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The potential of honey from Apis cerana in preventing chronic inflammation by downregulating the NF-κB pathway

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ABSTRACT

Chronic inflammation is an inflammatory condition that persists over a long period of time causing tissue damage and contributing to the development of chronic diseases such as arthritis, heart disease, or certain autoimmune conditions. The protein that plays a role in this regulation is the up-regulation of NF-κB, this is the aim of this research, namely to determine the potential of the compounds contained in A.cerana honey to bind with NF-κB. The research stages carried out were preparing the NF-κB protein structure database, protein preparation using the Yasara application, structure preparation and optimization using the Chemaxon application, as well as validating the molecular docking method and docking of compounds from A.cerana honey on the NF-κB protein using the PLANT application. The research results show that Ellagic acid, Taxifolin, Quercetin and Chrysin have affinity and form hydrogen bonds with the NF-κB protein. The binding energy between Ellagic acid, Taxifolin, Quercetin and Chrysin with the NF-κB protein respectively is 78,822; -64.89; -64,872 and -64,935. Ellagic acid, Taxifolin, Quercetin and Chrysin have potential anti-inflammatory activity through binding to the NF-κB protein, thereby preventing the inflammatory process.

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1. Introduction

Cardiovascular disease is a disease resulting from impaired function of the heart and blood vessels which caused 31% or around 17.5 million of the world's deaths in 2012. Of these deaths, 7.4 million were caused by coronary heart disease and 6.7 million by strokes. Coronary heart disease occurs due to narrowing of the coronary arteries so that there is no blood flow to the heart muscle. The underlying cause of coronary heart disease is atherosclerosis, which is chronic inflammation of the inner walls of the arteries resulting in thickening and decreased elasticity (Durkan et al., 2023).

Chronic hypercholesterolemia is one of the main causes of endothelial dysfunction. Chronic hypercholesterolemia will cause the accumulation of LDL in the intimal area. The presence of free radicals will cause the oxidation of LDL, called ox-LDL, which is able to activate endothelial cells, producing pro-inflammatory cytokines and adhesion molecules that stimulate infiltration of monocytes and T lymphocytes into the intimal area (Garas, 2017).

Cytokines will trigger the differentiation of monocytes into macrophages which will phagocytose ox-LDL to form foam cells which contribute to the formation of atherosclerotic plaque (Ermiş et al., 2023). In addition, pro-inflammatory cytokines are able to cause IκB phosphorylation so that NF-κB is released from the NF-κB-IκB complex to the nucleus to support the transcription process of pro-inflammatory genes. Based on the mechanism of plaque formation, research is being carried out to develop drugs for atherosclerosis, one of which is through a mechanism for preventing plaque formation by inhibiting the inflammatory process (P. Liu et al., 2022).

The compounds contained in honey are known to have pharmacological activity as anti-inflammatory and have activity in protecting cardiovascular disease. In the small intestine, glycosides from honey will be hydrolyzed to form aglycones more hydrophobic. This form will be more easily absorbed by passive diffusion and its bioavailability will be better compared to glycone so that the potential effect produced is also greater (Gantait et al., 2024).

The risk of using non-steroidal anti-inflammatories is that they often cause irritation to the stomach which can result in stomach pain, heartburn, or even gastric pain (Salsabila & Sudiono, 2022), reducing the blood's ability to clot, thereby increasing the risk of bleeding (Arfiandi et al., 2022). If used in the long term it can cause kidney problems, so it is necessary to search for compounds from nature that have the potential to act as anti-inflammatories (Agustina et al., 2015). Several studies regarding the anti-inflammatory activity of honey in inhibiting the inflammatory process (Yong et al., 2013) contain essential oils, flavonoids, caffeic acid, isoflavonoids, resin, sinapinic acid, chrysin, pollen, isoferulic acid (Naqvi et al., 2013). . Therefore, it is necessary to carry out research to determine the activity of the compounds contained in honey as anti-inflammatory in atherosclerosis based on their interaction with the NF-κB protein using the in *silico molecular docking method* . With this method, the binding affinity of the test *ligand* and the interactions that occur can be determined so that the potential of honey compounds as anti-inflammatory in atherosclerosis can be predicted.

2. Method

2.1 Materials and tools

The materials used in this research are the NF-κB protein with the code 4IDV and compounds contained in *Apis cerena* (Wu et al., 2022). The equipment used in this research was an Acer Aspire laptop and the software used was Yasara (Krieger & Vriend, 2014), Chemaxon (ChemAxon, 2016), Discovery Studio (ChemAxon, 2016) and PLANTS (ChemAxon, 2016).

