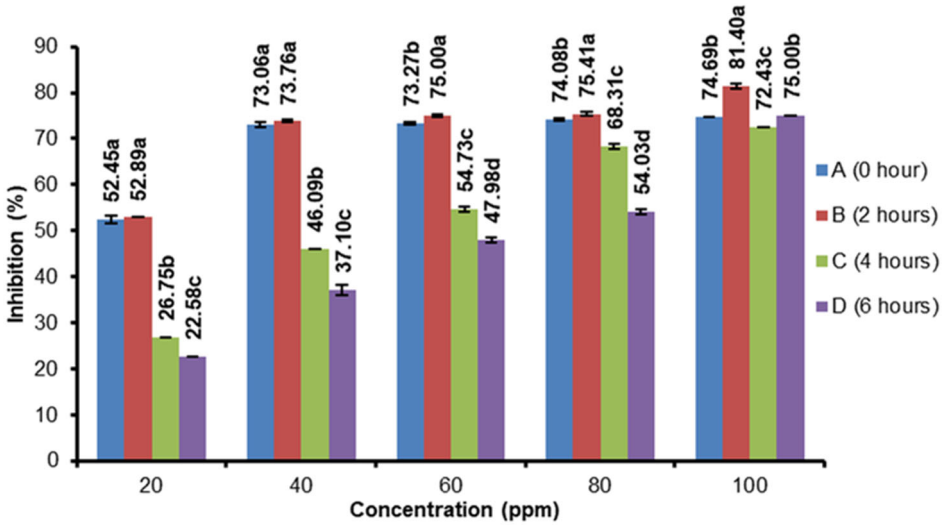

Fig 1. DPPH antioxidant activity profile of *Kawa Daun* beverage prepared with different smoking time

strong antioxidant activity. In contrast, sample C and D with the IC_{50} values of 53.56 and 64.37 ppm is classified as having strong antioxidant activity. The difference between percentage values for inhibition and IC_{50} values is due to the smoking time. The longer coffee leaves are smoked, the more likely it is that the bioactive substance of the coffee leaves will be damaged, affecting antioxidant activity. Several bioactive compounds are extremely temperature-sensitive and unstable.

The effect of temperature on bioactive components was reported–an increase in temperature that is not constant causes an increase in phenolic concentration to a specific temperature. However, the phenolic concentration then decreases with increasing smoking time and higher temperature [20]. The result was also in agreement with a previous study which reported that *in vivo* antioxidant activity of the coffee bean significantly decreased with the longer roasting time [21]. In another report, six flavonoids were reported to have antioxidant activities. The compounds, which consisted of rutin, naringin, eriodictyol, mesquitol, luteolin, and luteolin 7-O glucoside, were exposed to heat treatment (70–130 °C, 120 min). It was found that all were degraded at a different speed. The level of degradation increased with a higher temperature and longer heating duration [22]. Several factors may influence the phytochemical composition of plant samples and their biological activity, such as varieties, environmental changes during growth and harvest, post-harvest treatment, extraction, and sample preparation methods [23]. Sample preparation methods such as smoking and the brewing *Kawa Daun* beverage are also suggested to influence their antioxidant activity. The most recent study showed that DPPH radical scavenging activity, total phenolic content, and total procyanidins of C*offea arabica* L. leaf were significantly increased with a higher drying temperature [24].

Molecular Docking

As previously discussed, coffee leaves contain bioactive components that act as antioxidants. A recent study mentioned that bioactive components in coffee leaves come from the alkaloid group, i.e., caffeine, theobromine, theophylline, and trigonelline. It also comes from the phenolic compound group, i.e., caffeic acid, ferulic acid, sinapic acid, and chlorogenic acids (3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA). Quercetin, catechins, and kaempferol are examples of bioactive flavonoid phenolic compounds. Other phenolic compound group's bioactive components include xanthones, such as mangiferin and isomangiferin [25]. Because of the current advancements in science and technology, the exploration of the bioactive components present in coffee leaves focuses not only on the refreshing impact but also on the potential for antioxidant content, which can be detected early using *in silico* method. *In silico* method can be used to support the data resulting from *in vitro* experiment, or vice versa. In our study, *in vitro* method was used to provide initial information on the antioxidant activity of the *Kawa Daun* beverage. *In silico* method was then used to obtain detailed information on which compounds might associate with the antioxidant activity of the beverage.

