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In-silico Analysis of Bioactive Compounds from Staghorn Fern (*Platycerium bifurcatum* C. Chr.) as Anti-Inflammatory Drug Candidates for Inhibiting Cyclooxygenase-2 Enzyme

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Abstract
Inflammation can be suppressed through anti-inflammatory mechanisms by inhibiting the performance of the cyclooxygenase-2 enzyme, which results in decreased prostaglandin synthesis so that clinical signs of inflammation can be reduced. Anti-inflammatory mechanisms can be carried out by groups of NSAID drugs such as acemetacin, but continuous use can cause side effects, thus opening the way for the use of natural ingredients as alternative treatments such as *P. bifurcatum* C. Chr leaves. This study aims to analyze the potential of bioactive compounds in *P. bifurcatum* C. Chr leaves as anti-inflammatories based on their binding affinity values with the cyclooxygenase-2, biooral potential, ADMET, and toxicity tests through an in silico approach with molecular docking techniques. The results showed that in *P. bifurcatum* C. Chr leaves, gallic acid and quercetin compounds are present. The binding affinity value control acemetacin (control) is -7,9 kcal/mol, gallic acid is -6.3 kcal/mol, and quercetin is -8.8 kcal/mol. The acemetacin (control), gallic acid, and quercetin bind to the identical six amino acid residues of the cyclooxygenase-2, namely THR A:212, TYR A:385, HIS A:388, TRP A:387, ALA A:199, LEU A:390. Quercetin and gallic acid can be absorbed, distributed, metabolized, and excreted and do not show any health risk. They can be consumed orally safely and do not cause cancer in the long term, so that they can be candidates for anti-inflammatory drugs.

Keywords: in silico; anti-inflammatory; *Platycerium bifurcatum*; cyclooxygenase-2
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INTRODUCTION

Inflammation is part of the wound-healing process and is essential to eliminate microbial contaminants (Soliman and Barreda, 2023). However, inflammation can be prolonged due to incomplete. microbial clearance. Bacteria and endotoxins can cause an increase in pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α, thereby prolonging the inflammatory phase (Menke *et al.*, 2007; Schilrreff and Alexiev, 2022). During inflammation, arachidonate (AA) is synthesized by the enzyme cyclooxygenase-2 (COX-2) (Okano *et al.*, 1998; Gandhi *et al.*, 2017). The COX-2 enzyme is expressed in response to inflammatory stimuli at sites of tissue damage (Vanegas and Schaible, 2001). Inflammation can be suppressed through anti-inflammatory mechanisms by inhibiting the performance of the COX-2 enzyme, which results in decreased prostaglandin synthesis so that clinical signs of inflammation can be reduced (Jiang et al., 2014).

Anti-inflammatory mechanisms can be carried out by the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) group (Bindu *et al.*, 2020). The NSAIDs group, such as acemetacin, works by inhibiting the performance of the COX-2 enzyme, which causes prostaglandin synthesis to decrease so that clinical signs of inflammation can be reduced (Jiang et al., 2014; Ju et al., 2022). Improper use of NSAIDs over a long period can cause side effects in the form of impaired kidney function, edema, hypertension, and gastrointestinal bleeding (Lovell and Ernst, 2017). Society is aware of these dangers, so they have started to reduce the use of chemical drugs and switch to medicinal plants because they are considered cheap and have few side effects (Shoviantari et al., 2019). Several ethnobotanical studies have been conducted as an initial step to reveal the types of plants that the community has used as medicine. One of these plants is the staghorn fern (*Platycerium bifurcatum* C. Chr.) (Supriati *et al.* 2012).

The Owerii tribe of Imo, a state in Nigeria, uses staghorn fern to treat wounds. Staghorn fern leaf extract is also used for ethnomedical treatments such as edema and anti-inflammatory (Pemberton *et al*., 2003; Chinaka *et al*., 2018). Phytochemical research on a laboratory scale has proven that staghorn fern has bioactive compounds from secondary metabolites because it contains a total of phenols of 82.33 ± 0.30 mg GAE/g (Gallic Acid Equivalents) and contains a total of flavonoids of 648.67 ± 12.3 mg QE (Quercetin Equivalents) (Agbo et al., 2015).

