Original Article

TIPS Trends in Pharmaceutical Sciences 2023: 9(2): 113-126.

Comparison of MMP-9 Inhibition Activities of Phenolic Acids of *Sandoricum koetjape* Leaves by Molecular Docking

Susy Saadah[1*]; PhD, Silvester M. Tulandi[1]; MSc, Randi A. Rohman[2] ;MSc

[1]Department of Pharmaceutical and Food Analysis, Poltekkes Kemenkes Jakarta II, Indonesia
[2]Department of Biotechnology, Plant Production and Biotechnology Division, PT SMART Tbk, Indonesia.

Abstract

Sandoricum koetjape has been used for generations in traditional Indonesian medicine. The leaves were used to treat helminthiasis, cough, stomachache, diarrhea, bloating, leucorrhoea, colic, and fever in Indonesia. Identification of phenolic acids in the *Sandoricum koetjape* leaves was done by ultrahigh-pressure liquid chromatography (UPLC). Gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid were identified as phenolic acids found in *Sandoricum koetjape* leaf extracts. Heart disease, stroke, and cancer are the three noncommunicable diseases that kill the most people in Indonesia. Coronary artery disease, cardiovascular disease, cardiomyopathy, cancer, tumor, type 2 diabetes, and cholesterol have all been linked to MMP-9. This study aimed to determine the phenolic acids contained in the leaves of *Sandoricum koetjape* and to determine their inhibitory activity against the matrix metalloproteinase-9 (MMP-9). Molecular docking studies were carried out by the autodock 4.2 program integrated with the pyrx v.09.8 virtual screening tool. The chlorogenic acid in Sandoricum koetjape leaf extract binds more strongly than the other phenolic acids. Interacting between chlorogenic acid with MMP-9 on amino LEU187, LEU188, ALA189, HIS405, and TYR423. AdmetSAR and Protox II databases were used for physiochemical and ADMET properties. Chlorogenic acid is expected to have high oral bioavailability in humans, good intestinal absorption, and an equivalent distribution in the intestine and blood plasma. Chlorogenic acid's acute toxicity is also expected to be low. Chlorogenic acid is also non-toxic to the liver, immune system, mutagenic, and cytotoxic. *Sandoricum koetjape* phenolic acid, particularly chlorogenic acid, appeared to be an efficient MMP-9 inhibitor based on docking results.

Keywords: Chlorogenic acid, MMP-9, Molecular docking, Phenolic acids, *Sandoricum koetjape*.

Please cite this article as: Saadah S, Tulandi S.M, Rohman R.A. Comparison of MMP-9 Inhibition Activities of Phenolic Acids of *Sandoricum koetjape* Leaves by Molecular Docking. Trends in Pharmaceutical Sciences. 2023;9(2):113-126. doi: 10.30476/TIPS.2023.97645.1180

1. Introduction

Traditional medicines have been utilized for centuries to maintain health and treat a variety of diseases (1). Indonesia, as a mega biodiversity country, has a diverse range of medicinal plants (2). One of the traditional medicinal plants that are used in Indonesia is *Sandoricum koetjape* (3). The *S. koetjape* is taxonomically classified in the family *Meliaceae* (3). This medicinal plant is called Santol or Kecapi and has been traditionally used for the treatment of helminthiasis, cough, stomachache, diarrhea, bloating, leucorrhoea, colic, and fever, and is also consumed as a tonic after giving birth (3).

Sandoricum koetjape tissue used in this study was the leaf. Photosynthesis is the process by which plants synthesize food (4). The leaves of the *Sandoricum koetjape* are compound in alternating forms, stems up to 18 cm, pinnate with three leaflets, oblong to egg-shaped, rounded or slightly pointed at the base, tapering at the tip;

Corresponding Author: Susy Saadah, Department of Pharmaceutical and Food Analysis, Poltekkes Kemenkes Jakarta II, Indonesia.
Email: susy.saadah@poltekkesjkt2.ac.id

Received: 20/01/2023; Accepted: 01/06/2023

glossy green above, dull green below. The leaflets are long-stemmed, much longer than the side leaves (3). Many scientific studies on medicinal plants have been performed to support and discover the medicinal effects and mechanism of action based on scientific evidence (1). *Sandoricum koetjape*'s bioactivities have been reported in several scientific studies. *Sandoricum koetjape* seeds extract has inhibitory activity against P-388 leukemia cells (5). Furthermore, the total phenol content in the stems and leaves of *Sandoricum koetjape* was 1.4155 mg/g and 3.1469 mg/g, respectively (6). The major volatile compounds of the leaf extract of *Sandoricum koetjape* were 14,15-didehydro-cyclodecacyclotetradecene, 1H-cycloprop[e]azule-7-ol, and solanesol (7). The leaf of *Sandoricum koetjape* has xanthine oxidase inhibitory and antioxidant activities (8). *Sandoricum koetjape* fruit peel ethanol extract has anti-gingival inflammation properties after scaling (9). *In vitro* studies showed the pharmacological potential of *Sandoricum koetjape* extract including antioxidant, antibacterial, anticancer, antitumor, and insecticide activities (10). *Sandoricum koetjape* also has anti-inflammatory activity against tetradecanoylphorbol acetate (TPA) (11, 12). *Sandoricum koetjape* stem extract as anti-fungi activity against *Candida albicans* (13)

