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In Silico Study of the Activity of Bioactive Compounds from Cinnamon (Cinnamomum verum) Bark as Anti-inflammatory

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Abstract: Cinnamon bark (Cinnamomum verum) contains various bioactive compounds that can be used in drug development, one of which is anti-inflammatory. Inflammation is the body's defense response to the occurrence of an injury from objects unknown to the body, such as bacterial infections, trauma, autoimmune, viruses and toxins. The purpose of this study was to determine the content of bioactive compounds in cinnamon bark. This research method was carried out using the Dr. Duke's Phytochemical database. Duke's Phytochemical database to collect bioactive compounds in cinnamon bark, PubChem to download the ligand structure of bioactive compounds, PASS (Prediction of Activity Spectra for Substance) Online for screening the anti-inflammatory activity value of bioactive compounds, SEA and Swiss Target Prediction are used to determine the target protein, STRING is used to analyze the interaction between target proteins, Cytoscape is used to predict tissue topology, Pyrex is used for molecular docking, and Biovia Discovery Studio is used to visualize docking results. There are 16 cinnamon bark compounds that have a Pa value > 0.5. Based on the docking results, compounds that have a Pa value> 0.5. Based on docking results, beta caryopyhllene, caryopyhllene, fatty acid, and isocaryopyhllene compounds have PPARA activator mechanism with binding affinity of -7.4 kcal/mol, -7.9 kcal/mol, -7.8 kcal/mol, and -5.6 kcal/mol. Caffeic acid compounds have an MMP-9 activator mechanism with a binding affinity of -5.7 kcal/mol. Visualization of docking results shows that isocaryopyhllene and caffeic acid compounds have similar interactions and amino acid residues with control drugs so that they can be used as anti-inflammatory agents.

Keywords: Cucumber peels, antibacterial, *Staphylococcus epidermidis*, anti-acne cream

INTRODUCTION

Inflammation is the body's defense response to an injury from unknown objects, such as bacterial infections, trauma, autoimmune, viruses and toxins (Joshua et al, 2017). Diseases caused by inflammation include inflammation, asthma, sinusitis, hepatitis and tuberculosis. Changes in the inflammatory response from short-term to long-term can lead to chronic inflammation. Untreated chronic inflammation can lead to other chronic diseases such as cardiovascular disease, diabetes, arthritis and cancer (Libby, 2007; Zhou et al., 2016). A significant cause of death in the world is caused by chronic inflammatory diseases with more than 50% of deaths caused by inflammation (Furman et al., 2019). Inflammation is therefore an important disease to address. So far, the drugs for inflammation are NSAIDs (Nonsteroidal Anti-inflammatory Drugs) (Abdulla et al., 2013).

NSAIDs can produce anti-inflammatory effects by inhibiting prostaglandin synthesis. However, the use of NSAIDs or corticosteroid drugs can cause side effects such as abdominal pain, drowsiness, nausea, and dry mouth (Ocktavia, 2020). Due to the side effects of using these drugs, a relatively safe and effective treatment is needed, namely traditional medicine. Traditional medicine has been proven to play a major role in health (Batiha et al., 2020; Elbeshbishy et al., 2019).

One of the typical Indonesian herbal drinks that has many properties is wedang uwuh. The spices used in wedang uwuh have anti-inflammatory properties (Saras., 2023). One type of plant used for making wedang uwuh is cinnamon *(Cinnamomum verum). Cinnamon bark* has anti-inflammatory properties, due to the content of biologically active compounds contained in cinnamon bark including tannins, flavonoids, saponins, phenols, cinnamaldehyde, eugenol, and essential oils that can cure inflammation (Pramesti *et al.,* 2023).

Research by Annisa *et al.* (2021) ethanol extract of cinnamon bark has anti-inflammatory activity with an IC50 value of 57,412 µg/mL Research by Rahmila et al., (2021) topical administration of ethanol extract of cinnamon leaves with a concentration of 5%; 10%; and 20% has anti-inflammatory activity characterized by a decrease in the amount of exudate volume, inflammation diameter and the number of leukocyte types including segment neutrophils, rod neutrophils and monocytes. Research by Hong et al. (2012) Inhibition of in vitro and in vivo activity of cinnamon water extract was shown to inhibit TNF-α *(tumor necrosis factor alpha)* which TNF-α is the main cytokine in chronic acute inflammatory responses.

There are 3 types of methods commonly used to develop new drugs, including in vitro, in vivo and in silico (Kurniasih et al., 2022). The in-silico method is a method of approaching a real condition or situation into a computer simulation using a specific program with the aim of increasing the efficiency of the simulation and calculation process in designing drugs.

The results of the interaction between ligands and target proteins through molecular docking or in silico can produce affinity values and ligand interaction models for target proteins (Anjani *et al.,* 2022). Thus, this study was conducted to determine the content of bioactive compounds in cinnamon that have the potential as anti-inflammatory *in silico.*

METHODS

The research conducted is a descriptive and exploratory research with computer assistance *in silico.* The purpose of descriptive research is to describe the bioactive compounds of *cinnamon bark* and its target proteins related to anti-inflammatory. The purpose of exploratory research is to analyze the potential and mechanism of *cinnamon bark* bioactive compounds as anti-inflammatory.