Docking

This research began with redocking native ligands. The coordinates of the redocking ligand are obtained from the coordinates of the native ligand. To obtain these coordinates, protein preparation is first involved by removing water and ligands that are not related to the docking process such as NAG, glycerol and sulfate ions. The next stage is native ligand preparation. This native ligand preparation is carried out using Chemaxon software and the result is 10 native ligand conformers. The next stage is docking using PLANT software. The results of this docking are the docking score and the docking RMSD value. The next stage is docking of the compounds contained in *Apis cerena.* Apart from the docking score, other data obtained are the protein residues involved in the interaction and the type of interaction. To see this, Discovery Studio software is used.

Data analysis

The data analysis used in this research is the docking score and the formation of hydrogen bonds between the ligand and the NF-κB protein (Saputra, 2018)

3. Results and Discussion

Validation of the molecular docking method was carried out by docking the NF-κB protein without ligand with the native ligand which had been previously separated using the Yasara program. The results and visualization of the interaction validation of the molecular docking method can be seen in Figure 1. Based on the search for the native ligand coordinates, the native ligand coordinates are X: 16.6303; Y:13.7005 and Z:87.2809 with a radius of 11.9338 Ångström (Å). Based on redocking carried out on the native ligand, an RMSD value of 1.236 Å was obtained. This RMSD value is below 2 Å so it meets the ligand validation requirements (C et al., 2022). Therefore, the coordinates obtained in the redocking process can be used to carry out docking of ligands found in *A. cerana honey* .

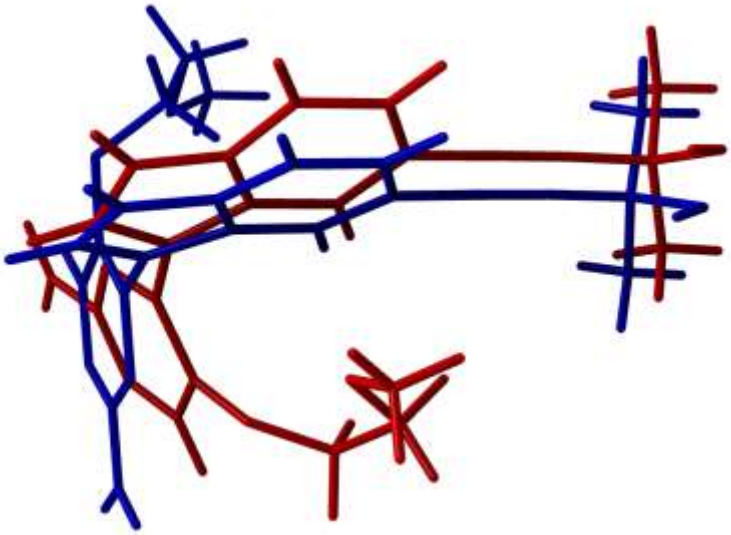


Figure 1. Ligand superpose

The native ligand is red and the redocking ligand is blue

Docking of compounds from *A.cerana honey* was carried out using the PLANTS program by setting the interaction site coordinates to be the same as the interaction site coordinates of the native ligand on the NF-κB protein. Results obtained from the docking process of *A.cerana compounds* with the NF-κB protein in the form of bond energy and amino acid residues which are formed using visualization from Discovery Studio. The results and visualization of the docking interactions that occur between the *A.cerana honey compound* and the NF-κB protein can be seen in tables 1 and 2 and figure 2.

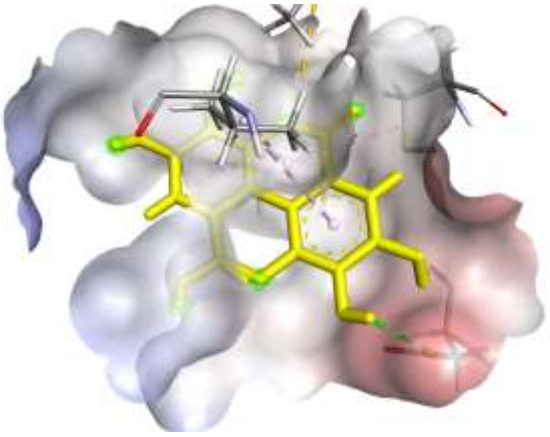
Table 1. Docking Score

Name	Docking score
native ligands	-83,511
Ellagic acid	-78,822
Taxifolin	-64.89
Quercetin	-64,872
Naringenin	-64,935
Chrysin	-64,935