Phenolic acid is composed of phenol (an aromatic ring with at least one hydroxyl substituent) and at least one organic carboxylic acid (14). Phenolic acids are common plant metabolites with bioactive properties that find use in functional food and animal feed formulations (15). Gentisic acid, a phenolic acid, has inhibitory α-amylase and α-glucosidase activity using *in silico* and *in vitro* tests (16). Koetjapic acid, a phenolic acid found in *Sandoricum koetjape* bark, has anticarcinogenic effects (12). Katonic acid, another phenolic acid from *Sandoricum koetjape*, was cytotoxic against ten cancer cell lines (17). Low molecular phenolic acids with antioxidant and antimicrobial properties include ferulic acid and coumaric acid (15). Caffeic acid and gallic acid have cytotoxic activity against human breast cancer cells, MCF-7, by inducing apoptosis (18).

The Ministry of Health of the Republic of Indonesia has mandated six health transformation programs by 2022, one of which is the transformation of the health resilience system (19). Non-communicable illnesses, stunting, TB, and mother-child health account for up to 60% of the transformation of the health system (20). The three non-communicable diseases that cause the highest number of deaths in Indonesia are heart disease, stroke, and cancer (19).

Matrix metalloproteinase-9 (MMP-9) is a protein that modulates cholesterol metabolism which could lead to coronary heart disease (21). MMP-9 is also used as a biological marker for inflammation and fibrosis in cardiovascular disease (22). MMP-9 is involved in the invasive metastatic potential of tumor cells (23). MMP-9 levels are higher in type 2 diabetics with coronary artery disease (24). Diabetes, smoking, increased lipids, and homocysteine contribute to oxidative stress leading to the activation of MMP-9 (25). MMP-9 is also associated with tissue destruction in several disease states such as dilated cardiomyopathy, arthritis, rheumatoid, and fibrotic lung disease (26).

Zymography showed that chlorogenic acid had a potent concentration-dependent inhibitory effect on MMP-9 activity (27). Chlorogenic acid also reduced the expression of MMP-9 at 72 hours after intracerebral hemorrhage (28). Chlorogenic acid showed an excellent binding affinity for MMPs (29). Caffeic acid inhibited the invasion of MCF-7 breast cancer cells by downregulating MMP-9 (30). Caffeic acid was able to inhibit MMP-9 gene expression and consequent decreased production of this enzyme (31). Caffeic acid, present in garlic, reduced UVB-induced fine line development by reducing matrix metalloproteinases (MMP) production (32). Ingestion of P-coumaric acid can significantly reduce MMP-9 gene expression during remyelination (33). Ferulic acid could inhibit vascular endothelial growth factor-induced vascular smooth muscle cell migration by inhibiting the MMP-9 mRNA expression (34). Gallic acid inhibited the migration and invasion of PC3 prostate cancer cells by downregulating MMP-9 and MMP-2 (35). Kombucha tea polyphenolic component demonstrated anticancer action by reducing MMP-9 in 786-O cells and MMP-2 in A549 cells (36). Green tea polyphenol Epigallocatechin-3-gallate (EGCG) reduced MMP-3 expression in

irradiation versus control groups (37). *Terminalia arjuna* polyphenols inhibit MMPs, PNFLCs, and SOCS3 (35).

Thus, the purpose of this study was to identify the type of phenolic acids in *Sandoricum koetjape* leaves and the prediction of MMP-9 inhibition by molecular docking.

2. Materials and methods

2.1. Plant materials

The leaves of *Sandoricum koetjape* were bought in February 2020 from a common natural market in Bogor, Indonesia. Taxonomical ID and verification were completed by Dr. Atik Retnowati at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences.

2.2. Phenolic acids analysis using UPLC

One gram of *Sandoricum koetjape* leaf sample was put into Erlenmeyer. Then it was dissolved with 50 ml of Methanol: HCl 2M 1:1 and then sonicated at 60 °C for 60 minutes. After that, filtered with Whatman paper. The filtrate is evaporated at 60 °C. The next step is solvent optimization using three different solvents. The first solvent uses ethyl acetate, the second solvent uses n-hexane, and the third solvent uses water. The residue obtained was mixed with 50 ml of distilled water and then extracted using 25 ml of each solvent and then extracted three times, the solvent phase was taken. The organic solvent was evaporated at 40 °C for 30 minutes. 2 mL of methanol was used to dissolve the residue and then filtered with a millipore. The obtained filtrate is injected into the Ultrahigh Performance Liquid Chromatography system. The mobile phase used was 2% acetic acid with methanol (82:18 v/v). The column used is Acquity UPLC BEH C18 1.7 m (2.1 x 50 mm) with a wavelength of 280 nm. The injection volume is 1mL, and the flow rate is 0.200 mL/min. Gallic acid, citric acid, caffeic acid, chlorogenic acid, p-coumaric acid, 4-hydroxybenzene acid, and ferulic acid are the standards used.