Collection and Unification of *cinnamon bark (Cinnamomum verum)* bioactive compounds

Bioactive compounds of *cinnamon bark* were collected from *Dr. Duke's Phytochemical* online database at https: Performing compound unification using the PubChem database accessed with the page https: When the compound is appropriate, then search for canonical SMILE.

Screening of bioactive compounds of cinnamon bark

Screening assay of bark bioactive compounds Way2Drug *Prediction of Activity Spectra for Substances* (PASS) Online database was used to analyze the potential anti-inflammatory activity of cinnamon bark compounds in relation to the structure of the compound and its biological activity. The PASS test was conducted using https://www.way2drug.com/passonline/. After that, canonical SMILES was entered and then clicked get prediction to find out the prediction of cinnamon bark bioactive compounds. The results of the analysis were collected and selected based on Pa values in the range > 0.5 which showed the highest potential in anti-inflammatory reactions.

Target protein prediction of cinnamon bark compounds

At the time of presiding can use Swiss Target Prediction which can be accessed through the page http://www.swisstargetprediction.ch/, Prediction of target proteins with *Similarity Ensemble Approach* (SEA) can be accessed through the page https://sea.bkslab.org/. the use of the *Swiss Target Prediction* database and SEA aims to determine the target protein of a compound based on the similarity of its structure. How to predict the target protein is by entering the canonical SMILE of the cinnamon bark bioactive compound.

Interaction Network Design of Target Protein and Bioactive Compound of Cinnamon Bark

Creating an interaction network between target proteins can be done using STRING through the page https://string-db.org/. On this page, click search then select multiple proteins and after that enter the protein name with the *organism homo sapiens*. Next, click search and wait for the *running process*. In the settings section, change the coefficient STRING correlation> 0.5 this figure proves the coefficient with great confidence, then click analysis and enrichment data there is an interaction of target proteins with KEGG *pathway enrichment.*

Design of Interaction Between Target Protein and Cinnamon Bark Compound

On this page cytoscape click STITCH and enter the bioactive compound of *cinnamon bark* in the column provided. Click search *network* to analyze the interactions that occur and wait for the *running process.* On the cytoscape menu at the top click apps - STRING - *query dit addition nodes to add target proteins* and enter all target proteins that have previously been analyzed using STRING. On next layout click *Grid layout - all nodes* to make the network neatly organized, click the *style* menu to set the position, color, size and type of *network* needed.

Topology Network Analysis with Cytoscape

Open the cytoscape application and enter the target protein that has been analyzed using STRING. Click *file - import - network* from file to enter all the target proteins in the filter menu on the right-hand side of the cytoscape page select *topology filter*. Click the *style* menu to adjust the position, color, size and type of network required on this page, select the tools menu and *analyze network* to analyze the network formed and obtain an analysis table in the form of *degree* values and also *betweenness centrality.*

Structure Preparation of Cinnamon Bark Compounds and Target Proteins

Compound structure preparation is carried out using PubChem which is accessed at https://pubchem.ncbi.nlm.nih.gov/ by copying the compound name that been obtained previously. Download the compound in the form of a 3D SDF file on the top right of the database page. Target protein structure preparation using RCSB Protein Data Bank accessed at https://www.rcsb.org/pdb/. The target protein name is entered in the search field. Many similar structures will be generated but the specifications are different. Evaluation of the target protein structure was carried out using PDBSum accessed at http://www.ebi.ac.uk/thorntonsrv/databases/pdbsum/. Click "procheck" for Ramachandran plot and G-factors. On the RCSB PDB page to download the selected protein structure in the form of a pdb format file. The protein structure is then cleaned using the Biovia Discovery Studio application downloaded from https://www.3ds.com/productsservices/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/

Molecular docking of Protein - Cinnamon Bark Bioactive Compound

The molecular docking process can be done to identify a bond between protein and ligand that forms the best intermolecular bond. The molecular docking process is done by using Pyrx tools Autodock vina wizard that can be downloaded. The steps taken to enter the protein structure by clicking the file after that click load molecule, right click on the protein name then selected autodock and make macromolecule to make the protein as a target receptor. Open the open babel menu to enter the ligand by inserting a new item and inserting a new ligand. ligand structures that are ready to be analyzed. Then minimize all. Right click again on the open babel table then convert all to autodock ligand and select the vina wizard menu then click start then select the macromolecule and ligand being analyzed then click forward.

Visualization of Molecular Docking Results

The result of docking visualized with bioavia has obtained data in the form of kcal/mol affinity value. Visualization of docking can be used with bioavia by selecting the view menu. Click hierarchy, then click the file menu and click open again to enter the docking results, right click on the ligand display, copy and paste on the protein structure. Click the ligand name then define ligand, select ligand interaction to know the interaction that occurs and the amino acid residues that have been formed, Display receptor surface on the H-bound bond. Right-click to add a label to the interaction that has occurred, after that click the reside object and attribute name then select show 2D diagram to produce 2D interactions that occur.