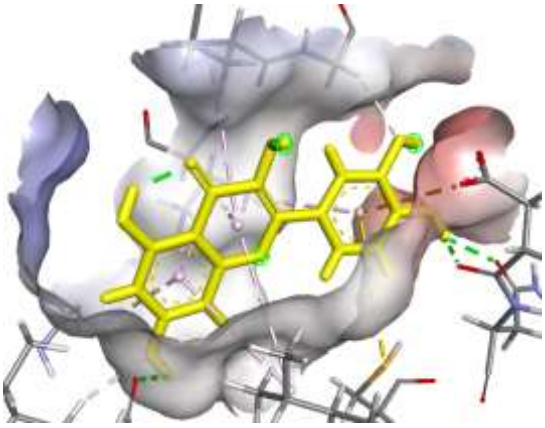
Table 2. Amino acid residues involved in interactions with NF-κB

Name	**Residue**
native ligands	ARG 405; ARG 408; VAL 414; ARG 416; ALA 427; LYS 429; GLU 440; CYS 444; VAL 453;ILE 467; MET 469; GLU 470;LEU 471; LEU 472; GLU 473; GLY 475; SER 476; GLN 479; LEU 522; CYS 533; ASP 534; PHE 535
Ellagic acid	ARG 408; GLY 409; VAL 414; ARG 416; ALA 427; MET 469; LEU 471; LEU 472; GLU 473; SER 476; GLN 479; ASP 519; ASN 520; LEU 522; CYS 533; ASP 534

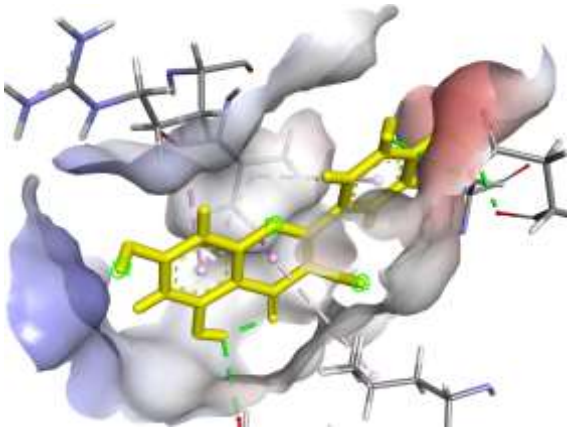
Taxifolin	ARG 405; ARG 408; GLY 409; VAL 414; ARG 416; ALA 427; MET 469; LEU 471; LEU 472; SER 476; LYS 517; ASP 519; ASN 520; LEU 522; CYS 533; ASP 534
Quercetin	ARG 405; ARG 408; GLY 409; SER 410; VAL 414; ARG 416; ALA 427; MET 469; GLU 470; LEU 471; LEU 472; GLU 473; SER 476; GLN 479; LYS 517; ASP 519; ASN 520; LEU 522; CYS 533; ASP 534
Naringenin	ARG 405; ARG 408; GLY 409; VAL 414; ARG 416; ALA 427; MET 469; GLU 470; LEU 471; LEU 472; GLU 473; SER 476; ASP 519; ASN 520; LEU 522; CYS 533; ASP 534
Chrysin	ARG 405; ARG 408; GLY 409; SER 410; VAL 414; ARG 416; MET 469; LEU 471; LEU 472; GLU 473; SER 476; ASP 519; ASN 520; LEU 522; CYS 533; ASP 534