2.3. Preparation of phenolics acid as a ligand

Each phenolic acid compound obtained from the database was searched in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) for its canonical structure and isomeric SMILE (simplified molecular-input line-entry system). The previously obtained canonical SMILE structure was entered as a keyword for searching ADMETSAR data using the LMMD AdmetSAR database version 2.0 (http://lmmd.ecust.edu.cn/admetsar2) and Protox version II (https://tox-new.charite.de/protox II/index.php?site=compound input).

2.4. Molecular docking and visualization

The RSCB PDB database ((https://www.rcsb.org/) MMP9 was used to obtain the 3D structures of the selected target proteins (PDB ID: 2ovx). Meanwhile, the PubChem database was used to obtain the 3D structure of each active compound of *Sandoricum koetjape* and control (pubchem.ncbi.nlm.nih.gov). Furthermore, the protein was prepared by removing water molecules in the Discovery Studio 2019 software, while the ligands were minimized using the Pyrx v software. 0.9.8. Docking is done using Autodock 4.2 which is integrated with Pyrx v.09.8. Docking is done by a targeted docking method with the Lamarckian GA algorithm. The size of the grid box is adjusted according to the position of the amino acid residues based on the literature. The docking results are obtained in the form of binding affinity or affinity energy resulting from the interaction of compounds with proteins. Furthermore, the interaction between the compound and the docked protein was visualized using the BioVia Discovery Studio 2019 software.

3. Results

3.1. Phenolic acids of Sandoricum koetjape leaves by UPLC

Figure 1 depicts the chromatogram obtained from the identification of phenolic acid in *Sandoricum koetjape* extracts using UPLC. Gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid were discovered in *Sandoricum koetjape* leaf extracts. As shown in Figure 2, caffeic acid was the major phenolic acid found in *Sandoricum koetjape* leaf extracts.

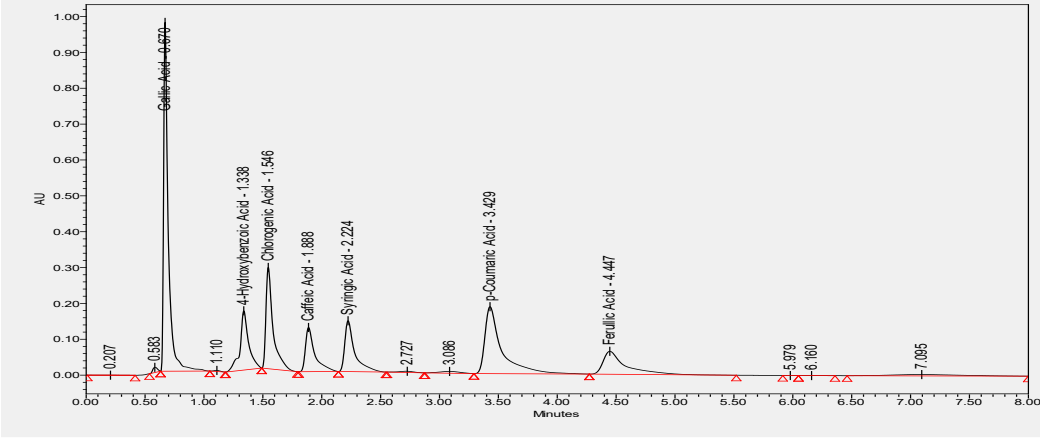


Figure 1. UPLC chromatogram of phenolic acids of extract of *Sandoricum koetjape* leaves.

3.2. In silico physiochemical parameters (ADME) prediction

The structure and characteristics of all studied molecules were presented in Table 1. The AdmetSAR v.2.0 database was used to obtain the Lipinski rule and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions by name, CASRN, and match search. AdmetSAR can predict around 50 ADMET endpoints with the QSAR model (Table 2), while the Protox II database was used to obtain Lipinski rule predictions but only provides Toxicity predictions from the entire ADMET (Table 3).