RESULT AND DISCUSSION

Collection and Screening of Cinnamon Bark Bioactive Compounds

The results of the collection of bioactive compounds of cinnamon bark obtained 48 compounds in table 1. Screening testing of bioactive compounds to find compounds with the intended activity. According to Malikhana et al., (2021) the PASS test value is more than 0, (Pa> 0.7) it can be said that the compound has very high biological activity. The value of Pa> 0.5 and less than 0.7 (0.5 < Pa> 0.7) can be said that the compound has pharmacological potential in the test. If Pa > 0.5 indicates the compound is said to have pharmacological activity with low potency.

Table 1. Compounds from Cinnamon Bark with Anti-inflammatory Activity

Compounds	Value Pa	Actity
(-)- Epicatechin	0,548	*Antiinflamatory*
2-Methoxy-cinnamaldehyde	0,560	*Antiinflamatory*
2-Vinylphenol	0,595	*Antiinflamatory*
Acetyleugenol	0,621	*Antiinflamatory*
Arabinoxylan	0,629	*Antiinflamatory*
Ascorbic acid	0,779	*Antiinflamatory*
Beta caryophyllene	0,745	*Antiinflamatory*
Beta carotene	0.690	*Antiinflamatory*
Beta pinene	0.611	*Antiinflamatory*
Borneol	0.538	*Antiinflamatory*
Bornyl acetate	0,580	*Antiinflamatory*
Caffeic acid	0,651	*Antiinflamatory*
Campester	0,502	*Antiinflamatory*
Caryophyllene	0,745	*Antiinflamatory*

Compounds	Value Pa	Actity
Catechins	0,548	*Antiinflamatory*
Cinnamaldehyde	0,596	*Antiinflamatory*
Cinnamic acid	0,656	*Antiinflamatory*
Cinnamic alcohol	0,515	*Antiinflamatory*
Cinnamyl acetate	0,699	*Antiinflamatory*
Cis ocimene	0,599	*Antiinflamatory*
Coniferaldehyde	0,561	*Antiinflamatory*
Coumarin	0,615	*Antiinflamatory*
Cumene	0,650	*Antiinflamatory*
Ethyl cinnamate	0,633	*Antiinflamatory*
Eugenol acetate	0,621	*Antiinflamatory*
Farnesol	0,643	*Antiinflamatory*
Fatty Acid	0,727	*Antiinflamatory*
Gamma terpineol	0,694	*Antiinflamatory*
Geraniol	0,643	*Antiinflamatory*
Geranyl acetate	0,747	*Antiinflamatory*
Humulene	0,747	*Antiinflamatory*
Isocaaryophyllene	0,745	*Antiinflamatory*
Isoeugenol	0,561	*Antiinflamatory*
Limonene	0,610	*Antiinflamatory*
Linalool	0,537	*Antiinflamatory*
Linalyl acetate	0,751	*Antiinflamatory*
Methyl cinamate	0,574	*Antiinflamatory*
P Coumaric Acid	0,641	*Antiinflamatory*
P cymene	0,644	*Antiinflamatory*
Phenylethyl-acetate	0,578	*Antiinflamatory*
Phytosterols	0,520	*Antiinflamatory*
Piperitone	0,546	*Antiinflamatory*
Sabinene	0,853	*Antiinflamatory*
Sesquiterpenes	0,794	*Antiinflamatory*
Stigmasterol	0,542	*Antiinflamatory*
Terpineol 4-OL	0,609	*Antiinflamatory*
Terpinolene	0,528	*Antiinflamatory*
Trans cinnamaldehyde	0,596	*Antiinflamatory*

Bioactive Cinnamon Bark Compounds with High Activity as Anti-inflammatory and their Target Proteins

Predicting target proteins from bioactive compounds of cinnamon bark can use the *Swiss Target Prediction* database and the Similarity Ensemble Approach characteristic of the species "*Homo Sapiens*". According to Diana (2019), target protein testing with the *Swiss Target Prediction* database is based on the same principle through reserve screening. A compound that is most affected by the target is indicated by a high probability bar that has a green color. If the probability value is high, it shows more accurate results. The results of testing compounds with target proteins that have a probability value >0.5 are in Table 2.

Table 2. Results of compounds with target proteins that have a probability value >0.5

Compounds	Target Protein
2-Methoxy-cinnamaldehyde	ADH1A, IDO1, MAOB, AOC3, CYP11B1, CYP11B2, MAPT, APP, CYP2A6, IGF1R, NR4A1, HSPA1A
Acetyleugenol	PTGS1
Ascorbic acid	GSK3B
Beta caryophyllene	PPARA, CNR2
Borneol	CA2, CA1, CA4, TRPM8
Caffeic acid	CA2, ALOX5, CA7, CA1, CA6, MMP9, CA12, MMP1, MMP2, PTPN1, CA14, CA9, CA5B, CA5A
Campesterol	HMGCR, CYP51A1, CYP17A1, AR