Molecular docking is used to examine the bioactivity of coffee leaves in the ligand and receptor complex. Molecular docking is essential for determining the conformation of the tested ligand interaction on the active site of the target protein receptor, as well as determining which tested ligand has the most significant negative affinity energy and interacts well with the target protein receptor. The human antioxidant receptor Peroxiredoxin V (PrxV) is chosen. It comprises essential cysteine residues as catalysts and thioredoxin as electron donors, which help scavenge peroxides and cellular metabolic responses to reactive oxygen species in the human body [7].

The docking results of 33 tested ligands and one native (control) ligand are well-ordered based on the ligand's binding strength to the receptor (Table 2). In Table 2, the top 10 ligands strongly bind the receptor. The binding strength is determined by whether the affinity energy value (kcal/mol) is more significant than the affinity energy value of the native ligand (control). If the affinity energy value is more negative, the ligand-receptor binding will be stronger. When the binding affinity energy is negative, it means that the bioactive

Table 2. The list of tested ligands and results of molecular docking between ligands and PrxV

No	Compounds identified in coffee leaves	References	PubChem CID	Affinity (kcal/mol)	Kd (M)
1	Xanthone	[6]	7020	-4.9	3.33E-04
2	Uric Acid	[5]	1175	-4.8	3.92E-04
3	Xanthosine	[3]	64959	-4.8	4.09E-04
4	Caffeine	[2,5,25]	2519	-4.6	5.23E-04
5	3-Methylxanthine	[2,5]	70639	-4.6	5.45E-04
6	7-Methylxanthosine	[3]	23724732	-4.6	5.68E-04
7	Theobromine	[2,6]	5429	-4.5	6.16E-04
8	Theophylline	[2,5]	2153	-4.5	6.16E-04
9	Caffeic Acid	[4]	689043	-4.5	6.42E-04
10	Xanthine	[2,5]	1188	-4.4	7.26E-04
11	Hypoxanthine	[6]	135398638	-4.4	7.26E-04
12	Methylxanthine	[2]	80220	-4.4	7.26E-04
13	7-Methylxanthine	[5]	68374	-4.4	7.26E-04
14	Allantoic	[5]	203	-4.4	7.57E-04
15	Allantoin	[5]	204	-4.3	8.56E-04
16	Ferulic acid	[11]	445858	-4.3	8.92E-04
17	Trigonelline	[24]	5570	-4.2	1.01E-03
18	Xanthosine-5-monophospate	[3]	73323	-4.2	1.05E-03
19	D1D	[7]	3MNG	-3.6	2.70E-03
20	5-caffeoylquinic acid (5-CQA)	[24]	5280633	-3.4	4.08E-03
21	Quercetin	[24]	5280343	-3.3	4.42E-03
22	3-caffeoylquinic acid (3-CQA)	[24]	102111217	-2.8	1.09E-02
23	Chlorogenic Acid	[24]	1794427	-2.7	1.29E-02
24	4-caffeoylquinic acid (4-CQA)	[24]	9798666	-2.4	1.94E-02
25	Mangiferin	[24]	5281647	0.0	1.00E+00
26	3,4-dicaffeoylquinic acid (3,4-diCQA)	[24]	5281780	2.8	9.16E+01
27	Dicaffeoylquinic acid (diCQA)	[24]	12358846	3.5	3.14E+02
28	3,5-dicaffeoylquinic acid (3,5-diCQA)	[24]	6474310	5.5	8.39E+03
29	Hyperoside	[24]	5281643	6.4	3.83E+04
30	4,5-dicaffeoylquinic acid (4,5-diCQA)	[24]	10324242	8.9	2.33E+06
31	Isomangiferin	[24]	5318597	13.9	8.24E+09
32	Rutin	[24]	5280805	26.3	5.54E+18

component of coffee leaves used as the tested ligand requires less energy to attach or interact with the receptor. After all, if the resulting affinity energy value is lower/negative, the tested ligand has a higher chance of interacting with the protein receptor [26], as can be seen in Table 2.