3.3. Prediction of Sandoricum koetjape leaves extract phenolic acid interaction with MMP-9

Figure 3 depicts the binding affinity of phenolic acids from the *Sandoricum koetjape* leaf extract on MMP-9. The affinity of phenolic acid binding to the leaves extract of Sandoricum koetjape is as follows: chlorogenic acid < caffeic acid< p-coumaric acid / ferulic acid < 4-hydroxybenzoic acid < gallic acid < syringic acid. The interaction residue of phenolic acids of *Sandoricum koetjape* leaf extract on MMP-9 was shown in Table 4. As shown in Figure 4, chlorogenic acid has Van der Waals interactions with MMP-9 on amino residues LEU187, ALA189, LEU397, GLN402, HIS405, PRO415, GLU416, LEU418, TYR420, MET422, TYR423, PHE425, GLU427, and PRO430. On the other hand, caffeic acid has Van der Waals interactions with MMP-9 on amino residues LEU188, LEU397, VAL398, GLU416, ALA417, LEU418, PRO421, TYR423, and THR426 (Figure 5). Figure 6 shows that almost all phenolic acids in *Sandoricum koetjape* leaf extract, except for 4-Hydroxy-

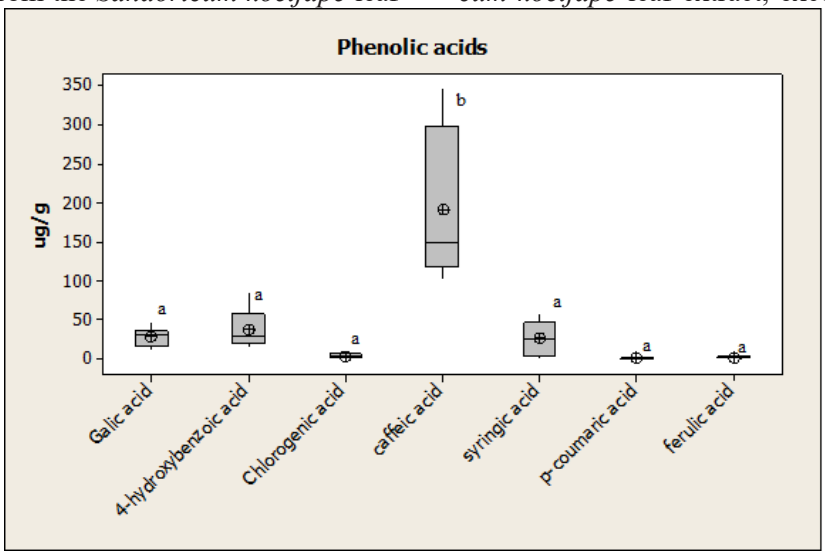
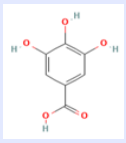
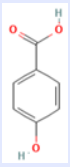
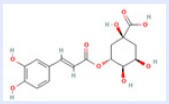
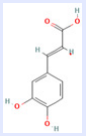
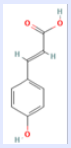
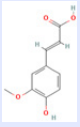


Figure 2. Phenolic acids on the extract of *Sandoricum koetjape* leaves.

Table 1. Structure and characteristics of phenolic acids of *Sandoricum koetjape* leaves extracts

Compound name	PubChem ID	Structure
Gallic acid	370	
4-Hydroxybenzoic acid	135	
Chlorogenic acid	1794427	
Caffeic acid	689043	
Syringic acid	10742	
p-Coumaric acid	637542	
Ferulic acid	445858	

benzoic acid, interact with LEU397 and VAL398.

4. Discussion

Sandoricum koetjape leaf extracts contained gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid (Figure 1). Caffeic acid was the most abundant phenolic acid found in *Sandoricum koetjape* leaf extracts, as shown in Figure 2. Caffeic acid is phenolic acid that contains two hydroxyl groups and is shown to inhibit LDL oxidation (38). The presence of a high caffeic acid content in *Sonneratia apetala* seed had beneficial effects on inflammatory responses and postpran-

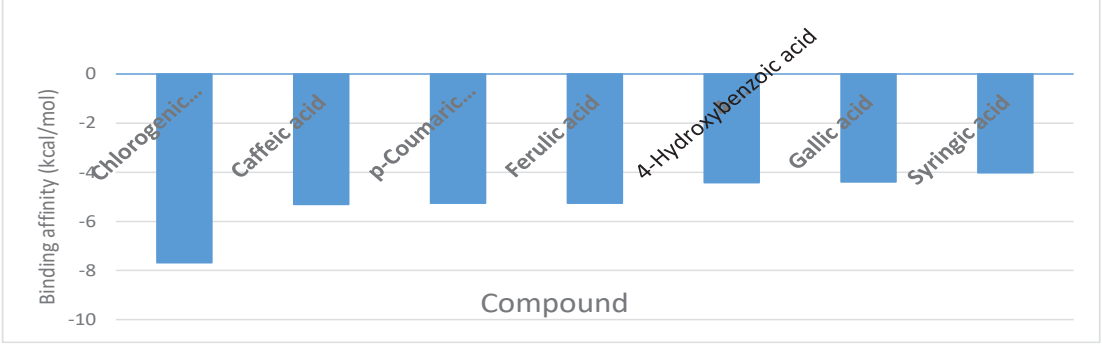


Figure 3. The binding affinity of phenolic acids of *Sandoricum koetjape* leaves extract on MMP-9