Compounds	Target Protein
Caryophyllene	PPARA, CNR2
Cinnamaldehyde	TRPA1
Cinnamic acid	HCAR2
Coumarin	CA2, CA7, CA1, CA3, CA6, CA12, CA14, CA9, CA4, CA13, CA5B, CA5A
Eugenol acetate	PTGS1
Fatty acid	FABP4, PPARA, FABP3, FABP5, PPARD, FABP2
Isocaryophyllene	PPAR, CNR2
Stigmasterol	CYP51A1, HMGCR, NR1H3, NPC1L1, CYP17A1
Trans cinnamaldehyde	TRPA1

Interaction Design of Bioactive Cinnamon Bark Compounds and Their Target Proteins as Anti-Inflammation

The purpose of PPI *(Protein-Protein Interaction)* design is to analyze the relationship between proteins involved in the occurrence of activity (Santoso *et al.,* 2018). To do the network design between target proteins is done using the STRING database. STRING is one of the web that has a function to analyze protein interactions. When using STRING i*n silico* tests, it will facilitate the prediction of query proteins and other proteins (Razali *et al.,* 2020). Nodes and edges are data obtained from STRING analysis. Nodes are proteins that play a role in biological activity, then edges are lines that connect nodes as a sign of interaction (Yuniastuti *et al.,* 2021). In the interaction network that has been tested with STRING at a high confidence setting (0.400) the interaction score of the protein interaction network construction results is shown in Figure 1. Of the 191 target proteins, the KEGG *pathway* was obtained with signaling pathways such as the PPAR signaling pathway, and the IL-17 *signaling pathway*.

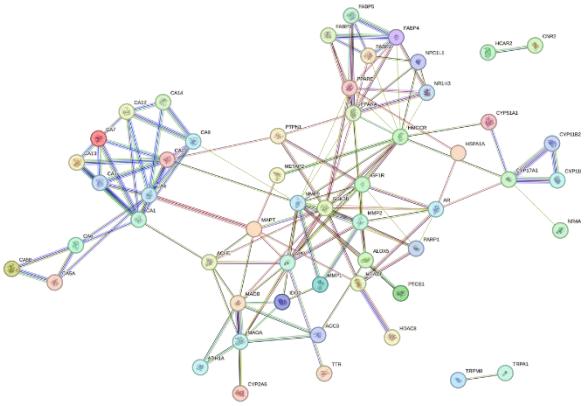

Figure 1. Protein interaction network in STRING. 4 out of 195 target proteins do not interact with other target proteins

Peroxisome proliferator activated receptors (PPARs) are DNA-binding nuclear hormone receptors that regulate inflammatory responses. Currently, three subtypes of mammalian PPARs have been identified, namely PPARA, PPARG, and PPARδ, which can also be referred to as PPARS (Etc *et al.,* 2019). Based on Athari's research (2019). Interleukin 17 (IL-17) is a cytokine that plays a major role in slow-type inflammatory reactions and also plays a role in the release of other inflammatory cytokines and chemokines such as IL-1, IL-6 TNF-α, and *macrophage inflammatory protein-2* (MIP-2). IL-17 cognate receptors identified so far are IL-17R, IL-17RH1, IL-17RL, IL-17RD and IL-17RE (Moseley *et al.,* 2003). Cytoscape is a database used to analyze interactions between compounds and target proteins (Hasibuan *et al.,* 2023). Cytoscape is used as a tool to expose the series of interactions between proteins (Leong *et al.,* 2021). In cytoscape there is a STITCH application. STITCH aims to analyze compound interactions and add target proteins using STRING (Amelia *et al.,* 2023). The network on the interaction of compounds and target proteins includes interactions of many compounds *(muli compound)* and many targets (*multi target).*

Orange nodes represent compounds in cinnamon bark. Blue color nodes illustrate the interaction between compounds and target proteins (Figure 2). The compounds from cinnamon bark that have target proteins are 9 compounds, namely caffeic acid, beta caryophyllene, coumarin, caryophyylene, acetyl eugenol, campesterol, fatty acid, isocaryophyllene, and stigmasterols.

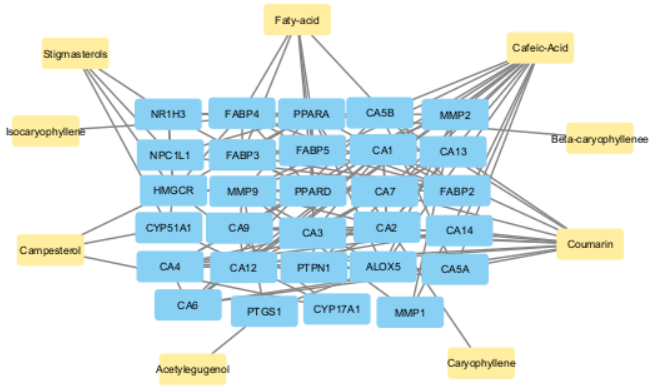

Figure 2. Visualization of raw data of compound and target protein interaction. Blue nodes indicate target proteins and yellow nodes indicate cinnamon bark compounds.

