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Molecular Docking of the *Cannabis sativa* L. Bioactive Compound Against Inflammation Induced by Cigarette Smoke Exposure

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Abstract. Cigarette smoke can modulate and increase chronic inflammation of the respiratory tract. The constituents of cigarette smoke-induced inflammation can activate several cell signaling pathways, including mitogen-activated protein kinases (MAPK), nuclear factor kappa-B (NF-κB), signal transducer and activator of transcription (STAT), and activatory protein-1 (AP-1). Cannabis plant (*Cannabis sativa* L.) contains many phytochemical compounds including cannabinoids, terpenes and phenolic compounds, which have potential in medicine, one of them is as an anti-inflammatory. This study aims to determine the interactions formed between bioactive compounds from cannabis plants in anti-inflammatory activity caused by cigarette smoke induction in the JAK/STAT and MAPK pathways through a study in silico. This research method was carried out in a descriptive exploratory manner usingonline databases such as PubChem, PASS online, SEA (Similarity Ensemble Approach) and Swiss Target Prediction, STRING. Docking simulation was carried out using PyRx 0.8. Data from software and web devices were analyzed descriptively and compared with control compounds. The docking results show that the compounds from *Cannabis sativa* L. can act asan anti-inflammatory in the context of cigarette smoke-induced inflammation. This is indicatedby the similarity of amino acid residues resulting from the interaction of the aspirin drug (control) with the anti-inflammatory receptor protein in the compound cannabidihydrophenanthrene with PDGFRA and KDR receptors and compounds cannabicyclol with AKT1 and KDR receptors. This is indicated by the presence of value affinityis low indicating a stable and strong bond.

Keywords: Anti-inflammatory, Molecular docking, *Cannabis sativa* L., Pathways JAK/STAT and MAPK.

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INTRODUCTION

Inflammation is a direct result of tissue injury that causes an imbalance in metabolic processes. During the inflammatory process, catabolic manifestations increase, namely proteolysis, decreased oxidative metabolism, or decreased cell space volume (Haramizu et al., 2011). Uncontrolled acute inflammation can become chronic and contribute to various chronic inflammatory diseases, such as arthritis, cardiovascular disease, cancer and diabetes (Libby, 2007; Zhou et al, 2016). Understanding of inflammatory response pathways and molecular mechanisms will better contribute to improving the prevention and treatment of inflammatory diseases (Chen et al., 2018). The JAK/STAT, MAPK, and NF-κB signaling pathways represent the most effective pathways to achieve optimal therapeutic response with minimal side effects.

Cigarette smoke can modulate and increase chronic inflammation of the respiratory tract. Direct activation of epithelial cells and immune cells in the oral respiratory tract inducessecretion of pro-inflammatory factors. This leads to repetitive chronic injury coupled with an abnormal tolerogenic response to antigens and colonization of coexisting pathogens can promote the development of auto-reactive immunity, which further perpetuates tissue injury and inflammation (Lee J et al, 2012). Cigarette smoke can alter many of the cell signaling pathways involved in cellular activation. Cigarette smoke constituents activate several cell signaling pathways, including mitogen-activated protein kinases (MAPK), nuclear factor kappa-B (NF-κB), signal transducer and activator of transcription (STAT), and activatory protein-1 (AP-1) all of which are involved in the regulation of inflammation, cell cycle, and other genes (Iles et al., 2005; Kroening et al, 2008; Liu et al., 2008; Smelter et al., 2010).

Cannabis sativa L. or better known as cannabis is a species of herb originating from Central Asia, which has been used in traditional medicine and as a source of textile fiber since ancient times. This plant is rich in phytochemicals that are used in

molecules of industrial interest, including cannabinoids, terpenes and phenolic compounds, as well as their biosynthetic routes (Andre et al, 2016). In a study by Wang et al., (2022) stated that the phytochemical compounds in *Cannabis sativa* L. in the form of CBD (cannabidiol) compared to DEX (dexamethasone) were able to suppress LPS-induced activation of the MAPK and NF- κB signaling pathways in RAW264.7 cells through different intracellular components indicating that CBD's anti-inflammatory biological mechanism differs from that of other immunosuppressants. Therefore, it is necessary to find new indications for the pharmacological use of cannabis products are justified by clues to search for targets within and outside the endocannabinoid system (Stasiłowicz et al., 2021).

Current drug development is accompanied by genomics, proteomics, bioinformatics, and efficient technology known as the in silico method. The in silico method not only speeds up the drug discovery process but can also prevent late-stage clinical failures thereby reducing substantial costs (Chikhale, 2020). Several previous studies stated that molecular docking is capable of screening a collection of compounds and calculating the strongest bonds between bioactive compounds in a plant and target proteins by various scoring methods. This method isused to explore a compound as a drug candidate and a specific protein as a molecular target.

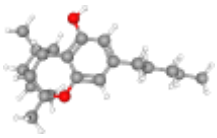
Based on the reasons above, this study aims to predict the potential of the bioactive compounds contained in *Cannabis sativa* L. as anti-inflammatory candidates caused by cigarette smoke by targeting the JAK/STAT and MAPK signaling pathways in silico.

METHODS

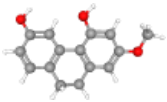
The tools needed are hardware and software. Hardware in the form of 1 set of Dell Corei3 and Acer Swift 3 Intel Corei3 laptops equipped with PyRx 0.8 software and BIOVIA Discovery Studio Visualizer. As well as PubChem, PASS Online, Vega ZZ, and PDB (Protein Data Bank) web servers.