Phenolic compounds have pharmacological properties such as superior antimicrobial, antioxidant, and anti-inflammatory (Liu et al., 2023). Gallic acid is a phenolic compound known to have many bioactivities such as antioxidants and anti-inflammatory (Kahkeshani et al., 2019). Flavonoids have many bioactivities, such as being anti-inflammatory (Saputri et al., 2020). Flavonoids can activate antioxidant pathways that produce anti-inflammatory effects (Maleki *et al*., 2019; Ysrafil *et al*., 2023). Flavonoids inhibit the secretion of arachidonic acid to reduce inflammatory reactions. Flavonoids such as quercetin can modulate the expression and activation of cytokines such as IL-1 and TNF-α. In addition, quercetin can inhibit the synthesis of cyclooxygenase-2, a pro-inflammatory enzyme (Al-Khayri et al., 2022).

However, research on the potential compounds in *P. bifurcatum* C. Chr plants as anti-inflammatory candidates has not yet been carried out. Therefore, it is necessary to conduct initial research to analyze the potential compounds in *P. bifurcatum* C. Chr plants as an anti-inflammatory by utilizing computational technology through an in silico approach. In silico represents a pragmatic methodology for drug development analysis, employing a computer-based system (Zloh and Kirton, 2018; Chikhale *et al*., 2021). The in silico approach can be carried out by molecular docking, a method in bioinformatics that can predict the binding between compounds (ligands) and proteins (receptors) at specific sites to find stable binding conformations (Ainsley et al., 2018). The in silico approach is effective in the discovery and design of compounds by analyzing affinity for targets, absorption properties, distribution, metabolism, excretion, and toxicity, as well as physicochemical characterization (Ekins *et al*., 2007; Roncaglioni *et al*., 2013). Based on the explanation above, this study aims to analyze the potential of compounds in the *P. bifurcatum* C. Chr plant as anti-inflammatory agents through an in silico approach using the target protein of the enzyme cyclooxygenase-2.

MATERIALS AND METHODS

This study uses an In silico approach as the molecular docking method. Bioactive compounds of *P. bifurcatum* C. Chr were obtained through LC-MS testing. The results of the bioactive compounds from the LC-MS test will be subjected to a virtual screening stage to determine their potential as candidates for anti-inflammatory drugs. The hardware used is a laptop with specifications of Intel (R) Celeron (R) N4020 CPU @ 1.10GHz, 8.00GB RAM, and Windows 10 64-bit as the operating system. The software used is PyMol, PyRx, LigPlot+, and Toxtree. This research was conducted from September 2023 to December 2023 at the Malangensis Herbarium, Universitas Negeri Malang.

Leaf extract of *P. bifurcatum* C. Chr using maceration method by soaking plant simplicial powder in 90% ethanol. Leaf extract of *P. bifurcatum* C. Chr was then identified its bioactive compounds through LC-MS test using Shimadzu LCMS-8040 LCMC, volume temperature set at 35℃, chromatography column through Shimadzu Shim Pack FC-ODS with dimensions of 2mm-150mm-3µm. The two highest bioactive compounds from the LC-MS test results will be further tested through virtual docking as candidates for anti-inflammatory drugs. Testing of the two highest bioactive compounds was performed based on research by Agbo *et al.* (2014), who found two highest compounds *in P. Bifurcatum* which were gallic acid and quercetin.

The initial stage carried out was to collect information from selected compounds in the form of CID, canonical smile, and 3D structure through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Sybil Data Files format (*.sdf). Information about the target protein was obtained through the UniProt web database. The UniProt ID of cyclooxygenase--2 enzymes was P35354. The 3D structure of the target protein cyclooxygenase two was downloaded from the Protein Data Bank web server (http://www.rcsb.org) with PDB ID 5IF9 in the Homo Sapiens Organism with XRay diffraction and a measurement resolution of 2.04 Å and stored in the PDB extension (*.pdb). After that, target protein structure was cleaned from other ligands and water molecules using PyMOL software.

The potential test of selected compounds for oral consumption was carried out by comparing the physicochemical properties with Lipinski's rules. Lipinski's criteria include: (1) Molecular weight ≤

500 daltons, (2) LogP ≤ 5, (3) H-bond donor ≤ 5, (4) H-bond acceptor ≤ 10, and (5) Molar Refractivity (40-130). Lipinski's five rules (https://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) including absorption, distribution, metabolism, excretion, and toxicology or ADMET tests was predicted using https://biosig.lab.uq.edu.au/deeppk/prediction. Toxicity tests of selected compounds was carried out using Toxtree software through the cramer rule, Kroes TTC decision tree, and carcinogenicity.