Table 2. Physiochemical properties of phenolic acids compound into the active site of MMP9

Entry	MW[a]	LogP[b]	HBD[c]	HBA[d]	TPSA[e]	nRB[f]
Gallic acid	170,12	0,5	4	4	97,99	1
4-Hydroxybenzoic acid	138,12	1,09	2	2	57,53	1
Chlorogenic acid	354,31	-0,65	6	8	164,75	4
Caffeic acid	180,16	1,2	3	3	77,76	2
Syringic acid	198,17	1,11	2	4	75,99	3
p-Coumaric acid	164,16	1,49	2	2	57,53	2
Ferulic acid	194,19	1,5	2	3	66,76	3
Rule of Lipinski	≤ 500	≤ 5	≤ 5	≤ 10	≤ 140	≤ 10

[a]Molecular Weight. [b]Logarithm of partition coefficient between n-octanol and water (LogP). [c]Number of hydrogen bond donors (HBD). [d]Number of hydrogen bond acceptors (HBA). [e]Topological polar surface area (TPSA). [f]Number of rotatable bonds (nRB).

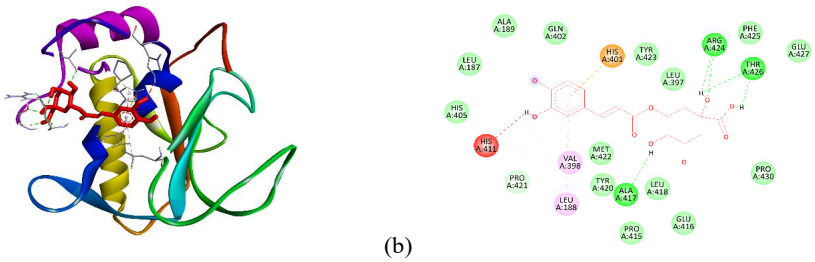
dial metabolic (39). Caffeic acid may have an antihypertensive effect by inhibiting ACE (40). Caffeic acid inhibits colon cancer growth by activating the AMP-activated protein kinase and phosphatidylnositide 3-kinase signaling pathways (38)

Caffeic and syringic acids both inhibit the growth of T47D human breast cancer cells (38). Beer's antioxidant activity is primarily attributed to syringic acid, caffeic acid, and ferulic acid (41). The potency of antiglycation of syringic acid, caffeic acid, and chlorogenic acid with the protein surface of human serum albumin (HSA) was investigated using molecular docking (14).

Organic free radicals, hydroxyl radicals, nitrogen sesquioxide, superoxide, and peroxynitrite have all been shown to be scavenged by caffeic acid and chlorogenic acid (38). Chlorogenic acid is the most frequently encountered caffeoyl ester, which is present in many vegetables and fruit, and also in coffee (42,43). Chlorogenic acid was found in *Prunus padus* L. flowers and leaves and has antioxidant activity (44). Chlorogenic acid inhibited porcine pancreatic α-amylase in the regulation of post-prandial hyperglycemia (45). Chlorogenic acid and ferulic acid have potential inhibitory activity against Covid-19 protease by molecular docking (46). Chlorogenic acid and caffeic acid showed equal antioxidant activity by trapping two peroxyl radicals and inhibiting copper-catalyzed human LDL peroxidation (38).

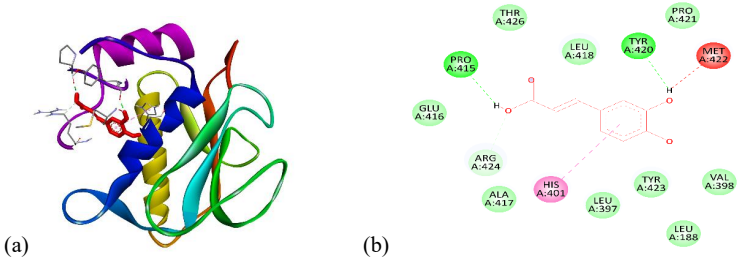
Table 2 shows the results of the physiochemical properties of phenolic acids in *Sandoricum koetjape* leaf extracts. The molecular weight (MW) of phenolic acids ranged from 138 to 355 Da (<500). The Log P values of phenolic acids ranged from -0.65 to 1.5 (<5) indicating that all phenolic acids had appropriate lipophilicity. Except for chlorogenic acid, most phenolic acids had acceptable limits of hydrogen bond properties (as donors or acceptors) and total polar surface area (TPSA). In general, the results show that all phenolic acids meet Lipinski's rule and can thus be consumed orally.