The materials used were 3D and 2D structures of the compounds Cannabicyclol, Cannabi dihydrophenanthrene, Cannabinerolic-Acid, Cannabigerol, Cannabinol, Cannabichromenic-Acid, Cannabichromene, Canabispiran, and Aspirin as controls downloaded from the PubChem database (https://pubchem. ncbi.nlm. nih.gov/) in *sdf format and 3D structures of several receptor proteins downloaded from the Protein Data Bank (https://www. rcsb.org/search) in *pdb format.

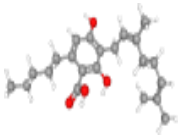
These receptor proteins include Serine/ threonine-protein kinase AKT (AKT1), Serine/threonine-protein kinase mTOR (MTOR), Epidermal growth factor receptor erbB1 (EGFR), Platelet-derived growth factor receptor alpha (PDGFRA), Macrophage colony stimulating factor receptor (by homology) (CSF1R), Vascular endothelial growth factorreceptor 2 (KDR), Vascular endothelial growth factor receptor 1 (FLT1), PI3-kinase p85-alpha (PIK3R1), PI3-kinase p110-alpha (PIK3CA).



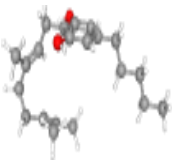
Cannabicyclol



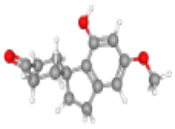
Cannabidihyd rophenanthrene



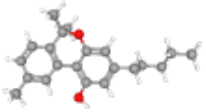
Cannabinerolic-Acid



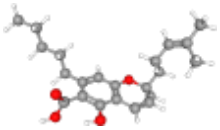
Cannabigerol



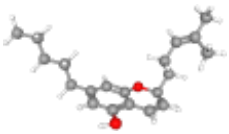
Cannabispiran



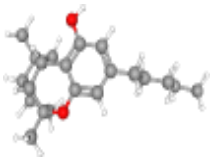
Cannabinol



Cannabichromenic-Acid



Cannabichromene



Cannabicyclol

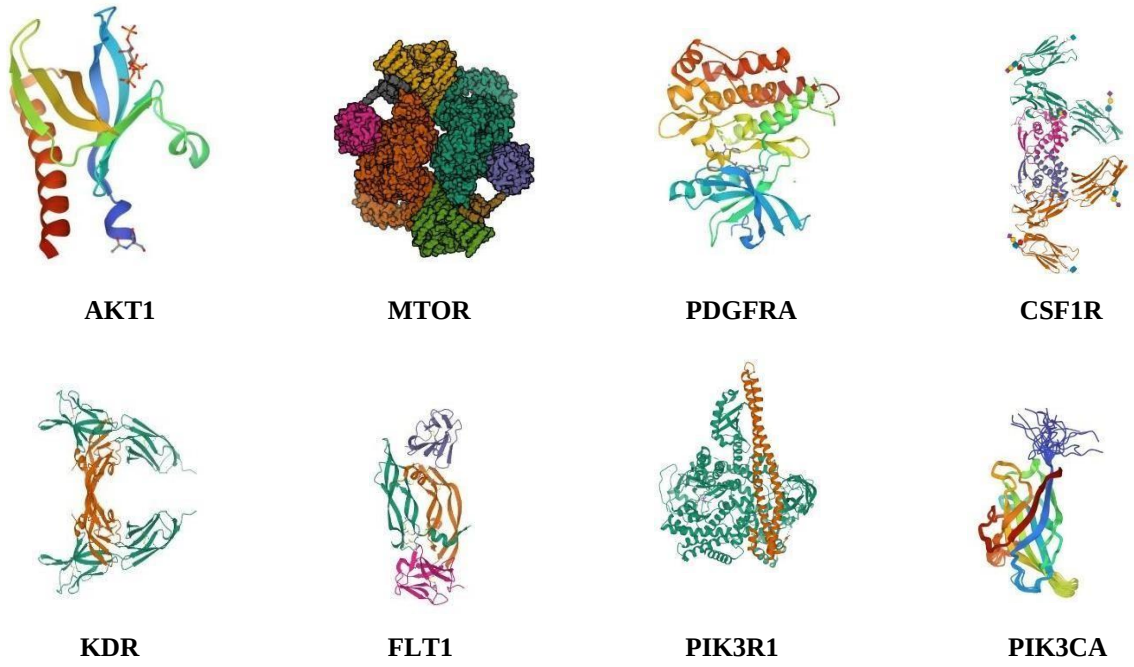


Figure 1. Ligands and receptors

Collection and Screening of Bioactive Compounds

Collection of compounds was performed using the Dr Duke database https://phytochem.nal.usda.gov/phytochem/search. The collected compounds were thenunified by collecting the SMILE of each compound using PubChem on the https://pubchem.ncbi.nlm.nih.gov/page. Screening is carried out to select compounds that have potential activity as anti-inflammatories using the http://www.pharmaexpert.ru/passonline/page.

Determination of Target Proteins and Signaling Pathways Affected

Determination of the target protein is carried out by the SEA test on the http://sea.bkslab.org/ page or Swiss Target Prediction on the http://new.swisstargetprediction.ch/ page to predict targets on compounds that have the highest probability. In this test, uniprot collection was carried out to determine the signaling pathways between target proteins which would later be used in the STRING test. In the STRING test phase, the anti-inflammatory signaling pathway can be seen through the KEGG Pathways analysis on the https://string-db.org/ page.