Topology Analysis of Protein Interaction Networks

Topology analysis is a quantitative analysis conducted to determine the key proteins involved in the mechanism of a disease (Hasanah *et al.*, 2018). *Degree* and *betweenness centrality* are some of the parameters considered in *topology analysis* (Ren *et al.*, 2016). Degree is a parameter that shows the results of interactions that there are several links between nodes. Nodes are high d*egree* values which are considered central nodes and have a role in transmitting signals in the network (Nowakowsaka *et al.*, 2022). Betweenness centrality shows the number of shortest paths based on the number of nodes.

And is known to represent the capacity of nodes to relate to other components of the network (Azuaje et al., 2010). The results of topological analysis were visualized to the size and color according to the parameter values (Figure 3). Based on the results of network topology analysis, there are 6 proteins that have the highest value based on degree and betweenness centrality (Table 4). The six proteins consistently occupy proteins that are regulated by cinnamon bark compounds and play a role in anti-inflammatory mechanisms.

Table 3. Top 6 Proteins Highest Degree and Betweenness Centrality Values

No.	Namae Protein	*Degree*	*Betweenness Centrality*
1.	PPARA	12	0,3482
2.	MMP-9	9	0,8822
3.	HMGCR	9	0,2616
4.	CA-4	8	0,6481
5.	CA-2	8	0,3650
6.	CA-9	8	0,3350

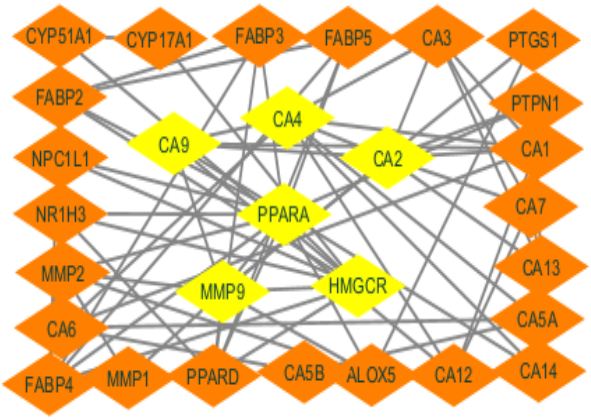

Figure 3. Results of topological analysis of protein interaction networks based on degree and betweenness centrality

In silico study of Molecular Tethering of Bioactive Cinnamon Bark Compounds

Molecular Docking of Compound and Target Protein

Before conducting molecular tethering docking analysis, the target protein was prepared first. Target protein preparation aims to separate the native ligand; each target protein is obtained protein structure without *native ligand* and *native ligand* structure. The target protein used must contain native ligand with inhibitory activity against the target protein (Setyawati *et al.,* 2022). In this study, 2 target protein structures were used including PPARA, and MMP-9.

The HMGCR target protein cannot be analyzed further, because research conducted by Wang (2015) in testing chronic HMGCR / HMG-CoA reductase inhibitor treatment against dysglycemia explains that statin class drugs are used as HMGCR / HMG-CoA inhibitors to reduce cholesterol levels. Then the target proteins CA-2, CA-4, and CA-9 also cannot be analyzed further, because the research conducted by Aslam (2020) explained that CA inhibitors are drugs used to treat glaucoma, hypertension, heart failure, and epilepsy Then the CA target protein is considered part of the diuretic class of drugs. PDBSum facilitates structural analysis based on Ramchandran's Procheck and G-factors values (Laskowski *et al.,* 2018).

Protein structures with good quality and can be used for docking research are characterized by the value of the most favored regions in the Ramachandran graph of 90% and the G-factors value is characterized by an overall average value >-0.5 (Tri., 2023). PDBSum which was initially analyzed by entering the codes that have been obtained from RSCB obtained data in Table 4.

Table 4. Statistics of Ramchandran Protein Target

Protein Target	*Procheck Ramchandran (Most Favoured Regions)*	*G-factors (Overal average)*
PPARA	91.00%	0.24
MMP9	90.00%	0.15

At the time of docking, ligands in the form of cinnamon bark compounds that have the highest degree and betweenness centrality values were used. The results of molecular docking produce binding affinity values and RMSD values between compounds and target proteins (Table 5). Binding affinity testing is done with the aim of testing the strength of molecular biology interactions (Octavia *et al.,* 2018). The smaller binding affinity value indicates that the protein and the compound have high activity (Tyas et al., 2018).

Evaluating the difference in docking orientations obtained from co-crystallized poses is the benefit of *Root-mean square deviation* (RMSD) (Triwulansari et al., 2022). There are 3 descriptions regarding the classification of unequal RMSD when docking, namely: 1.) a good network when the RMSD value is < 2.0 Å; 2.) an acceptable network when the RMSD value is between 2.0 and 3.0 Å; 3.) a poor network when the RMSD ≥ 3.0 Å (Ramírez *et al.,* 2018). Drug compounds that are proven to be efficacious as anti-inflammatories are one of the docking processes. In PPARA and MMP-9 target proteins, dexamethasone was used as a comparator. Dexamethasone is a corticosteroid class drug used as an anti-inflammatory and immunosuppressant (Indrianita *et al.,* 2013).