The preparation stage was performed by converting selected compounds and drug (control) file extension into PDB format. The next blind docking stage uses PyRx software with AutoDock Vina (http://pyrx.sourceforge.io). All ligands (selected compounds) and target proteins (macromolecules) were converted into AutoDock Macromolecule and AutoDock Ligand Format (pdbqt). The docking results including affinity values (kcal/mol), binding site locations, and visualization of protein-ligand interactions. The coordinates of the center of interaction between ligand and protein were X: 25.5239, Y:31.5717, Z: 197.6774, and the dimensions (area of coverage of the molecule in Angstroms), X:166.9051, Y: 221.4428, Z: 216.2536. Visualization of docking results using PyMOL. The visualization results using PyMOL were in the form of a 3D structure to see the binding position of the selected compound and control drug on the target protein. Furthermore, 2D visualization was carried out using LigPlot+ software to see the active binding site of the selected compound and control drug located on the same amino acid residue and determine the type of interaction between hydrogen bond distance and hydrophobicity.

RESULTS

The results of the LC-MS test showed 87 bioactive compounds in the leaves of *P. bifurcatum* C. Chr, as shown in Figure 1. The chromatogram results show that the gallic acid and quercetin compound groups have the most significant percentage in the leaves of *P. bifurcatum* C. Chr—the composition of the gallic acid and quercetin compounds in the LC-MS results (Table 1). Gallic acid occupied the highest peak because it contained the highest composition, 2.83258%. Quercetin occupied the second-highest peak, containing composition of 2.23165%. Furthermore, the two compounds were evaluated its anti-inflammatory activity based on Probability activity (Pa) and Probability in activity (Pi) values. The gallic acid compound had Pa value of 0,640 and Pi value of 0,003. Quercetin compound had Pa value of 0,689 and Pi value of 0,017. Both compounds was found to have Pa > Pi values. Both compounds had Pa values ranging from 0,5 < Pa <0,7 (Table 2).

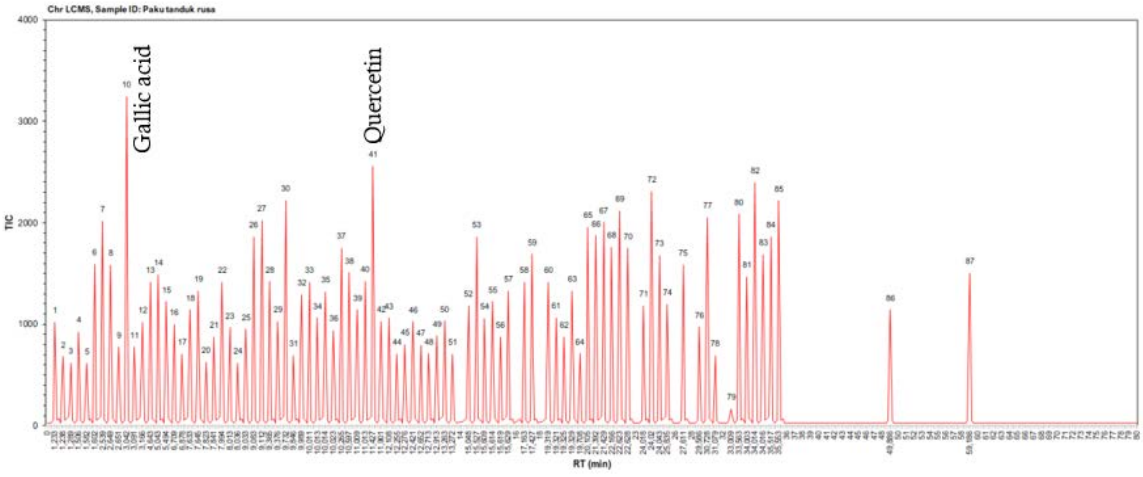

Figure 1. LC-MS Chromatogram of *P. bifurcatum* C. Chr

Tabel 1. Peak of mass spectrum chromatogram of *P. bifurcatum* C. Chr

No.	Compound	Peak Number	Composition (%)
1.	Gallic acid	10	2.83258
2.	Quercetin	41	2.23165

Tabel 2. Potential of bioactive compounds of *P. bifurcatum* C. Chr as antiinflammatory

No.	Compound	Potency	Pa Value	Pi Value
1.	Gallic acid	Antiinflammatory	0,640	0,003
2.	Quercetin	Antiinflammatory	0,689	0,017

Based on the results of the LC-MS test, two compounds were obtained and used for molecular docking. Simplified Molecular Input Line Entry System (SMILES) and CID of each compound was obtained through PubChem. The results of exploring the two compounds as test ligands are presented in Table 3—protein cleaning from water and all unnecessary ligands using PyMOL software. Figure 2 shows the 3D protein structure before (A) and after cleaned (B).