Preparation of Ligands (Bioactive Compounds) and Target Proteins

The 3D structures of cannabis plant compounds were downloaded using the Pubchem database which were later used as ligands. Then the ligands were prepared using the PyRx application to be minimized and formatted to *pdbqt.

The 3D structure of the target protein was downloaded using the RCSB PDB databaseand then prepared using the Vega ZZ application. The preparation process aims to remove water molecules, natural ligands and unnecessary residues, as well as adding hydrogen molecules.

Molecular Docking and Visualization

Molecular docking is performed to bind bioactive compounds (ligands) to target proteins (receptors). The docking process was carried out using Autodock vina on the PyRx 0.8 application. Protein molecules were added as macromolecules in *pdbqt format, then prepared ligand molecules were added in *pdbqt format and then ran vina. The result of docking between the ligand and the receptor is indicated by the binding affinity value.

The next stage is visualization using the Discovery Studio Visualizer application in 2 dimensions (2D) and 3 (3D), so that in this analysis the bond between the ligand and the receptor can be identified.

RESULTS AND DISCUSSION

Ligand and Target Protein Preparation

The predetermined ligands were then searched for by Canonical SMILE. The next stepwas to test PASS Online. In the analysis of PASS Online

results, what is considered is the valueof Pa or potential activity. A Pa value > 0.7 indicates that the compound has a high potential to actually become a bioactive compound in vitro and/or in vivo experimental tests and at the same time has a high degree of similarity to drug compounds with the same bioactivity. The value of 0.5 < Pa < 0.7 indicates that the compound has a high potential to actually become a bioactive compound in in vitro and/or in vivo experimental tests and at the same time has the potential to become a new scaffold for the development of new drug compounds with the relevant bioactivity. And the Pa value <0.5 indicates that the compound has a low potential to become a bioactive compound in in vitro and/or in vivo experimental tests and at the same time has a low potential to be successful (Chellilah, 2008). The development of anti-inflammatory drug compounds described in Table 1.

Table 1. Ligand & Protein Preparation and Unification

No	Compound	Canonical SMILE	Pa	Activity
1.	Cannabicyclol	CCCCCC1=CC(=C2C3C4C(C3(C)C)CCC4(OC2=C1)C)O	0.242	Anti- inflammatory
2.	Cannabidihydrophenanthrene	COC1=CC2=C(C(=C1)O)C3=C(CC2)C=CC(=C3)O	0.536 0.440	Intestinal anti-inflammatory Anti-inflammatory
3.	Cannabinerolic-Acid	CCCCCC1=CC(=C(C(=C1C(=O)O)O)CC=C(C)CCC=C(C)C)O	0.732 0.384	Anti- inflammatory Intestinal anti-inflammatory
4.	Cannabigerol	CCCCCC1=CC(=C(C(=C1)O)CC=C(C)CCC=C(C)C)O	0.666 0.346	Anti- inflammatory Intestinal anti-inflammatory
5.	Cannabinol	CCCCCC1=CC(=C2C(=C1)OC(C3=C2C=C(C=C3)C)(C)C)O	0.500 0.228	Anti- inflammatory Intestinal anti-inflammatory
6.	Cannabichromenic-Acid	CCCCCC1=CC2=C(C=CC(O2)(C)CCC=C(C)C)C(=C1C(=O)O)O	0.578 0.284	Anti- inflammatory Intestinal anti-inflammatory
7.	Cannabichromene	CCCCCC1=CC(=C2C=CC(OC2=C1)(C)CCC=C(C)C)O	0.508 0.215	Anti- inflammatory Intestinal anti-inflammatory
8.	Canabispiran	COC1=CC2=C(C(=C1)O)C3(CCC(=O)CC3)CC2	0.442 0.429	Anti- inflammatory Intestinal anti-inflammatory

Target protein determination is carried out by the SEA test or Swiss Target Prediction to predict targets for compounds that have the highest probability of compounds based on similarities in 2D and 3D structures (Gfeller et al., 2014). In this test, Uniprot collection was carried out to determine the signaling pathways between target proteins which would later be used in the STRING test. In the STRING test phase, the anti-inflammatory signaling pathway can be seen through KEGG Pathways analysis. The results of this analysis obtained the JAK /STAT signaling pathways and NF-κB signaling pathways which have a role in the anti- inflammatory mechanism. Target proteins are marked in red and blue, which include Serine/threonine-protein kinase AKT (AKT1), Serine/threonine-protein kinase mTOR (MTOR), Epidermal growth factor receptor erbB1 (EGFR), Platelet-derived growth factor receptor alpha (PDGFRA), Macrophage colony stimulating factor receptor (by homology) (CSF1R), Vascular endothelial growth factor receptor 2 (KDR), Vascular endothelial growth factor receptor 1 (FLT1), PI3-kinase p85-alpha (PIK3R1), PI3-kinase p110- alpha (PIK3CA).