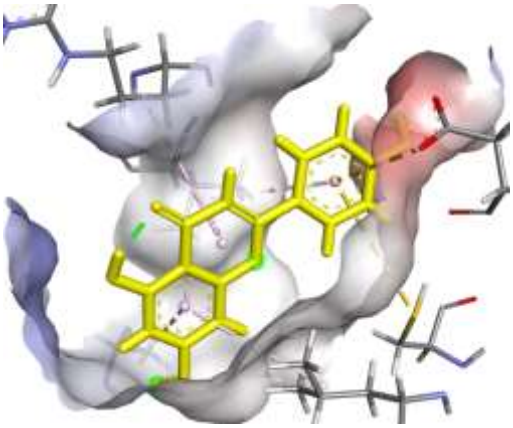
Ellagic acid



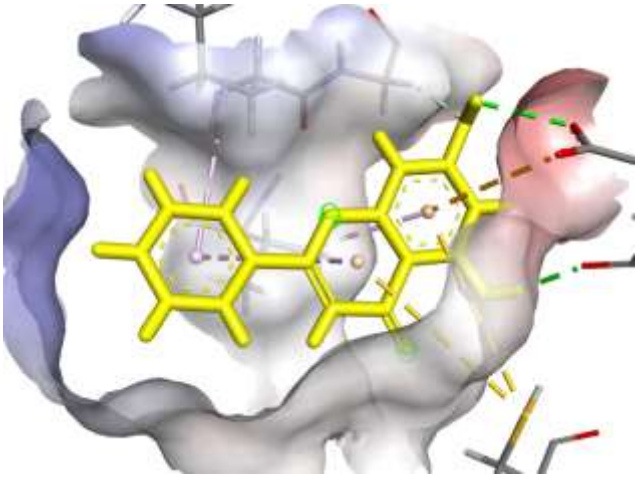
Quercetin



Taxifolin



Naringenin



Chrysin

Figure 2. 3D structure of the interaction between compounds and NF-κB

The bond between the ligand and the amino acid residue is a hydrogen bond because this bond determines the stability of the bond and the energy required for the reaction to occur. Hydrogen bonds occur at a maximum distance of 3.4 Å (Bitencourt-Ferreira et al., 2019). The hydrogen bonds formed between compounds from *A. cerena honey* and the NF-κB protein are formed between Ellagic acid and ASP19 with a distance of 2.64658 Å . Taxifolin forms bonds with residues LEU472 and ASP519 at a distance of 2.93734 Å and 2.70821 Å. Quercetin binds to LEU472 with a distance of 2.73634 Å, ASP519 (2.0979 Å), ASN510 (2.3531 Å), GLY409 (2.5789 Å), LEU471 (2.7103 Å). Chrysin forms ASP519 and GLY409 bonds at a distance of 2.06565 Å and 2.1463 Å.

Besides hydrogen bonds, other bonds are also formed, namely PI-sufur and electrostatic. There are only two residues involved in the Pi-sulfur reaction, namely MET569, CYS533. Ellagic acid forms a bond with MET569 at a distance of 5.96213 Å. CYS533 with quercetin with a distance of 5.1949 Å , Naringenin 5.43709 Å and Chrysin 5.96927 Å. The electrostatic bond between residue ASP519 and Quercetin at a distance of 4.12685 Å, Naringenin at a distance of 4.20753 Å and Chrysin at a distance of 4.16494 Å (Zhou & Pang, 2018).

Docking of compounds contained in A. cerena honey to the active site of the NF-κB protein was carried out using the PLANT program by setting the interaction site coordinates to be the same as the interaction site coordinates of the native ligand on the protein. The results obtained from the docking process between compounds from honey and the NF-κB protein are in the form of bond energy and hydrogen bond data in the 10 best conformations. The bond energy shows the affinity between Ellagic acid, Taxifolin, Quercetin and Chrysin and the protein. The smaller the bond energy obtained, the more stable the bond formed.

Based on the bond energy values obtained, it shows that the compounds Ellagic acid, Taxifolin, Quercetin and Chrysin have potential anti-inflammatory activity because they have affinity and form hydrogen bonds with the NF-κB protein (Hadi et al., 2023; Zhou & Pang, 2018). The bond that occurs between Ellagic acid, Taxifolin, Quercetin and Chrysin with the NF-κB protein is able to inhibit the transcription of pro-inflammatory genes (Saputri et al., 2016; Sutomo & Pratama, 2020).

NF-κB is an Important Transcription Factor in Various Cellular Processes (T. Liu et al., 2017). The NF-κB family of transcription factors has an important role in cellular responses to various stimuli, such as stress, cytokines, free radicals, heavy metals, ultraviolet radiation, oxidized LDL, and bacteria or viruses (antigen). NF-κB plays a key role in regulating the immune response to infection (κ light chain is an important component of immunoglobulins) (Chen et al., 2023). Dual Roles of NF-κB: Immunity and Disease. Improper regulation of NF-κB is associated with various diseases, including cancer,

inflammatory and autoimmune diseases, septic shock, viral infections, and immune system disorders. On the other hand, NF-κB is also involved in important processes such as synaptic plasticity and memory (Mukherjee & Gopalakrishnan, 2023).

4. Conclusion

Ellagic acid, Taxifolin, Quercetin and Chrysin have potential anti-inflammatory activity because they have docking scores below -50 and form hydrogen bonds with the NF-κB protein so they can prevent chronic inflammatory processes using *in silico* methods. This research is still limited to computational model research so laboratory research using in vitro and in vivo anti-inflammatory methods is needed to validate this computational test. Research on anti-inflammatory drug compounds is needed as complementary drugs to analgesic drugs circulating in the community.

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