Table 2 reveals that 32 bioactive components serve as ligands in the human receptor of Peroxiredoxin V (PrxV). Several compounds had more negative affinity energy than the D1D control ligand, namely xanthone, uric acid, xanthosine, caffeine, 3-methylxanthine, 7-methylxanthosine, theobromine, theophylline, caffeic acid, xanthine, hypoxanthine, methylxanthine, 7-methylxanthine, allantoic, allantoin, ferulic, trigonelline, xanthosine-5-monophospate (XMP), hydroxycinnamic acid, and sinapic acid. The affinity of a small molecule ligand for a macromolecule receptor is commonly used to define a compound's biological activity. Factors that may affect the ligand's affinity include electrostatic interactions between the ligand and acceptor, the

contributions of solvation and desiccation, and the steric complementation of the two binding partners. Changes in the number of degrees of freedom and changes in the structure of the ligand and acceptor during complex formation generate additional effects. Understanding the determining enthalpic and entropic contributions to binding is an initial step for predicting affinity [27].

Virtual simulation can be used to understand the characteristics of a molecule based on its structure and microscopic interactions between other molecules. Most ligand-binding interactions can be classified as binding affinity or affinities. The affinity value reflects whether a ligand can be tethered or connected to the receptor. At the affinity energy, the strength of the binding interaction between the protein and its ligand can be observed. The more negative value gives the greater chance of ligand to bind to the receptor, and vice versa. After acquiring the affinity energy, the tethering results are analyzed using the Ligplot software. Ligplot is a computer program that generates 2-dimensional (2D) graphics by automatically representing the interaction of protein-ligands [28]. Fig. 2 depicts the 2D structure of the top 10 most robust ligands with antioxidant capability.

Visual analysis is performed by comparing the positions and interactions between the ligands and protein residues on the PrxV receptor. The analysis is carried out to observe the type of interaction and the residues involved. PyMOL software was used to see the binding distance. Generally, the ligands have occupied the appropriate active site at the PrxV receptor. A comparison of the molecular binding results between the top 10 ligand compounds and the most negative PrxV receptor can be seen in Table 3. Amino acid residues that interact using hydrogen bonding on native ligands are Glycine 46 and Threonine 44. The same things were also found in some test ligands such as caffeic acid hydrogen-bonded amino acid residues, such as Threonine 147 and Arginine 127. Based on the results of the hydrophobic interaction analysis, there was no significant difference between the tested and native ligands; the amino acids that appeared mainly were the same.

The affinity energy produced by the D1D-PrxV native ligand complex is -3.6 kcal/mol, which is lower than the energy produced by the top ten strongest PrxV-ligands. The lower energy value suggests that the natural D1D ligand has a weaker binding than the top ten ligand-receptor complexes, namely xanthones, uric acid, xanthosine, caffeine, 3-methylxanthine, 7-methylxanthosine, theobromine, theophylline, caffeic acid, and xanthine. Table 2 shows that the interaction between the xanthone complex and the PrxV receptor has the most negative affinity energy value (-4.9 kcal/mol) and the lowest Kd value (3.33E-04). Based on