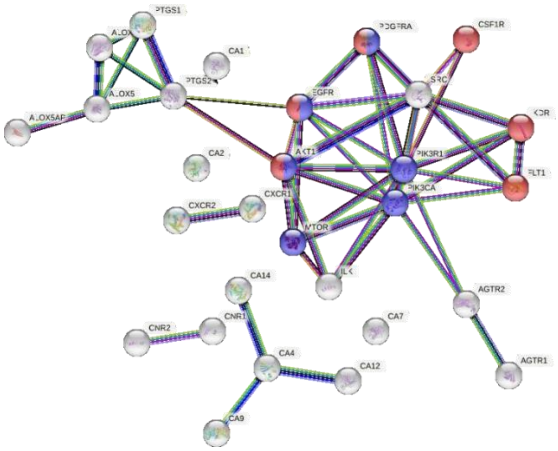


Figure 2. STRING Pathways

Figure 2 shows the results of the STRING test, the red protein is a protein in the MAPKsignaling pathway, and the blue protein is in the JAK/STAT signaling pathway. The protein network results use a minimum required interaction score of 0.7 with only showing high confidence results. This result is the result of screening because by forming a protein network,it is known that the proteins that enter into the pathway that will be carried out in research. After knowing the types of proteins, the compounds that affect these proteins can be identifiedbased on the results from the previous collection using the SEA test or SwissTarget Prediction.Therefore, it is possible to determine the compounds used by the ligands and the proteins usedby the receptors for the molecular docking stage.

Molecular Docking and Visualization

Docking results provide various types of scoring or assessment. The scoring function is needed to distinguish valid binding conformations by separating the wrong conformations tobe sorted from the correct macromolecular-ligand conformations (Sliwoski et al., 2014). Molecular docking results between cannabis plant compounds and target proteins are indicated by binding affinity values (Table 2).

Table 2. Affinity Binding Results

No	Receptors	Ligan	*Binding Affinity (*kkal/mol)
1.	Serine/threonine-protein kinase AKT (AKT1)	Cannabicyclol Cannabidihydrophenanthrene Control	-6.2 -6.4 -5.5
2.	Serine/threonine-protein kinase mTOR (MTOR)	Cannabinerolic-Acid Control	-6.4 -5.1
3.	Epidermal growth factor receptor erbB1 (EGFR)	Cannabidihydrophenanthrene Cannabigerol Cannabinol Control	-6.5 -5.5 -7.2 -5.1
4.	Platelet-derived growth factor receptor alpha (PDGFRA)	Cannabidihydrophenanthrene Control	-9.0 -6.9
5.	Macrophage colony stimulating factor receptor (by homology) (CSF1R)	Cannabichromenic-Acid Control	-6.5 -5.5
6.	Vascular endothelial growth factor receptor 2 (KDR)	Cannabichromene Cannabicyclol Cannabidihydrophenanthrene Control	-5.5 -5.6 -5.7 -4.5
7.	Vascular endothelial growth factor receptor 1 (FLT1)	Cannabidihydrophenanthrene Control	-6.2 -4.7
8.	PI3-kinase p85-alpha (PIK3R1)	Canabispiran Control	-5.8 -5.3
9.	PI3-kinase p110-alpha (PIK3CA)	Canabispiran Control	-5.7 -5.0

In the AKT1 receptor binding affinity of cannabidihydrophenanthrene (-6.4 kcal/mol) and cannabicyclol (-6.2 kcal/mol) was higher than the control. The cannabinerolic-acid compound has a binding affinity of -6.4 kcal/mol which indicates a higher binding affinity value than the control at the MTOR receptor. At the EGFR receptor, the compounds cannabidihydrophenanthrene, cannabigerol, and cannabinol each had a binding affinity of -6.5; -5.5; -7.2 kcal/mol respectively which indicated a higher binding affinity value than the control. The cannabidihydrophenanthrene compound has a binding affinity of -9.0 kcal/mol, which means it has a higher binding affinity value than the control at the PDGFRA receptor. At the CSF1R receptor, the cannabichromenic-acid compound had a binding affinity of -6.5 kcal/mol which was higher than the control. Similar to the previous receptor, in the KDR receptor, cannabichromene compounds (-5.5 kcal/mol),

cannabicyclol (-5.6 kcal/mol), and cannabidihydrophenanthrene (-5.7 kcal/mol) have higher binding affinity than with control. Furthermore, at the FLT1 receptor, the cannabidihydrophenanthrene compound (-6.2 kcal/mol) has a higher binding affinity than the control. Followed by PIK3R1 receptors with canabispiran compounds (-5.8 kcal/mol) and PIK3CA with canabispiran compounds (-5.7 kcal/mol) which showed higher binding affinity than the controls. The results of molecular docking in this study showed that the compounds screened in cannabis plants had a higher binding affinity than thedrug compounds used as controls.

The control used in this study was aspirin. Aspirin is a non-steroidal anti-inflammatorydrug (NSAID) that is widely used in patients with coronary artery disease and transient ischemic attack (TIA) (Millard and Hernandez, 2018). (Saputri et al., 2016) stated that the smaller the value of the binding affinity, the higher the binding affinity between the receptor and the ligand, and conversely, the greater the value, the lower the binding affinity, the bond affinity between the receptor and the ligand. The size of the binding affinity value is influenced by various kinds of interactions that are formed, for example hydrogen bonds, van der waals interactions, and hydrophobic bonds at different amino acid residues (Table 3).