Table 3. Ligands from *P. bifurcatum* leaves used for the molecular docking test process

No.	Coumpund	CID	SMILES
1.	Acemetacin (Control)	1981	CC1=C(C2=C(N1C(=O)C3=CC=C(C=C3)Cl)C=CC(=C2)OC)CC(=O)OC C(=O)O
2.	Gallic acid	370	C1=C(C=C(C(=C1O)O)O)C(=O)O
3.	Quercetin	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O

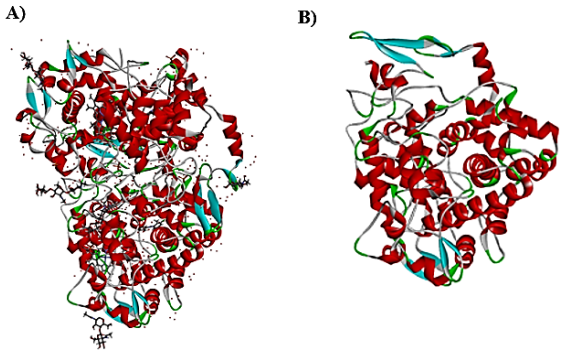

Figure 2. 3-D structure of (A) uncleaned protein of cyclooxygenase 2, and (B) cleaned cyclooxygenase 2

The results of the biooral (physicochemical) tests based on parameters of Lipinski's Rule Of Five, including molecular weight, HBA, HBD, partition coefficient (Log P), and molar refractivity, are shown in Table 4. Acemetacin and quercetin tested met Lipinski rule, but gallic acid showed a lower molar refractivity value than the minimum standard.

Table 4. Lipinski Test Results

No.	Parameter	Lipinski's Rule	Acetemetacin	Gallic Acid	Quercetin
1.	Molecular mass	≤500 Dalton	415	170	302
2.	Hydrogen bond donor	≤5	1	4	5
3.	Hydrogen bond acceptor	≤10	6	5	7
4.	LogP	≤5	3,47	0,50	2,01
5.	Molar Refractivity	40-130	106,7	38,39	74,05

Pharmacokinetic tests determine a compound's absorption, distribution, metabolism, excretion, and toxicity. Parameters for determining the pharmacokinetic properties of a compound consist of (1) absorption categorized as good with a value between 70–100% and poor 0–20% and (2) distribution consisting of distribution volume and blood-brain barrier (Log BB). The distribution volume is low if the log VDss value is < -0.15 and high if > 0.45. The compound can pass through the blood-brain barrier if it has a Log BB value > 0.3 and cannot be appropriately distributed if Log BB < -1, (3) Metabolism (CYP2D6 substrates and inhibitors), (4) Excretion and (5) Ames toxicity. The results of Table 3 show that gallic acid and quercetin could be absorbed well by the intestines because they have a value of >80%. However, quercetin couldn't penetrate the brain and reach the target. Gallic acid and quercetin resulted in negative values for the Ames test, meaning they had low mutagenic effects, closely related to carcinogenicity. Carcinogenicity is very important for toxicity testing because it harms human health. Table 3 shows that the compound has a negative value for carcinogenicity, so it is safe for humans.

Table 4. Pharmacokinetic Test of Bioactive Compounds of *P. bifurcatum* C. Chr

Compound	Parameter						
	Absorption	Distribution		Metabolism		Excretion	Toxicity
	Intestinal absorption (%)	VDss (Log L/kg)	Log BB	Substrate CYP2D6	Inhibitor CYP2D6	Total Clearance (log ml/min/kg)	Ames
Acemetacin (control)	89,60	0,87	0,11	No	No	0,33	Safe
Gallic acid	85,50	-1,1	-0,9	No	No	9,83	Safe
Quercetin	86,60	0,14	-1,7	No	No	8,91	Safe

A toxicity test using Toxtree software is used to determine compounds based on three methods, namely the. Based on the Cramer rule method, Kroes TTC decision tree, and carcinogenicity successively show that acemetacin, gallic acid, and quercetin are included in the class I category, "substance would not be expected to be safety concern," and "negative for genotoxic and nongenotoxic carcinogenicity." Based on the toxicity test using the Cramer rule method, Kroes TTC decision tree, and carcinogenicity, it can be concluded that acemetacin, gallic acid, and quercetin can be consumed orally safely and do not cause cancer in the long term. (Table 5).