Table 5. Results of ligand interaction with target protein

Ligand Bonds - Target Protein	***Binding Affinity* (kkal/ mol)**	**RMSD**
Dexamethasone (kontrol) - PPARA	-7.0	0.0
Beta caryophyllene – PPARA	-7.4	0.0
Caryophyllene – PPARA	-7.9	0.0
Fatty acid - PPARA	-7.8	0.0
Isocaryophyllene - PPARA	-5.6	0.0
Dexamethasone (kontrol) – MMP-9	-6.9	0.0
Caffeic acid – MMP-9	-5.7	0.0

Based on the results of docking interaction between PPARA with beta caryophyllenee, caryophyllene and fatty acid respectively produced binding affinity values of -7.0 kcal/mol, -7.4 kcal/mol, and -7.9 the three proteins have low binding affinity values. In contrast to isocaryopyhllene which has a binding affinity value of -5.6 kcal/mol. While the MMP-9 target protein targeting caffeic acid compounds obtained a binding affinity.

Visualization of Molecular Docking Results of Compounds and Target Proteins

Biovia discovery studio visualizer application is used to view the interactions and also the amino acid residues that play a role and the interactions obtained (Ullaya *et al.*, 2021). The similarity between interactions and amino acid residues created is an important pattern with the similarity of the pharmacological effects produced by the presence of a ligand when occupying the active site on the receptor (Junaidin *et al.*, 2022). Target protein and ligand interactions include hydrogen bonds, hydrophobic bonds, electrostatic bonds, and van der waals bonds (Pantsar *et al.*, 2018).

The parameters considered during docking analysis are the similarity between amino acid residues and the bond that occurs between the ligand and the receptor. The tested ligand interacts with amino acid residues and similar bond types indicating that there is similarity in activity, because it has a similar type of interaction (Rizky *et al.*, 2020). The results of docking visualization were carried out on compounds and target proteins PPARA, and MMP9 with the following results:

***Peroxixme Poliferator Activated Receptor* Alpha (PPARA)**

PPARA is a fatty acid-activated transcription factor that has a nuclear receptor. PPARA plays a role in inflammation. PPARA regulates proliferation or contributes to wound healing or inflammation. Activation of PPARA results in anti-inflammatory effects (Dubrac *et al.*, 2015),

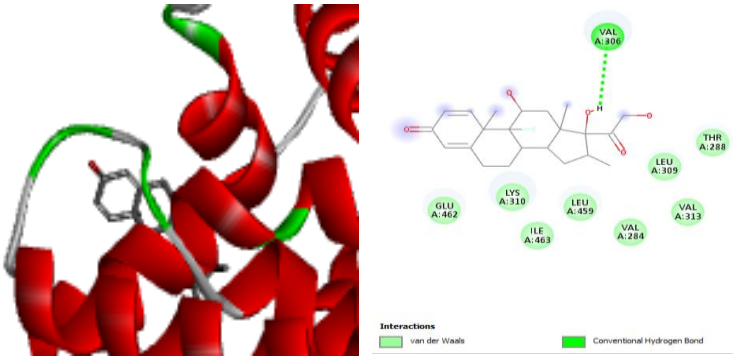

Figure 4. 2D and 3D visualization of the interaction between PPARA and Dexamethasone ligand.

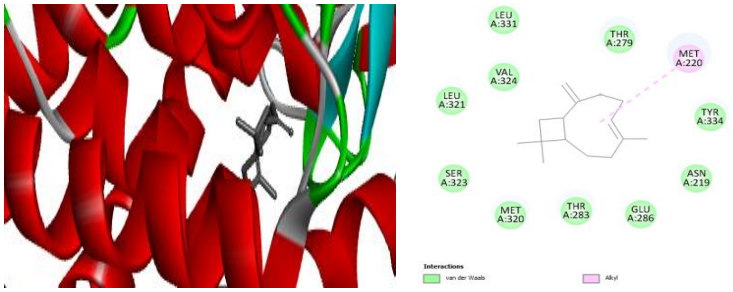

Figure 5. 2D and 3D visualization of the interaction between PPARA and beta caryophyllene ligand

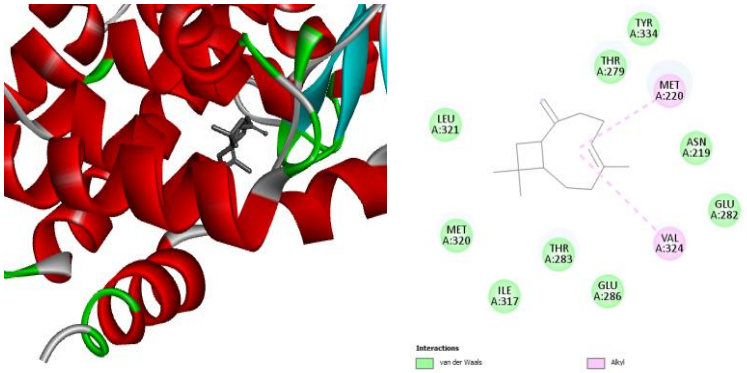

Figure 6. 2D and 3D visualization of the interaction between PPARA and Caryopyhllene ligand