Table 3. Ligand-Receptor Amino Acid Residues

No	Ligand -macromolecule	Van der Waals interactions	Hydrophobic Bonds			Hydrogen Bonds	Distance (Å)
			Pi Bonds	Alkyl Bonds	Carbon Hidrogen Bonds	Residue	
1.	AKT1-Cannabicyclol (-6.2 kcal/mol)	Pro A:57 Ala A:56 Tyr A:43 Glu A:45 Gln A:53 Pro A:47	-	Lys A:44	-	Leu A:58 (N-HN)	1.02019
2.	AKT1-Cannabidihydrophenanth Rene (-6.4 kcal/mol)	Arg A:30 Leu A:16 Val A:100	Glu A:101 Trp A: 15	Pro A:29	His A:17	His A:99 (N-HN)	1.01981
3.	AKT1-Control (-5.5 kcal/mol)	Pro A:47 Gln A:53 Tyr A:43 Pro A:57 Lys A:44	-	-	-	Ala A:56 (N-HN) Glu A:45 (N-HN) Leu A:58 (N-HN)	1.01957 1.01982 1.02019
4.	MTOR-Cannabinerolic-Acid (-6.4 kcal/mol)	Glu E:1045 Glu E:1023 Val E:1013 His E: 1015 Leu E: 1012 Unk E:1162	-	Lys E: 1048 Leu E:1053 Leu E:1066 Leu A:1056	-	Leu E:1049 (N-HN) Gly E:1010 (N-HN) His E:1014 (N-HN)	1.02034 1.01972 1.01974
		Ser E:1052 Gln E:1011 His E:1007		Leu E:1059			
5.	MTOR-Control (-5.1 kcal/mol)	Ile C:278 Ser C:272 Gln C:21 Phe C:313	Tyr C:277	Leu C:8 Ala C: 22 Val C:279 Leu C:289 Val	-	-	-

No	Ligand -macromolecule	Van der Waals interactions	Hydrophobic Bonds			Hydrogen Bonds	Distance (Å)
			Pi Bonds	Alkyl Bonds	Carbon Hidrogen Bonds	Residue	
				C:6			
6.	EGFR-Cannabidihydrophenanthrene (-6.5 kcal/mol)	Val C:213 Gln C:276 Tyr C:220 Gln C:218	Val C:275	Leu C:215 Phe C:214	-	-	-
7.	EGFR-Cannabigerol (-5.5 kcal/mol)	Gln C:218 Val C:213 Leu C:277 Val C:275 Pro C:289 Arg C:290 Pro C:274	Tyr C:220	Leu C:215 Phe C:214	-	Gln C:276 (N-HN)	1.02034
8.	EGFR-Cannabinol (-7.2 kcal/mol)	Val C:213 Phe C:214 Gln C:218 Gln C:276	Tyr C:220	Arg C:290 Trp C:291 Val C:292 Val C:275 Leu C:215	-	Pro C:289 (N-HN)	1.02004
9.	EGFR - Control (-5.1 kcal/mol)	Asn C:308 Ser C:305 Ala C:310 His C:304 Cys C:303 Asp C:331	-	-	-	Thr C:309 (N-HN) Leu C:311 (N-HN) Arg C:302 (N-HN) Asn C:329 (N-HN)	1.02002 1.02004 1.02045 1.02045
10.	PDGFRA-Cannabidihydrophenanthrene (-9.0 kcal/mol)	Met A:66 Glu A:93 Gly A:98 Cys A:163	Leu A:153 Val A:25	Val A:76 Lys A:45 Phe A:165 Ala A:43 Leu A:17 Tyr A:94	Thr A:92	Cys A:95 (N-HN)	1.02039
11.	PDGFRA-Control (-6.9 kcal/mol)	Leu A:17 Leu A:153 Tyr A:94 Ala A:43 Val A:76 Val A:44 Ile A:90 Met A:66	Val A:25 Phe A:165	Cys A:163 Lys A:45		Thr A:92 (N-HN) Glu A:93 (N-HN)	1.02028 1.01982
12.	CSF1R-Cannabichromenic-Acid (-6.5 kcal/mol)	Gln B:354 Met B:334 Ile B:350 Arg B:339 Asn B:343 Asp B:342	-	Val A:351 Ile B:348 Ala B:347 Leu B:358	-	Phe B:340 (N-HN) Met B:338 (N-HN) Glu B:335 (N-HN)	1.01993 1.01976 1.02007
13.	CSF1R -Control (-5.5 kcal/mol)	Phe B:327 Phe B:364 Phe B:404 Asn	-	Lys B:324 Leu B:328	-	Thr B:365 (N-HN) Lys B:406 (N-HN)	1.02044 1.0197