Table 5. Toxicity test of bioactive compounds *P. bifurcatum* C. Chr using Toxtree

No.	Compound	*Cramer Rule*	*Kroes TTC Decision Tree*	*Carcinogenicity*
1.	*Acemetacin*	*Low (Class I)*	*Substance would not be expected to be safety concern*	*Negative for genotoxic and nongenotoxic carcinogenicity*
2.	*Gallic acid*	*Low (Class I)*	*Substance would not be expected to be safety concern*	*Negative for genotoxic and nongenotoxic carcinogenicity*
3.	*Quercetin*	*Low (Class I)*	*Substance would not be expected to be safety concern*	*Negative for genotoxic and nongenotoxic carcinogenicity*

Based on the results of the docking analysis in Table 6, the binding affinity value of gallic acid was -6.3 kcal/mol while quercetin was -8.8 kcal/mol. This finding shows that the affinity value of quercetin is smaller than acemetacin (Table 6).

Table 6. Affinity values of bioactive compounds in *P. bifurcatum* C. Chr

No.	Compound (Ligand)	Binding Affinity (kkal/mol)
1.	*Acemetacin* (control)	-7,9
2.	Gallic acid	-6,3
3.	Quercetin	-8,8

The docking visualization results show that acemetacin, gallic acid, and quercetin have the same binding site for cyclooxygenase 2 (Figure 3). The analysis of the interaction between acemetacin, gallic acid, and quercetin on cyclooxygenase two can determine the type of hydrophobic bond, hydrogen bond, and hydrogen bond distance (Å) between the compound and cyclooxygenase 2 (Figure 4).

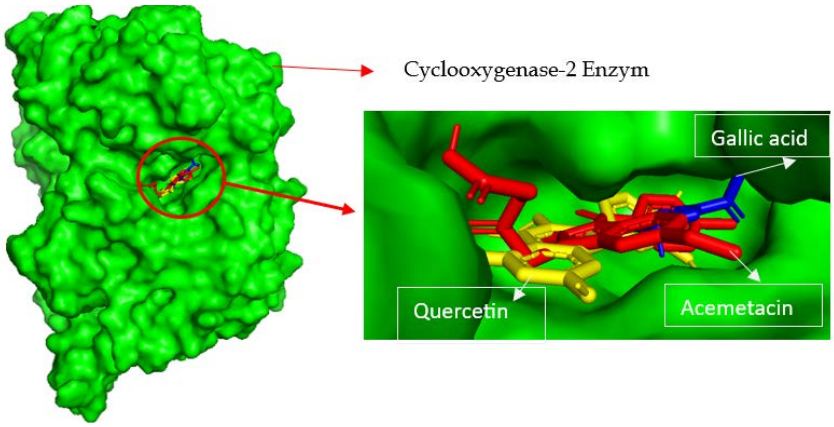

Figure 3. Docking visualization of acemetacin, gallic acid, and quercetin against cyclooxygenase 2