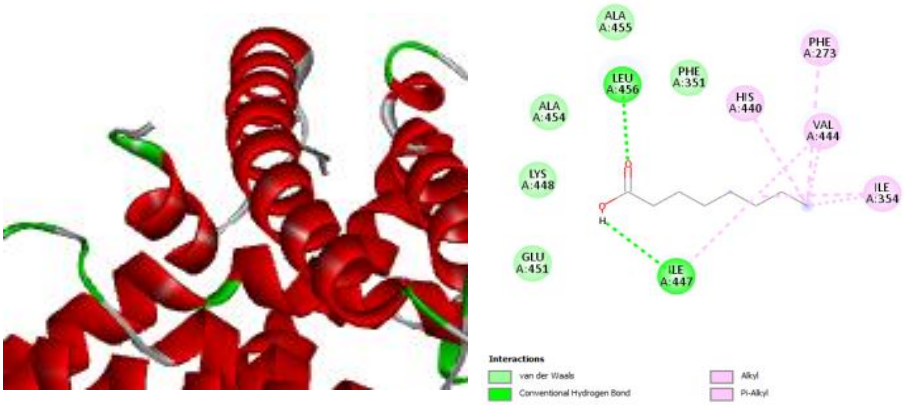

Figure 7. 2D and 3D visualization of the interaction between PPARA and Fatty acid ligand.

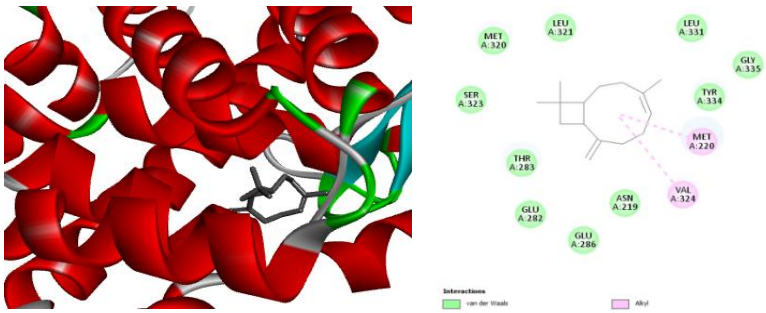

Figure 8. 2D and 3D visualization of the interaction between PPARA and isocaryopyllene ligand

The results of amino acid bond interactions between PPARA target proteins and ligands can be seen in Table 6. 2D interactions obtain data in the form of amino acid residues and interactions formed. Of the four compounds that have been visualized using the biovia discovery studio visualizer, there is 1 compound that has similarities and also amino acid residues when compared to the control drug dexamethasone issocaryopyhllene compound (Figure 8. C). The mechanism of action of the issocaryopyhllene compound, which is identical to the control drug, is shown in Figure 8. The results of amino acid bond interactions between PPARA target proteins and ligands can be seen in Table 6.

Table 6. Interactions formed between PPARA and ligands

Bond Name	Bond Van der Waals	Bond Hydrophobic Alkyl	Bond Hydrogen
Dexamethasone – PPARA	GLU: 462 LYS: 310 ILE: 463 VAL: 284 VAL: 313 LEU: 309 **THR: 288**	-	VAL: 306
Isocaryopyhllene - PPARA	LEU: 321 LEU: 331 GLY: 335 TYR: 334 ASN: 219 GLU: 286 GLU: 282 **THR: 288** SER: 323 MET: 320	MET: 220 VAL: 324	-

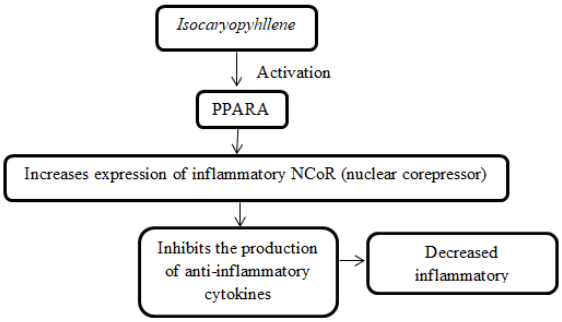

Figure 9. Concept map of the mechanism of isocaryopyhllene in reducing inflammation

Matrix Metalloproteinase-9 (MMP-9)

MMP-9 is one of the enzymes that plays an important role during the progressive process of inflammation (Chrestella et al., 2010). MMP-9 activation provides anti-inflammatory effects in various inflammatory conditions such as skin redness (Mardiyantoro et al., 2010). Increased MMP-9 activity in endometriosis can facilitate invasion resulting in the formation of endometriosis lesions, where endometriosi lesions trigger chronic inflammatory reactions that can lead to pain (Kumalasari et al., 2018). In the docking test of the MMP-9 target protein analyzed with the control drug dexamethasone. The results of amino acid bond interactions between MMP-9 target proteins and ligands can be seen in the table 7 below.