No	Ligand -macromolecule	Van der Waals interactions	Hydrophobic Bonds			Hydrogen Bonds	Distance (Å)
			Pi Bonds	Alkyl Bonds	Carbon Hidrogen Bonds	Residue	
		B:407					8
		Asp B:367 Cys B:408 Cys B:321 Lys B:325 Phe B:412					
14.	KDR- Cannabichromene (-5.5 kcal/mol)	Ser A:38 Phe A:35 Asp A:22 Pro A:28	Phe A:24	Lys A:36 Ile A:34 Ile A:31	-	-	-
15.	KDR- Cannabicyclol (-5.6 kcal/mol)	Asn A:50Gln A:10	TyrA:9	Tyr A:13 MetA:6 PheA:5	-	-	-
16.	KDR- Cannabidihydrophenanthrene (-5.7 kcal/mol)	Asn A:50Tyr A:13Gln A:10	TyrA:9	MetA:6 PheA:5	-	-	-
17.	KDR-Control (-4.5kcal/mol)	Gln A:10Tyr A:13Asn A:50 Phe A:5	TyrA:9	MetA:6	-	-	-
18.	FLT1- Cannabidihydrophenanthrene (-6.2 kcal/mol)	Thr X:266 Leu X:265 Lys X:231Pro X:233	-	Leu X:264	-	Lys X:230 (N-HN) Phe X: 232(N-HN)	1.02029 1.01972
19.	FLT1- Control (-4.7kcal/mol)	Glu V:52Asn V:50Leu V:54 Gly V:47Glu V:55	-	CysV:48	Lys V:95	Asp V:51(N-HN) Cys V:49(N-HN) Cys V:56(N-HN)	1.02019 1.02006 1.02004
20.	PIK3R1- Canabispiran (-5.8 kcal/mol)	Ile B:103Thr B:55	PheB:52	AlaB:51 AlaB:48	-	Arg B:99(N-HN)	1.01961
21.	PIK3R1-Control (-5.3kcal/mol)	Leu B:96Cys B:63Gln B:66Thr B:65Tyr B:69Ile B:89Asn B:92 Tyr B:93	-	-	-	Gln B:62(N-HN)	1.01965
22.	PIK3CA-Canabispiran (-5.7 kcal/mol)	Ser A:6 Thr A:112AspA:111 Phe A:107Arg A:75 Pro A:71 Ile A:70	Leu A:113	-	-	-	-
23.	PIK3CA-Control (-5.0 kcal/mol)	Val A:86 His A:96Cys A:97 Leu A:99	AspA:27	Ile A:28 Lys A:87 Ile A:28	-	Glu A:95 (N-HN)Ser A:85 (N-HN)	1.01998 1.01939

The results of molecular docking were visualized with the Biovia Discovery Studio software so that amino acid residues and chemical bonds formed indicated the binding site of a protein targeted by the ligand. The similarity of the interaction of amino acid residues in the docking results between the ligand and the control indicates that the ligand has the potential to be a substitute for control (Chamata et al., 2020).

The results of visualization of the AKT1 receptor protein with the compound cannabicyclol showed van der Waals interactions, hydrophobic bonds, and hydrogen bonds at the amino acid residue Leu58 with a distance of < 2Å which indicated that the bonds formed were getting stronger. The results of visualization of the AKT1 receptor protein with the cannabidihydrophenanthrene compound showed van der Waals interactions, hydrophobic bonds, and hydrogen bonds on the His99 amino acid residue with a distance of <2Å, i.e. 1.01981 Å. The results in Table 3 show that only the cannabicyclol

compound showed the same interaction with the control after docking with AKT1, namely the amino acid residues Pro47, Gln53, Tyr43, Pro57, Lys44, Ala56, Glu45, and Leu A:58. The cannabicyclol compound can inhibit AKT so that it acts as an anti-inflammatory. AKT1-specific inhibition can provide a therapeutic approach to reduce vascular permeability and excessive leukocyte migration during acute inflammatory reactions (Di Lorenzo et al., 2009).

Visualization of MTOR with cannabinerolic-acid compounds showed van der Waals interactions, hydrophobic bonds, and hydrogen bonds in the amino acid residues Leu1049 (1.02034 Å), Gly1010 (1.01972 Å), and His1014 (1.01974 Å). In the visualization results, thereis no similarity between the amino acid residues and the control at the binding site which is the area of protein binding to the ligand that will affect the conformation and function of the protein (Arwansyah et al., 2014). This is because a ligand will seek the most stable conformation on the active site of the target protein.

EGFR visualization results with cannabidihydrophenanthrene compounds showed onlyvan der Waals interactions and hydrophobic bonds. This was followed by interactions with the cannabigerol compound which showed van der Waals interactions, hydrophobic bonds, and hydrogen bonds at the amino acid residue Gln276 at a distance of 1.02034 Å. Similar to the previous compound, the cannabinol compound also has three interactions at once with hydrogen bonds at the Pro289 amino acid residue 1.02004 Å. However, none of the three compounds showed the same amino acid residues as the control.

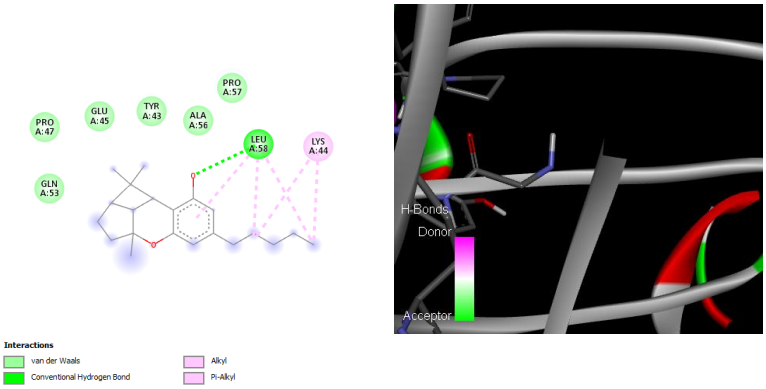
The visualization results of PDGFRA with the cannabidihydrophenanthrene compound show three interactions at once namely van der Waals interactions, hydrophobic bonds, and hydrogen bonds at the Cys95 amino acid residue 1.02039Å apart. The results in table 3 show that the binding affinity of this compound with PDGFRA is the highest binding affinity value compared to all docking results. In addition, this compound showed the same interaction withthe control after docking with PDGFRA, namely the amino acid residues Met66, Glu93, Cys163, Leu153, Val25, Val76, Lys45, Phe165, Ala43, Leu 17, and Thr92. According to Golen(2017) states that inhibition of PDGFRA is used for targeting anti-inflammatory therapy in inflammatory breast cancer. CSF1R visualization results with cannabichromenic-acid compounds showing van der waals, hydrophobic bonds, and hydrogen bonds in the amino acid residues Phe340 (1.01993 Å), Met338 (1.01976 Å), and Glu335 (1.02007 Å). In the visualization results of this compound there is no similarity of amino acid residues with the control.