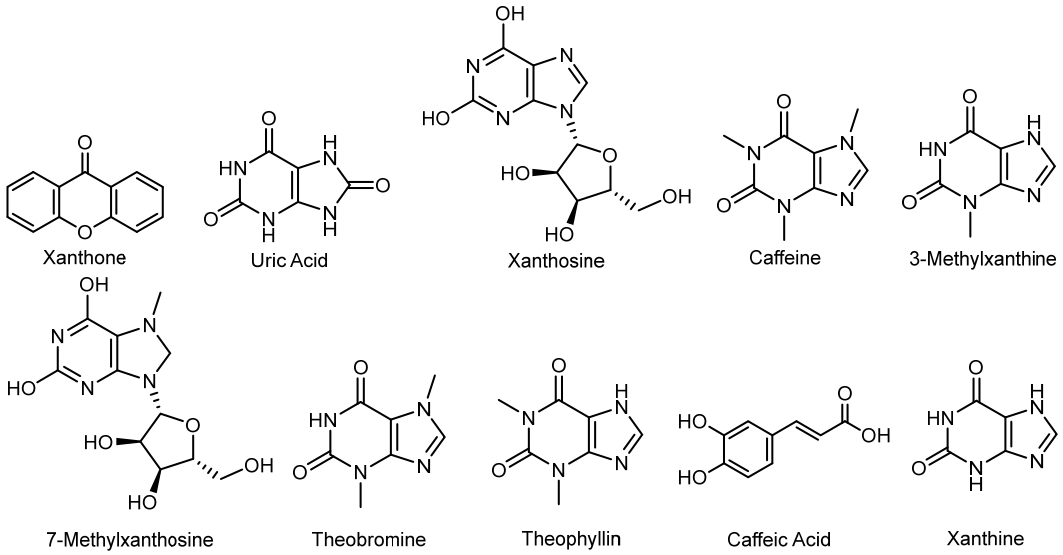

Fig 2. The 2D Structure of top 10 antioxidant ligands

Table 3. Comparison of strongest ligand-receptor complex compounds interaction

No	Complex compound ligand-receptor	Affinity (kcal/mol)	Hydrophobic interactions		Hydrogen bond	
			Total interactions	Amino acid residues	Total bond	Amino acid residues
1	Native ligand D1D-PrxV (Control)	-3.6	6	Proline 40, Threonine 147, Threonine 44, Phenylalanine 120, Cysteine 47, Arginine 127	2	Glycine 46, Threonine 44
2	Xanthone-PrxV	-4.9	7	Arginine 127, Leucine 116, Leucine 149, Glycine 46, Phenylalanine 120, Isoleucine 119, Threonine 147	1	Threonine 147
3	Uric acid-PrxV	-4.8	4	Proline 45, Phenylalanine 120, Threonine 147, Threonine 44	5	Threonine 44, Threonine 147, Glycine 46, Arginine 127, Cysteine 47
4	Xanthosine-PrxV	-4.8	7	Proline 45, Phenylalanine 120, Isoleucine 119, Glycine 148, Glycine 46, Leucine 149, Threonine 44	3	Threonine 44, Threonine 147, Arginine 127
5	Caffeine-PrxV	-4.6	6	Leucine 116, Leucine 149, Proline 40, Phenylalanine 120, Threonine 44, Threonine 147	3	Glycine 46, Threonine 147, Arginine 127
6	3-Methylxanthine-PrxV	-4.6	3	Cysteine 47, Phenylalanine 120, Threonine 147	4	Glycine 46, Cysteine 47, Threonine 147, Arginine 127
7	7methylxanthosine-PrxV	-4.6	6	Proline 40, Proline 45, Leucine 149, Glycine 46, Cysteine 47, Phenylalanine 120	3	Threonine 44, Threonine 147, Arginine 127
8	Theobromine-PrxV	-4.5	6	Threonine 147, Proline 40, Proline 45, Cysteine 47, Phenylalanine 120, Leucine 116	3	Glycine 46, Threonine 44, Threonine 147
9	Theophylline-PrxV	-4.5	4	Threonine 44, Threonine 147, Phenylalanine 120, Cysteine 47	3	Threonine 147, Arginine 127, Glycine 46
10	Caffeic acid-PrxV	-4.5	6	Proline 40, Proline 45, Cysteine 47, Isoleucine 119, Phenylalanine 20, Threonine 147	4	Threonine 44, Threonine 147, Arginine 127, Glycine 46
11	Xanthine-PrxV	-4.4	6	Threonine 44, Threonine 147, Phenylalanine 120, Proline 40, Proline 45, Arginine 127	3	Threonine 44, Cysteine 47, Glycine 46

this value, it can be concluded that the hydrophobic interaction and hydrogen bonding between the xanthones ligand and the receptor is firmer as compared to those of other tested ligands, including D1D control. The interaction between rutin and the PrxV receptor, on the other hand, has the highest positive affinity energy value (26.3 kcal/mol) and the highest Kd value (5.54E+18). This high-affinity energy value implies that the hydrophobic interactions and hydrogen bonds formed between the ligand and the receptor are weaker than those of other tested ligands including D1D control.