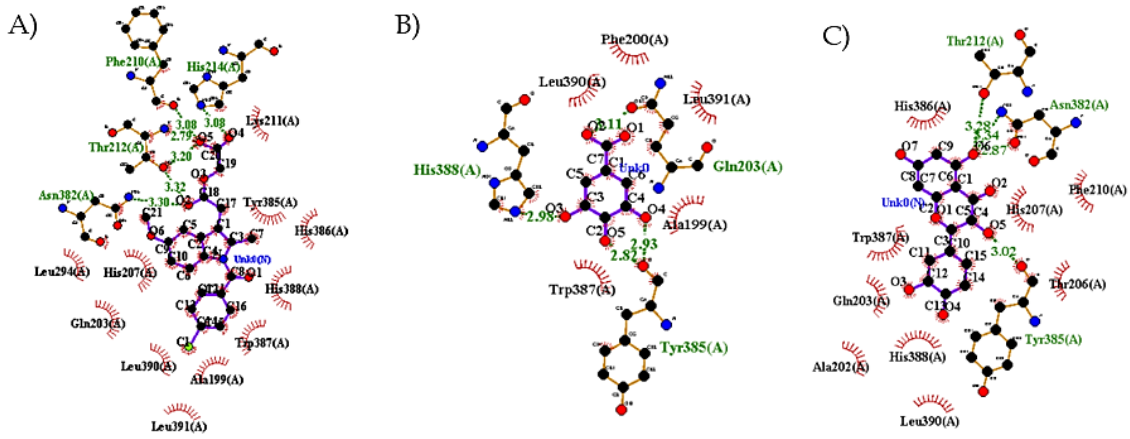

Figure 4. Type and distance of bioactive compounds of *P. bifurcatum* C. Chr to cyclooxygenase 2. (A) Acemetacin; (B) Gallic acid; and (C) Quercetin.

Acemetacin's control compound has 15 amino acid residues that bind to cyclooxygenase 2. Four amino acid residues have hydrogen bonds and 11 hydrophobic bonds. Three amino acid residues that have hydrogen bonds with Cyclooxygenase 2 are HIS A: 214 (3.08 Å), ASN A: 382 (3.30 Å), THR A: 212 (3.10Å), PHE A: 210 (3.08 Å). The bioactive compound gallic acid showed 46.70%, while quercetin 86.70% showed the similarity of amino acid residues with the control. Acemetacin, gallic acid, and quercetin have identical amino acid residues, namely THR A:212, TYR A:385, HIS A:388, TRP A:387, ALA A:199, LEU A:390 (Table 7).

Table 7. Amino acid residues and types of bonds between bioactive compounds of *P. bifurcatum* c. Chr against cyclooxygenase 2

No.	Compound	Amino Acid Residue	Types of Bonds	Hydrogen Bond Distance (Å)	Amino Acid Residue Similarity to Control
1.	*Acemetacin* (control)	HIS A:214	Hydrogen	3,08	100%
		ASN A:382	Hydrogen	3,30	
		THR A:212	Hydrogen	3,10	
		PHE A:210	Hydrogen	3,08	
		TYR A:385	Hydrophobic		
		LYS A:211	Hydrophobic		
		HIS A:386	Hydrophobic		
		HIS A:388	Hydrophobic		
		TRP A:387	Hydrophobic		
		ALA A:199	Hydrophobic		
		LEU A:391	Hydrophobic		
		LEU A:390	Hydrophobic		
		GLN A:203	Hydrophobic		
		LEU A:294	Hydrophobic		
		HIS A:207	Hydrophobic		
2.	*Gallic acid*	GLN A:230	Hydrogen	3.11	46,70%
		TYR A:385	Hydrogen	2,87	
		HIS A:388	Hydrogen	2,98	
		PHE A:200	Hydrophobic		
		LEU A:391	Hydrophobic		
		ALA A:199	Hydrophobic		
		TRP A:387	Hydrophobic		
		LEU A:390	Hydrophobic		
3.	*Quercetin*	THR A:212	Hydrogen	3,28	86,70%
		ASN A:382	Hydrogen	1,94	
		ALA A:199	Hydrogen	2,27	
		TYR A:385	Hydrophobic		
		LEU A:390	Hydrophobic		
		LEU A:391	Hydrophobic		
		PHE A:210	Hydrophobic		
		GLN A:203	Hydrophobic		

TRP A:387	Hydrophobic
HIS A:207	Hydrophobic
HIS A:388	Hydrophobic
HIS A:386	Hydrophobic
HIS A:214	Hydrophobic

DISCUSSION

There are 87 compounds identified from LC-MS from *P. bifurcatum* C. Chr plant. Gallic acid and quercetin compounds have the most significant composition compared to other compounds (Table 1). This result follows previous research, which stated that the phenolic compounds gallic acid and flavonoid quercetin are abundant in *Platycerium bifurcatum* C. Chr leaves (Agbo *et al*., 2014). The results of the Prediction of Activity Spectra for Substances (PASS) (Table 2) shows simultaneous predictions for many types of biological activities based on the structure of drug-like compounds. The predicted activity in PASS is indicated by the Probability of being active (Pa) and inactive (Pi). The Pa and Pi values criteria state that a compound shows activity if Pa > Pi. If the Pa value > 0,7, then the compound is experimentally quite high and has activity as a drug. If the value is 0,5 < Pa < 0,7, then the compound is experimentally relatively low, and the possibility of its activity as a drug is slight. However, if Pa < 0,5, the compound is experimentally minimal and does not show activity as a drug (Lagunin *et al*., 2018). The gallic acid compound has a Pa value of 0,640 and a Pi value of 0,003. Quercetin compound has a Pa value of 0,689 and a Pi value of 0,017. Both compounds have Pa > Pi values. This result shows that gallic acid and quercetin are active anti-inflammatory drug candidates. Both compounds have Pa values ranging from 0,5 < Pa <0,7. Based on this, the gallic acid and quercetin compounds are experimentally relatively low, and the possibility of their activity as drugs is slight, but they still show pharmacological activity as anti-inflammatory drugs.