The visualization results of KDR with cannabichromene, cannabicyclol, and cannabidihydrophenanthrene compounds showed only two interactions, namely van der Waals interactions and hydrophobic bonds. The results in table 3 only contain cannabicyclol and cannabidihydrophenanthrene which showed the same interaction with the control after belaying with KDR. In the cannabicyclol compound, the similarities are in the amino acid residues Asn50, Gln10, Tyr9, Tyr13, Met6, and Phe5. As for the cannabidihydrophenanthrene compound, the similarities are found in the amino acid residues Asn50, Tyr13, Gln10, Tyr9, Met6, and Phe5. KDR is a receptor for vascular endothelial growth factor (VEGF). The bindingformed between KDR and VEGF can lead to increased angiogenesis, and thus, recruitment ofperipheral leukocytes to the inflamed synovium, which reduces the growing synoviocyte load by supplying oxygen and nutrients needed for tissue metabolism. This will regulate the development of the inflammatory process (Yoo et al., 2009). In the research results of Ugur etal (2018) that VEGF levels were found to be high in smokers, this is used to increase awarenessin the community about the dangers of the inflammatory effects of smoking in the human body.

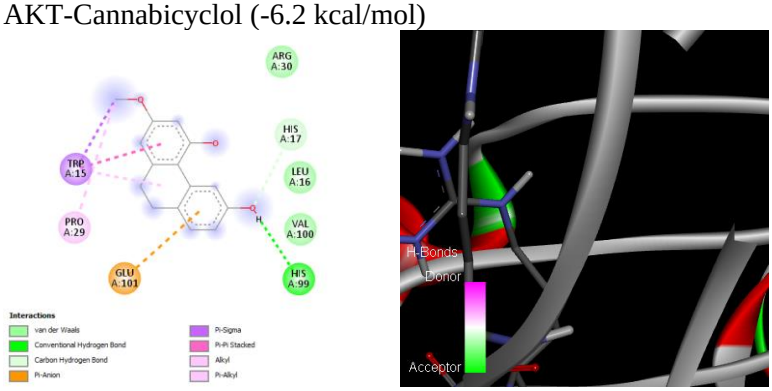
The visualization results of FLT1 with cannabidihydrophenanthrene compounds which show three interactions at once, namely van der waals, hydrophobic bonds, and hydrogen bondsin the amino acid residues Lys230 (1.02029Å) and Phe232 (1.01972 Å). In the visualization results of this compound there is no similarity of amino acid residues with the control. The visualization results of PIK3R1 with the canabispiran compound show van der Waals interactions, hydrophobic bonds, and hydrogen bonds at the Arg99 amino acid residue which is 1.01961 Å apart. However, the visualization results of PIK3CA with canabispiran compounds only show van der Waals interactions and hydrophobic bonds. In the results of thetwo visualizations of this compound with the PIK3R1 and PIK3CA receptors, there were no amino acid residues in common with the control.

Visualization results show that compounds from *Cannabis sativa* L. can act as anti-inflammatories in the context of cigarette smoke-induced inflammation. This is indicated by the similarity of amino acid residues resulting from

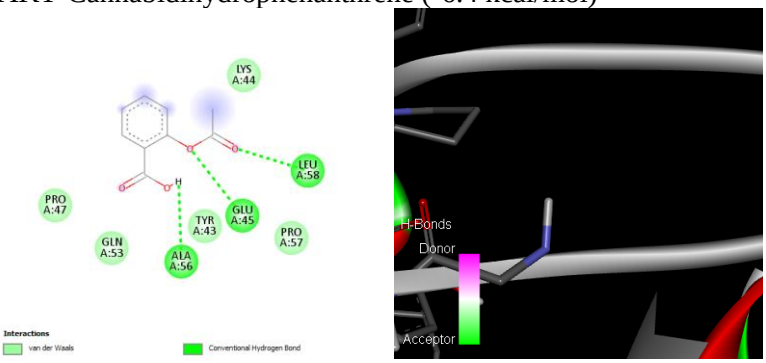
the interaction of aspirin (control) with anti-inflammatory receptor proteins, namely the cannabidihydrophenanthrene compound with PDGFRA and KDR receptors and the cannabicyclol compound with AKT1 and KDR receptors. The results of visualization in three dimensions (3D) and two dimensions (2D) can be seen in (Figure 3). This is in line with previous studies which have proven that the bioactive compounds in *Cannabis sativa* L. have anti-inflammatory effects (Kopustinskiene et al., 2022). Based on the in silico study, it can be explained that the cannabidihydrophenanthrene and cannabicyclol are bioactive compounds contained in *Cannabis sativa* L. as anti-inflammatory candidates caused by cigarette smoke by targeting the JAK/STAT and MAPK signaling pathways. The novelty of this study is the proof that these compounds can be useful as anti-inflammatories, including inflammation due to exposure to cigarette smoke.