In Silico ADMET (adsorption, distribution, metabolism, excretion, and toxicity) properties of phenolic acids in *Sandoricum koetjape* leaf extracts are presented in Table 3. HIA analysis indicated that all phenolic acids illustrated well-ab-



(a) (b)

Figure 4. Interaction Chlorogenic acid on MMP-9 (a) 3D visualization (b) amino residues interaction.



(a) (b)

Figure 5. Caffeic acid on MMP-9 (a) 3D visualization (b) amino residues interaction.

sorbed compounds that cause easily absorbed from the intestine to the bloodstream. The blood-brain barrier (BBB) protects the brain from exogenous substances. The ability of a drug to cross into the brain is an important factor to consider when reducing side effects and toxicities or improving the efficacy of drugs with pharmacological activity in the brain. When the BBB is greater than 0.3, molecules can pass quickly through the blood-brain barrier (47). Table 3 shows that the BBB values of phenolic acids range from 0.3005 to 0.8938, implying that all of the phenolic acids in *Sandoricum koetjape* leaf

Human oral bioavailability is a tool for selecting potential drug candidates and rejecting those with a lower likelihood of success during the early stages of drug discovery and development (48). Table 3 shows that phenolic acid HOB values range from 51.43 to 71.43, implying that all phenolic acids have high human oral bioavailability (HOB > 50).

Protox II is a web server that predicts small molecule toxicity such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. Drug-induced liver injury is one of the most concerning safety aspects in drug development research, and it also increases the drug attrition rate. The compound that caused at least one pathological or physiological hepatic event was deemed hepatotoxic and was strongly linked to liver disruption (47). Table 3 shows that none of the phenolic acids are hepatotoxic.

Carcinogenicity was the toxicological endpoint that raised the most concerns about human health (49). Phenolic acids with inactive prediction were deemed non-carcinogenic. There are three phenolic acids has potential carcinogenicity, i.e. gallic acid, chlorogenic acid, and p-coumaric acid. Mutagenesis is also associated with carcinogenesis, according to a variety of evidence, including mutation spectra and tumor genomic sequencing. The ability to cause permanent mutations in the DNA sequence is referred to as mutagenicity (50). According to Table 3, none of the phenolic acids are mutagenic.

Immunotoxicity testing is another important method for determining product safety. Immunotoxicity testing is essential not only for product safety assessment but also for product market authorization (51). Chlorogenic acid and ferulic acid

Table 3. *In Silico* ADMET of phenolic acids compound into the active site of MMP9.

Entry	HIA[a]	BBB[b]	HOB[c]	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	LD50 (mg/kg)	Toxicity Class
Gallic acid	98.02	0.7143	71.43	Inactive	Active	Inactive	Inactive	Inactive	2000	4
4-Hydroxybenzoic acid	98.35	0.7623	51.43	Inactive	Inactive	Inactive	Inactive	Inactive	2200	5
Chlorogenic acid	90.20	0.3005	70.00	Inactive	Inactive	Active	Inactive	Inactive	5000	5
Caffeic acid	96.45	0.4365	64.29	Inactive	Active	Inactive	Inactive	Inactive	2980	5
Syringic acid	99.08	0.8938	67.14	Inactive	Inactive	Inactive	Inactive	Inactive	1700	4
p-Coumaric acid	97.11	0.7372	60.00	Inactive	Active	Inactive	Inactive	Inactive	2850	5
Ferulic acid	97.74	0.8504	65.71	Inactive	Inactive	Active	Inactive	Inactive	1772	4

[a]Human Intestinal Absorption (HIA). [b]Blood Brain Barrier (BBB). [c]Human Oral Bioavailability (HOB).