The visualization results of the molecular docking between the top 10 most potent ligand compounds and the PrxV receptor can be seen in Fig. 3. The involvement of the similar amino acid residue indicates the extent of the ligand-binding tendency to its receptor. The results of the tenth complex of the coffee leave tested ligands showed almost the same binding ability as the D1D control ligands

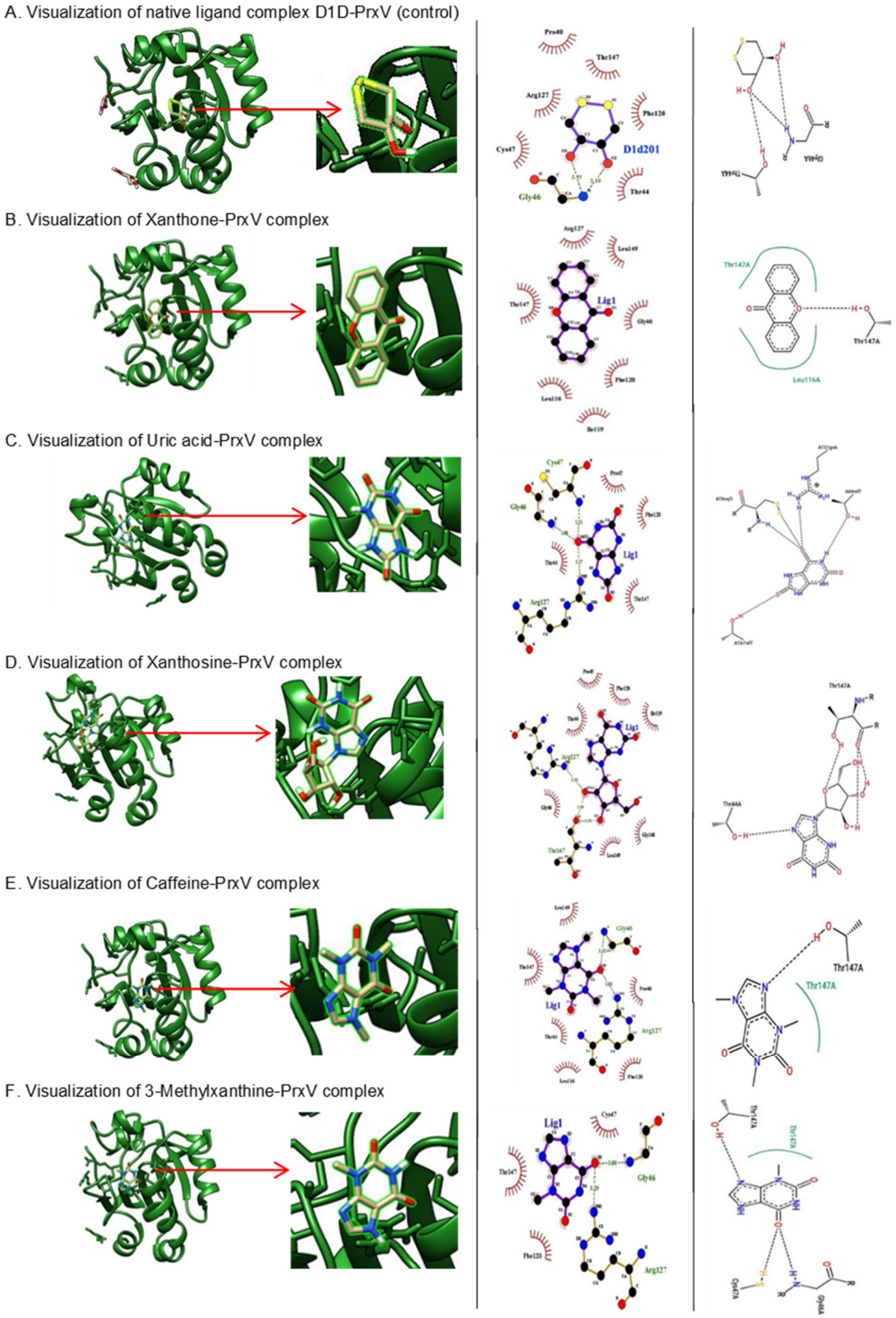

Fig 3. (A-K) Visualization of the 10 strongest ligand-receptor complexes using PyMOL Molecular Graphics System Software Version 2.4.0 and LigPlot and evaluated through www.swissadme.ch