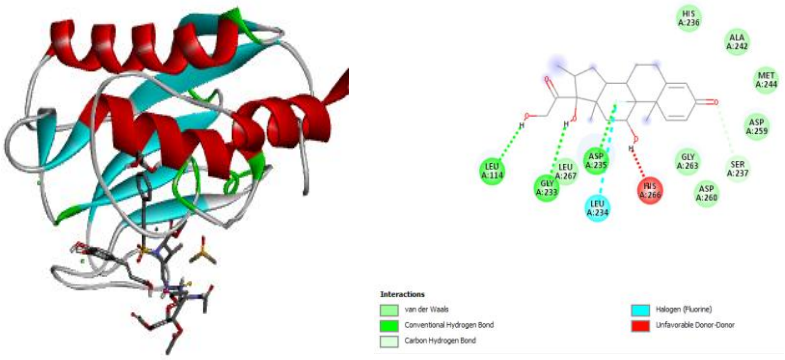

Figure 10. 2D and 3D visualization of the interaction between MMP-9 and dexamethasone ligand.

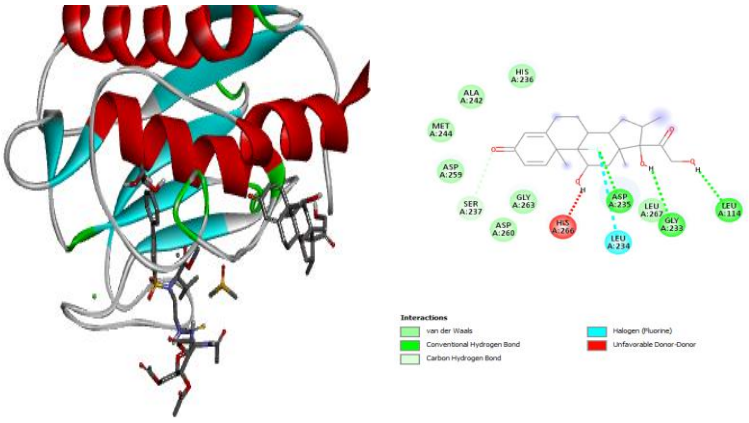

Figure 11. 2D and 3D visualization of the interaction between MMP-9 and caffeic acid ligand

Table 7. Interactions formed between MMP-9 and ligands

Name Bond	Bond Van der Waals	Bond Halogen	Bond Hydrogen

Dexamethasone – MMP9	**HIS: 236** **ALA: 242** **MET: 244** **ASP: 259** **ASP: 260** GLY: 263	**LEU: 234**	LEU: 114 **GLY: 233** ASP: 235
Caffeic acid – MMP-9	**HIS: 236** **ALA: 242** **MET: 244** **ASP: 259** **ASP: 260** LEU: 267	**LEU: 234**	SER: 237 LEU: 267 ASP: 235 **GLY: 233** LEU: 114

This compound produces van der Waals interaction results on 5 amino acid residues, namely HIS: 236, ALA: 242, MET: 244, ASP: 259, and ASP: 260. This compound has bond interactions that are similar to the control drug dexamethasone, this indicates that the compound tested had the same activity as the control drug dexamethasone. The mechanism of action of the *caffeic acid* compound which is identical to the control drug is in figure 11.

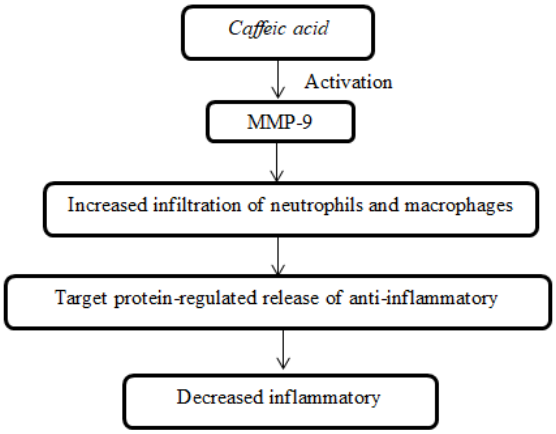

Figure 12.Concept map of the mechanism of caffeic acid in reducing inflammation

CONCLUSION

Cinnamon bark compounds have a role as anti-inflammatory agents. Based on the results of the PASS Online test with a Pa value > 0.5, 16 compounds were found. These compounds are 2-methoxy-cinnamaldehyde, acetyleugenol, ascorbic acid, beta caryopyhllene, borneol, caffeic acid, campesterol, caryopyhllene, cinnamaldehyde, cinnamic acid, coumarin, eugenol acetate, fatty acids, isocaryopyhllene, stigmasterols, and trans cinnamaldehyde. A total of 5 cinnamon bark compounds target PPARA and MMP-9 proteins which play a role in anti-inflammatory mechanisms. Based on the docking results, the isocaryopyhllene compound has a mechanism as a PPARA activator with a binding affinity value of -5.6 kcal/mol. The caffeic acid compound has a mechanism as an MMP-9 activator with a binding affinity value of -5.7 kcal/mol. Other compounds also have similar interactions and amino acid residues with the control drug so they can be used as candidates for anti-inflammatory agents that target the PPARA and MMP-9 proteins.

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

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