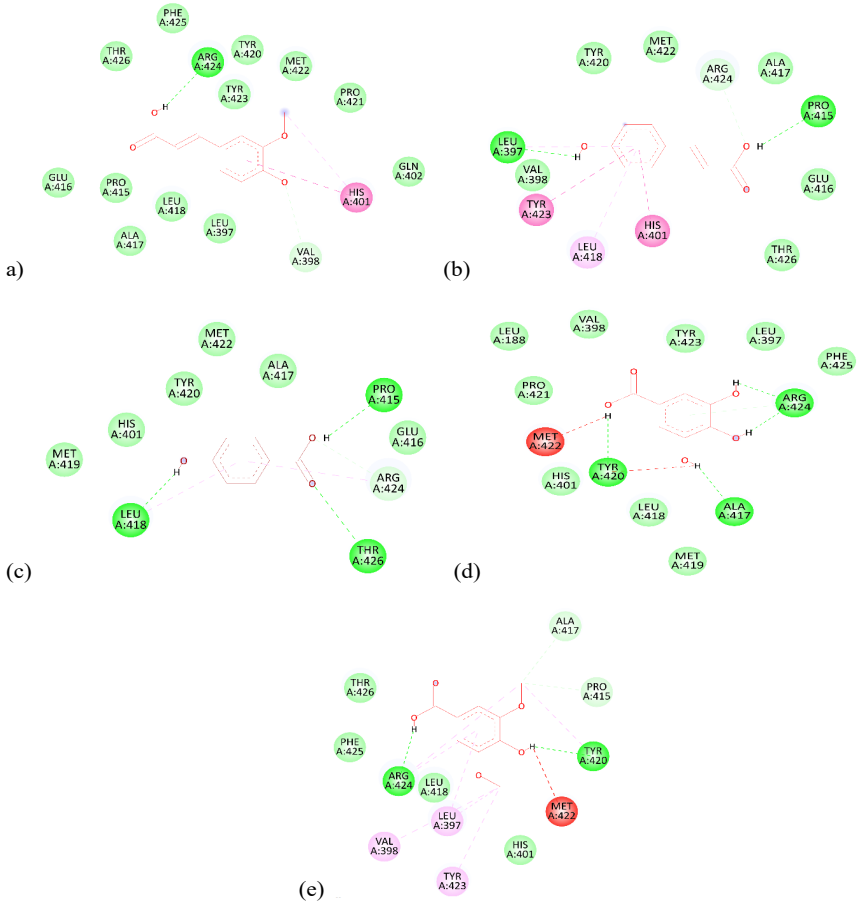


Figure 6. Interaction with (a) ferulic acid (b) p-coumaric acid (c) 4-hydroxybenzoic acid (d) gallic acid, and (e) syringic acid.

are two phenolic acids that may be immunotoxic. Additionally, cytotoxicity is the assay that determines physiological effects at the cellular level (52). None of the phenolic acids are cytotoxic, according to Table 3. Based on research conducted by Jin et al., chlorogenic acid does not have cytotoxic to the growth of Hep3B cells (27).

A prospective phenolic acid's potential toxicity must be evaluated. The LD50 (median lethal dose) is used to calculate the dose of a test substance that kills 50% of animals in a given species. It is used to calculate the potential dangers of chemicals to humans (53). The lethal dosage value can be used to calculate the acute toxicity and relative toxicity of various compounds (47). Three phenolic acids belong to category 4 (LD50 = 300 to 2000 mg/kg), such as gallic acid, syringic acid, and ferulic acid, which means that those phenolic acids are slightly toxic. Four phenolic acids belong to category 5 (LD50 = 2000 to 5000 mg/kg), such as 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, and p-coumaric acid, which means they are low acute toxicity.

Molecular docking studies were performed to predict and analyze the affinity bonds of phenolic acids from *Sandoricum koetjape* leaves extract on MMP-9 protein. Molecular docking is a virtual environment that allows complex details of ligand-protein interactions to be supported (14). The binding affinity of phenolic acids from *Sandoricum koetjape* leaves extract on MMP-9 is depicted in Figure 3. The affinity of phenolic acid binding to *Sandoricum koetjape* leaves extract is as follows: chlorogenic acid < caffeic acid< p-coumaric acid / ferulic acid < 4-hydroxybenzoic acid < gallic acid < syringic acid. *In silico* molecular docking studies, caffeic acid and ferulic acid have a stronger binding tendency on MMP-9 than gallic

Table 4. Interaction residue of phenolic acids of *Sandoricum koetjape* leaves extract on MMP-9

Ligand	Chemical ID	Interaction residue			
		Van der Waals	Hydrogen bond	Pi alkyl	Alkyl
Chlorogenic acid	1794427	LEU187, ALA189, LEU397, GLN402, HIS405, PRO415, GLU416, LEU418, TYR420, MET422, TYR423, PHE425, GLU427, PRO430,	ALA417, ARG424, THR426	LEU188, VAL398	-
Caffeic acid	689043	LEU188, LEU397, VAL398, GLU416, ALA417, LEU418, PRO421, TYR423, THR426	PRO415, TYR420	-	-
Ferulic acid	445858	LEU397, GLN402, PRO415, GLU416, ALA417, LEU418, TYR420, PRO421, MET422, TYR423, PHE425, THR426	ARG424	HIS401	-
p-Coumaric acid	637542	VAL398, GLU416, ALA417, TYR420, MET422, THR426,	LEU397, PRO415	LEU418	-
4-Hydroxybenzoic acid	135	HIS401, GLU416, ALA417, MET419, TYR420, MET422	PRO415, LEU418, THR426	ARG424	-
Gallic acid	370	LEU188, LEU397, VAL398, HIS401, LEU418, MET419, PRO421, TYR423, PHE425	TYR420, ARG424	ARG424	-
Syringic acid	10742	HIS401, LEU418, PHE425, THR426	TYR420, ARG424	LEU397	VAL398, TYR423

acid, according to Atale *et al*., 2021 (54).

The results of molecular docking of the MMP9 protein with phenolic acids from *Sandoricum koetjape* showed that chlorogenic acid had the highest binding affinity value compared to other phenolic acids tested in this study. Chlorogenic acid-MM9 has a higher binding affinity than the minimum standard (-7 kcal/mol). This indicates that the bond formed between chlorogenic acid-MMP9 is relatively stable and has the potential to have the same strong bond as the control.