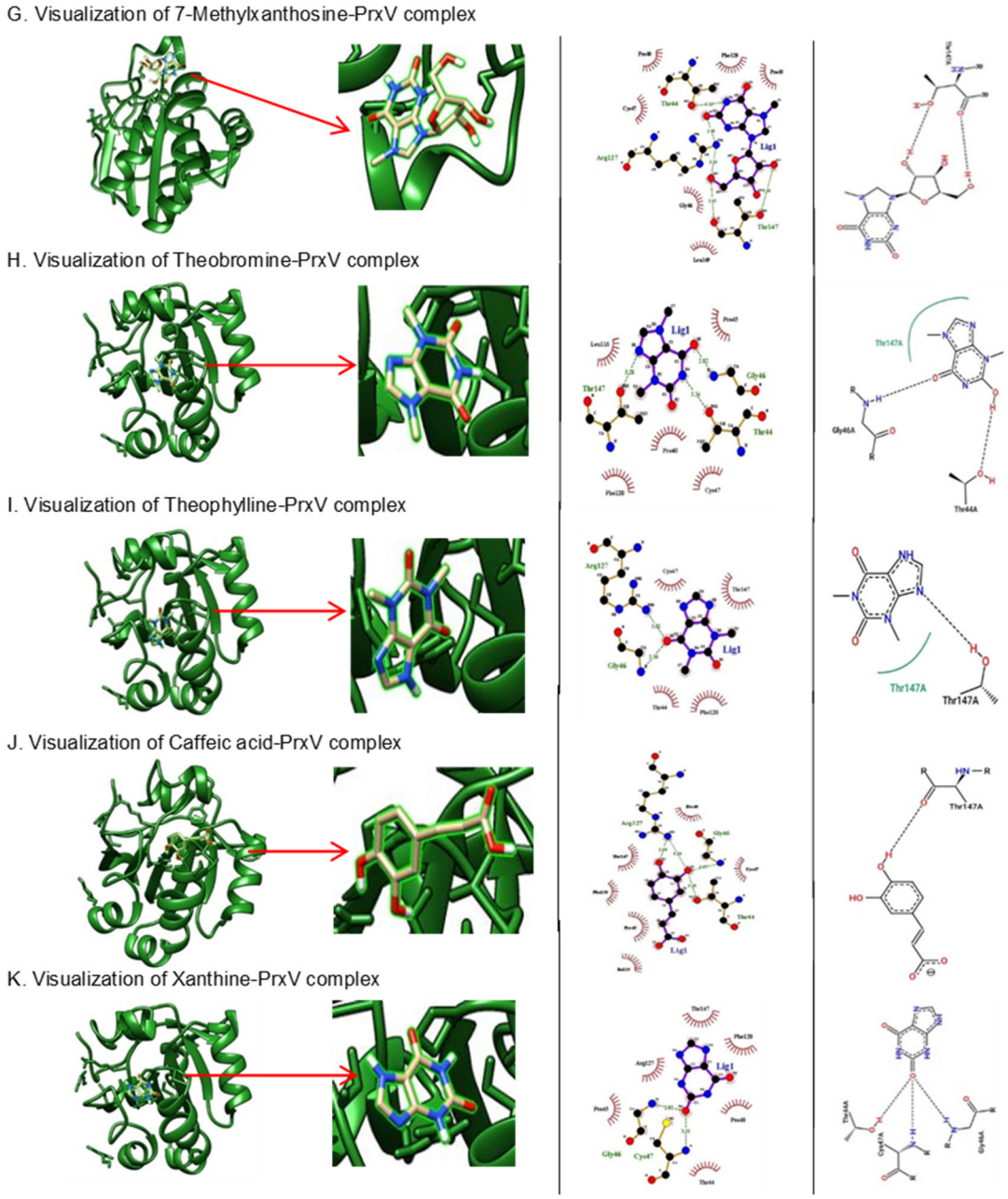

Fig 3. (A-K) Visualization of the 10 strongest ligand-receptor complexes using PyMOL Molecular Graphics System Software Version 2.4.0 and LigPlot and evaluated through www.swissadme.ch (*Continued*)