Table 4 shows that gallic acid and quercetin compounds meet the criteria for oral bioavailability because they have a molecular weight of less than 500 Daltons. This result shows that both compounds do not take long to diffuse through the cell membrane, so the body quickly absorbs them. The number of hydrogen donors and hydrogen acceptors describes that the higher the hydrogen bond capacity, the higher the energy required for absorption (Weni et al., 2020). The number of hydrogen bond donors and acceptors of gallic acid and quercetin compounds complies with the criteria, so it does not require much energy for the absorption process. The range of Log P values that meet Lipinski's rule is -4 to 5. Log P values that are too negative result in a molecule being unable to pass through the lipid bilayer membrane (Lipinski, 2004). The Log P value of gallic acid and quercetin is ≤ 5. This result shows that both compounds dissolve in nonpolar solvents to penetrate the lipid bilayer membrane. The molar refractivity value indicates the total polarizability of a drug molecule (Ruswanto et al., 2022). Only quercetin compounds comply with the MR value criteria, while the MR value of gallic acid is lower than the minimum limit of the rule. Thus, it can be concluded that only quercetin compounds comply with the five Lipinski rules so that they can be consumed orally. Although gallic acid compounds do not comply with the five Lipinski rules and cannot be consumed orally, these compounds can be given sublingually or by injection.

Acemetacin, gallic acid, and quercetin have intestinal absorption values above 80%. These tree compounds are suggested to be good and can be absorbed by the body well. A compound is said to have a low distribution volume if the log VDss value is <-0.15 and high if> 0.45. The higher the VDss value, the greater the drug distributed to the tissue than plasma (Pires *et al*. 2015). The VDss of gallic acid and quercetin are both > -0.15, thus both are low log VDss and tend to remain in blood plasma, meaning lower drug doses are required to achieve a given plasma concentration (Krüger et al., 2020).

Compounds can penetrate the blood-brain barrier with a Log BB value > 0.3 and cannot be appropriately distributed if Log BB <-1. The Log BB value of gallic acid > 0.3 while quercetin < - 1. Gallic acid can penetrate the blood-brain barrier, while quercetin quercetin slightly penetrates the blood-brain barrier. Negative BBB values can be overcome by simultaneous prediction between log BB and log PS. The prediction of log BB or log PS individually is inaccurate, so it must be done simultaneously to produce a positive BBB. However, in vivo testing is still required (Lanevskij et al., 2012).

Gallic acid and quercetin compounds are not substrates and inhibitors of CYP2D6, so they are not metabolized by P450. The clearance parameter is drug elimination. The rate of drug elimination depends on concentration (Smith et al., 2019). The greater the clearance value, the greater the elimination rate, so the rate of drug elimination from the body becomes faster. The total clearance value of gallic acid is higher than acemetacin and quercetin. This result shows that gallic acid has a faster

elimination rate. Overall, it can be concluded that gallic acid and quercetin can be absorbed and distributed in tissues rather than plasma and pass through the blood-brain barrier.

The Threshold of Toxicological Concern (TTC) is a pragmatic risk assessment tool that establishes a threshold value for human exposure to all chemicals. The TTC is the exposure level that poses no significant risk to human health (Kroes et al., 2004; Patlewicz et al., 2018). Based on the toxicity test, it can be concluded that acemetacin, gallic acid, and quercetin can be consumed orally safely and do not cause cancer in the long term.