Chlorogenic acid has Van der Waals interaction with HIS405. On the other hand, ferulic acid, 4-hydroxybenzoic acid, gallic acid, and syringic acid interact with HIS401. It is known that HIS405 and HIS401 are chelate-catalytic zinc ions (55). Crucial histidine residues of the metal-binding domain prevent Zn2+ binding to the active site and thus inhibit MMP activity (54).

Chlorogenic acid also has Van der Waals interaction with MMP-9 on amino residues LEU187, ALA189, LEU397, GLN402, HIS405, PRO415, GLU416, LEU418, TYR420, MET422, TYR423, PHE425, GLU427, and PRO430 as shown in Figure 4. It is known that LEU187 and TYR423 was the active site of MMP-9 (56). TYR423 also has Van der Waals interaction with other phenolic acids, such as caffeic acid, ferulic acid, and gallic acid. In addition, syringic acid has alkyl interaction with TYR423. Chlorogenic acid also interacts with ALA189 through Van der Waals interactions. ALA189 is known to be an active-site cleft residue with a side chain that is not exposed to solvent (55).

Several studies have been conducted on the inhibition of MMP 9 by chlorogenic acid. Isolated chlorogenic acid from *Euonymus alatus* has shown a strong and selective inhibited the proteolytic activity of MMP-9 in a concentration-dependent manner (27). Chlorogenic acid treat-

ment confers neuroprotection against intracerebral hemorrhage by inhibiting MMP-9 expression (28). Chlorogenic acid fits into the active site of MMPs and also interacts with active site amino acid residues present in the MMPs through hydrogen bonding (29).

On the other hand, caffeic acid has Van der Waals interaction with MMP-9 on amino residues LEU188, LEU397, VAL398, GLU416, ALA417, LEU418, PRO421, TYR423, and THR426 as shown in Figure 5. PRO421 has been proposed to be essential for MMP-9 peptide hydrolysis (57). Donors and acceptors of inter-main chain hydrogen bonds to substrates or inhibitors are provided by PRO421, LEU188, and TYR423 (26). LEU188 acts as a hydrogen bond donor to the functional group of ligands (26). LEU188 residue also interacts with chlorogenic acid, caffeic acid, and gallic acid. It is known that the active sites of MMP-9 were PRO421 and TYR423 (56). PRO421 and TYR423 also act as hydrogen donor-acceptor in a few ligand conformations (26). TYR423 also has Van der Waals interaction with ferulic acid.

Caffeic acid inhibited the invasion of MCF-7 breast cancer cells by docking and interacting in the binding site of MMP-9 (30). Caffeic acid has been shown to decrease MMP production thereby reducing the appearance of fine lines caused by UVB radiation (32). Caffeic acid was able to down-regulated MMP-9 gene expression in lipopolysaccharide-activated human monocytes (31).

Almost all phenolic acids in *Sandoricum koetjape* leaves extract, except 4-Hydroxybenzoic acid, have interaction with LEU397 and VAL398 as shown in Figure 6. LEU397 and VAL398 are specific to MMP9 (26). Ingestion of P-coumaric acid can significantly reduce MMP-9 gene expression during remyelination (33). Ferulic acid could inhibit vascular endothelial growth factor-induced vascular smooth muscle cell migration by inhibiting the MMP-9 mRNA expression (34). Gallic acid was shown to attenuate the enzyme activities of MMP-9 which is involved in the degradation of the extracellular matrix and plays important roles in PC3 prostate cancer cells migration and invasion (35).

5. Conclusion

The seven identified phenolic acids in *Sandoricum koetjape* leaf extract are gallic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid. Chlorogenic acid binds more strongly than the other phenolic acids found in *Sandoricum koetjape* leaf extract. Chlorogenic acid interacts with MMP-9 on amino residues LEU187, LEU188, ALA189, HIS405, and TYR423, and is predicted to occupy MMP-9 active sites. Chlorogenic acid is also expected to have low acute toxicity. Chlorogenic acid is expected to have good intestinal absorption and to be distributed similarly in the intestine and the blood plasma. Chlorogenic acid is expected to have good intestinal absorption, be distributed similarly in the intestine and blood plasma, and have a high human oral bioavailability. Chlorogenic acid is also not hepatotoxic, immunotoxic, mutagenic, cytotoxic, or has low acute toxicity. Further, an *in vivo* study to be conducted to assess the inhibitory activity of *Sandoricum koetjape* phenolic acid is to down-regulate MMP-9 activity.

Acknowledgement

The authors would like to thank the Directorate General of Health Workforce, Ministry of Health, Republic of Indonesia, for funding this research. The authors declare that they have no conflicts of interest.

Conflict of Interest

None declared.

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