since they generally had the same binding sites in both hydrogen bonding and hydrophobic interactions. This similarity indicated that the ten bioactive components of coffee leaves have almost the same or even better antioxidant activity than the D1D control ligand. The affinity energy value determines the strength of the ligand-receptor complex interaction. If the affinity energy of the tested ligand is lower than that of the control ligand, the results can be utilized to make an early prediction about the ligand's potential as an antioxidant. The ligands and bound residues are not stationary during the molecular docking simulation. Because the interactions influence each other, both are constantly in motion.

Consequently, these interactions will cause the atom to move from its initial position. The resulting interaction of the ligand and receptor can be either attraction or

repulsion. The bound residue tends to experience a slight shift due to the influence of the ligand [29].

The results of this research imply that the binding of these ten active compounds to the active site of PrxV, namely the active 2-Cys, will lead to an increase in the work selectivity of PrxV in catalyzing the reduction process of hydrogen peroxide substrate (H_2O_2) to become water (H_2O). Due to oxidative stress because of excessive ROS production, endogenous antioxidants must receive additional exogenous antioxidants from outside the body, such as bioactive components in the form of phenolic compounds, alkaloids, and flavonoids, which can be obtained from food or beverage intakes. The bioactive components of coffee leaves, such as xanthones, caffeine, and chlorogenates, can increase the activity of PrxV's enzymatic antioxidant. Coffee leaf's phenol molecules can capture ROS and can form chelates with metals that encourage the generation of ROS, hence preventing lipid peroxidation and DNA damage. The –OH group, which can contribute one molecule of hydrogen to make the ROS stable, is the active group that generally works as a catcher and inhibitor of free radical processes. Many –OH groups in coffee leaf polyphenols are multifunctional. They can react with free radicals as reducing agents, free radical scavengers, metal chelating agents and boosting the work of the PrxV enzyme [30].

Finally, the results of our study emphasize the potential of coffee leaves as a source of antioxidant compounds. However, the composition of these antioxidant compounds might be changed when the leaves are subjected to different processing methods, especially those with prolong exposure to high temperature, as presented in this study. Several other recent reports also indicated similar trends, not only to the antioxidant activity of the coffee leaves but also to its anti-inflammatory activity [31-33]. *In vivo* antioxidant assessment of coffee leaf extract in experimental animals and human studies is also not as extensive as that of coffee beans, which should be highlighted in the future research direction.

■ CONCLUSION

The best antioxidant activity of the *Kawa Daun* beverage was obtained in *Kawa Daun* with smoking times of 0 and 2 h (on the value of IC_{50} < 20 ppm). The results of molecular docking between the PrxV receptor and the studied ligands of 33 bioactive components of coffee leaves reveal that ten compounds have the most significant potential as antioxidants based on the most negative affinity energy, such as xanthone, uric acid, xanthosine, caffeine, 3-methylxanthine, 7-methylxanthosine, theobromine, theophylline, caffeic acid, and xanthine with affinity energy of -4.9, -4.8, -4.8, -4.6, -4.6, -4.6, -4.5, -4.5, -4.5, and -4.4 kcal/mol, respectively. Those top 10 ligands have more vital interaction from the control ligand of D1D, of which ligand has affinity energy (-3.6 kcal/mol). This study provided scientific evidence for using *Kawa Daun* is a traditional functional beverage. A short smoking time (2 h) is recommended for better antioxidant performance of the beverage. Further research to confirm the potential antioxidant of *Kawa Daun* by *in vivo* method is recommended.

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