Based on the results of the docking analysis in Table 6, the binding affinity value of gallic acid (-6.3 kcal/mol) is greater than the binding affinity value of quercetin (-8.8 kcal/mol). This result states that quercetin has the highest affinity and does not require much energy to bind the cyclooxygenase two enzymes, but it is not very different from gallic acid. The binding affinity value is a parameter of the ability of the compound to bind to the target protein (Thafar et al., 2019). A higher binding affinity indicates that the compound requires less energy to bind to its target protein (Ádám et al., 2017). Gallic acid is a bioactive compound that can act as an anti-inflammatory that inhibits sPLA2, as an antioxidant agent, and as a cyclooxygenase-2 inhibitor (Toyama et al., 2022). In silico screening of quercetin against important inflammatory molecular targets, cyclooxygenase-2 was performed using the DS 2.1 tool, resulting in a binding affinity -2.583 and has excellent efficacy in an anti-inflammatory activity that can be used to treat inflammatory diseases (Preethi, 2023). Based on this, it can be predicted that the gallic acid and quercetin compounds have the potential to be anti-inflammatory.

The visualization results show that gallic acid and quercetin are located close to each other in the same binding site when bound to cyclooxygenase-2 (Figure 2). Cyclooxygenase 2 enzyme is specific because the conformation of amino acid residues in the active site stabilizes the substrate specifically. The active site of this enzyme is a pocket that generally fills a relatively small part of the entire enzyme and is usually filled with water when not binding to the substrate (Ju et al., 2022). Based on this, the compounds gallic acid and quercetin can inhibit the performance of the cyclooxygenase-2 because they have the same active site as the acemetacin control, so they are predicted to be able to inhibit the production of arachidonic acid.

The binding site, interaction, and bond distance of gallic acid and quercetin compounds can be seen in Table 7, and the amino acid residues can be seen in Figure 5. Gallic acid and quercetin bind to the identical eight amino acid residues of the cyclooxygenase-2. The binding site reveals amino acid residues critical in developing interactions between ligands and macromolecules. Consequently, it is important to examine the amino acids that contribute to developing interactions that result in pharmacological effects (Ikhtira et al., 2023; Koban et al., 2022). Three amino acid residues have hydrogen bonds, namely HIS A: 388, which has a hydrogen bond to gallic acid (2.98 Å); GLN A: 203, which has a hydrogen bond to gallic acid (4.11 Å); TYR A: 385 has a hydrogen bond to gallic acid and quercetin (2.93 Å and 3.00 Å). One amino acid residue with an unfavorable bond to gallic acid is TRP A: 387. Four amino acid residues with hydrophobic bonds are ALA A: 199, LEU A: 391, LEU A: 390, and HIS A: 207. The interaction between compounds and amino acid residues will be more stable if they have hydrogen bonds (Mardianingrum et al., 2021). Hydrogen bonds have weak interactions if the hydrogen bond length is > 1.85 Å (Varma et al., 2010). The smaller the hydrogen bond distance between the ligand and the amino acid residue on the receptor, the greater the affinity strength of both (Fadhilah and Tjahjono, 2012).

The hydrogen bond distance of gallic acid and quercetin compounds is > 1.85 Å. This result shows that the hydrogen bond interaction is weak, but the presence of hydrogen bonds that form indicates the increasing complexity of the structure. Hydrophobic bond interaction also determines the ligand's stability to the receptor. The presence of this bond will reduce the interaction between nonpolar residues and water, which will cause damage to the protein structure and loss of enzyme activity (Lins and Robert, 2020). The similarity of hydrophobic interactions on the identical amino acid residues between acemetasine (control) and two compounds (gallic acid and quercetin) and the hydrogen bonds found in both compounds indicate that the interaction between the ligand and the target protein is quite strong. Based on this, it can be predicted that gallic acid and quercetin compounds have the potential to be anti-inflammatory.

CONCLUSION

Gallic acid and quercetin compounds contained in staghorn fern plants have the potential as anti-inflammatory as antagonists of the cyclooxygenase-2 because they have stable binding affinity

values of gallic acid - 6.3 kcal/mol while quercetin -8.8 kcal/mol. The acemetacin (control), gallic acid, and quercetin bind to the identical six amino acid residues of the cyclooxygenase-2, namely THR A:212, TYR A:385, HIS A:388, TRP A:387, ALA A:199, LEU A:390. Based on the in silico approach, quercetin and gallic acid can be absorbed, distributed, metabolized, and excreted, and do not show any health risks, can be consumed orally safely, and do not cause cancer in the long term so they can be candidates for anti-inflammatory drugs. However, in vitro and in vivo validation and standardization are required to ensure reliable predictions.

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CONFLICT OF INTEREST

There is no conflict of interest.

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