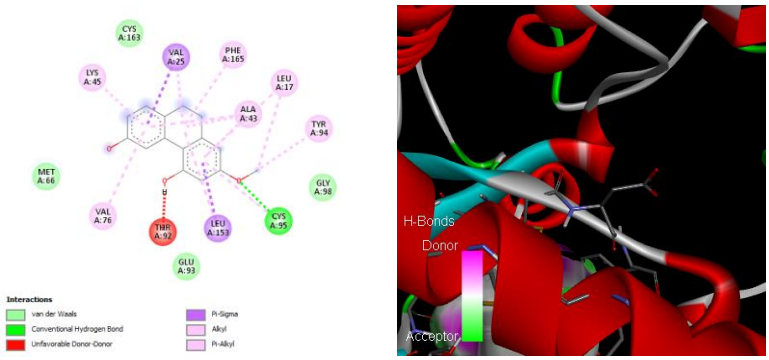
AKT-Cannabicyclol (-6.2 kcal/mol)



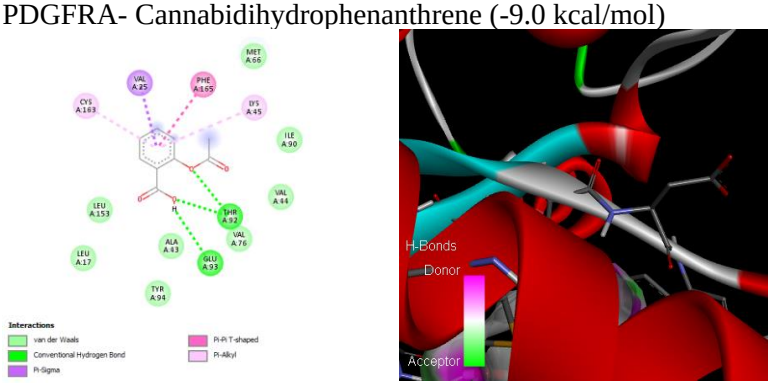
AKT-Cannabidihydrophenanthrene (-6.4 kcal/mol)



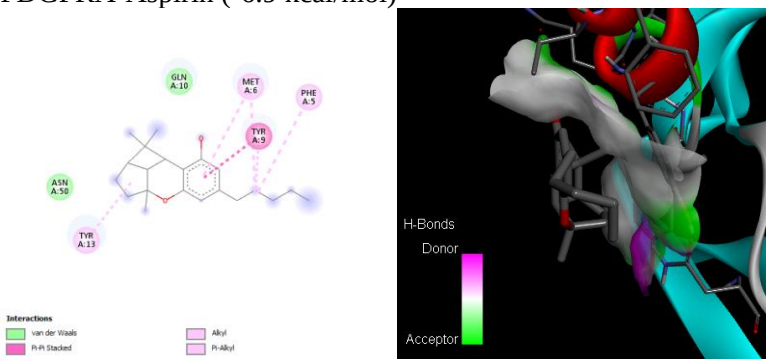
AKT-Aspirin (-5.5 kcal/**mol)**



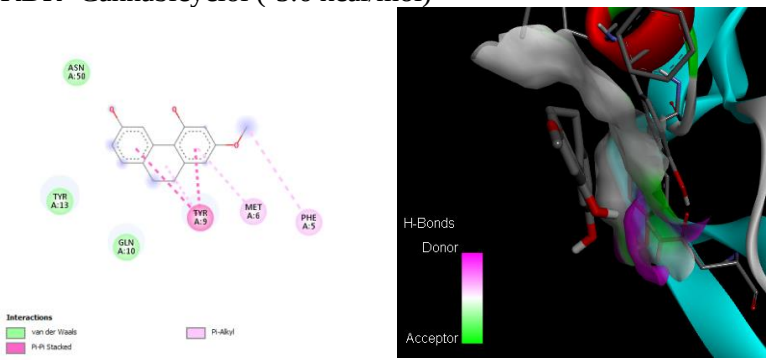
PDGFRA- Cannabidihydrophenanthrene (-9.0 kcal/mol)



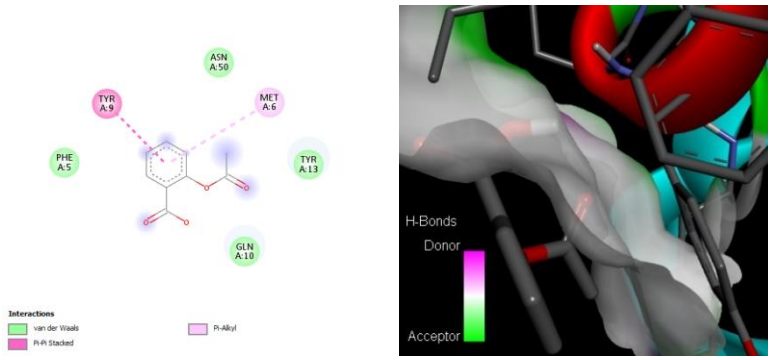
PDGFRA-Aspirin (-6.9 kcal/mol)



KDR- Cannabicyclol (-5.6 kcal/mol)



KDR- Cannabidihydrophenanthrene (-5.7 kcal/mol)



KDR-Aspirin (-4.5 kcal/mol)

Figure 3. 2D and 3D visualization of ligand compounds with proteins

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the cannabidihydrophenanthrene and cannabicyclol contained in Cannabis plant have high potential to be used as anti-inflammatory drug candidates in inflammation caused by cigarette smoke. It through the JAK/STAT and MAPK signaling pathways by binding to the receptor protein Serine/threonine- protein kinase AKT (AKT1), Platelet-derived growth factor receptor alpha (PDGFRA), and Vascular endothelial growth factor receptor 2 (KDR). Therefore, it is necessary to carry out further research in vitro to determine the anti-inflammatory effect due to exposure to cigarette smoke.

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