



In Silico Study on Anti-inflammatory Effect of Bioactive Compounds of Velvet Bean (*Mucuna pruriens* L. (DC.)) Leaves Against NF- κ B Activation Pathway

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ABSTRACT: Activation of nuclear factor- κ B (NF- κ B) receptors potently enhances pro-inflammatory cytokines production thereby promoting inflammatory reactions. NF- κ B receptor inhibition is one of the targets in overcoming inflammatory reactions. One of the natural ingredients that have the potential as an anti-inflammatory is velvet bean leaf extract (*Mucuna pruriens* L. (DC.)) which is used by the local people of West Sumatra. This study aimed to investigate the anti-inflammatory mechanism of the bioactive compounds detected in velvet bean leaf extract against NF- κ B (1U36) activation pathway using an in silico approach. The phytochemical analysis of the ethanolic extract of velvet bean leaves was performed using GC-MS. Subsequently, their potential bioactivities were explored using PASS online test, preparation of ligands, receptors, and molecular docking. The results showed that the 34 compounds contained in the miang bean leaf extract had anti-inflammatory bioactivity with a probability activity value of >0.7 based on PASS online. The docking results showed that the highest binding affinity value was found in artemin (-7.5 kcal/mol) which was higher than the standard anti-inflammatory drug ketoconazole (-7.2 kcal/mol). Therefore, one of the mechanisms of miang bean leaves as an anti-inflammatory is due to the inhibitory action of artemin on the NF- κ B activation pathway.

Keywords: artemin; in silico; nuclear factor- κ B; pro-inflammatory cytokines.

ABSTRAK: Aktivasi reseptor *nuclear factor- κ B* (NF- κ B) secara potensial meningkatkan produksi sitokin pro-inflamasi sehingga mendorong reaksi inflamasi. Inhibisi reseptor NF- κ B ini menjadi salah satu target dalam mengatasi reaksi inflamasi. Salah satu bahan alami yang berpotensi sebagai anti-inflamasi yaitu ekstrak daun kacang miang (*Mucuna pruriens* L. (DC.)) yang digunakan oleh masyarakat lokal Sumatera Barat. Penelitian ini bertujuan untuk mengetahui mekanisme antiinflamasi senyawa bioaktif pada ekstrak daun kacang miang terhadap jalur aktivasi NF- κ B (1U36) menggunakan pendekatan in silico. Analisis fitokimia ekstrak etanol daun kacang miang dilakukan dengan menggunakan GC-MS. Selanjutnya, potensi bioaktivitasnya dieksplorasi menggunakan PASS online (*Prediction of Activity Spectra for Substances*), dilanjutkan dengan uji kelayakan ligan menggunakan *Lipinski Rule of Five Test*, preparasi ligan (Biovia Discovery Studio), dan reseptor (PyMol), dan *molecular docking* menggunakan PyRx. Hasil penelitian menunjukkan bahwa 34 senyawa yang terdapat pada ekstrak daun kacang miang memiliki bioaktivitas antiinflamasi dengan nilai aktivitas probabilitas $>0,7$ berdasarkan PASS online. Hasil docking menunjukkan bahwa nilai *binding affinity* tertinggi ditemukan pada artemin (-7,5 kkal/mol) yang lebih tinggi dari obat anti inflamasi standar ketoconazole (-7,2 kkal/mol). Oleh karena itu, salah satu mekanisme daun kacang miang sebagai antiinflamasi yaitu akibat aksi penghambatan artemin terhadap jalur aktivasi NF- κ B.

Kata kunci: artemin; in silico; NF- κ B; sitokin proinflamasi.

Introduction

Activation of NF- κ B receptors causes the production of proinflammatory cytokines that promote the inflammatory reaction. The process of inhibiting the activation of NF- κ B becomes a target to overcome the inflammatory reaction that occurs excessively [1,2]. Inflammation is a positive response of the body to tissue damage to overcome the infection, repair tissue damage, and restore tissue homeostasis [3]. However, if the inflammatory response occurs excessively it will also cause

acute tissue damage [4]. One of the traditional medicinal ingredients that have the ability as an anti-inflammatory is the leaf of the velvet bean (*Mucuna pruriens* L.(DC.)). Velvet bean leaf extracts of 400 mg/kg BW were effective in overcoming inflammation in mice. Even though it is effective as an anti-inflammatory, the mechanism of velvet bean leaf extract as an anti-inflammatory is not known [5,6].

Therefore, through in silico

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studies, it is possible to predict the anti-inflammatory mechanism of velvet bean leaves on the NF- κ B receptor signaling pathway (downstream inflammatory pathway). These receptors were chosen because of their downstream part of the inflammatory pathway. When the NF- κ B receptor is activated, it will stimulate the nucleus to produce inflammatory cytokines [7]. In silico study is a computational simulation method using certain applications and web tools to predict the activity of compounds in new drug discovery efforts. The in silico method used is molecular docking between the receptor and the ligand [8]. In silico studies have several advantages when compared to in-vitro and in-vivo studies, they are effective in predicting compounds that are thought to be efficacious as drugs, do not require high costs, and save time. This study aimed to determine the anti-inflammatory mechanism of the bioactive compounds in the extract of velvet bean leaves against the activation pathway of NF- κ B (1U36) using an in silico approach.

Research Methods

Tools

The tool used is an Acer Aspire 4750 laptop (Intel Core i3-2310M Processor, 2.10 GHz, 2 GB SDRAM). While the software used is PyRx 0.8 (by Sourceforge), Biovia Discovery Studio 2021 (by Dassault Sytemes), PyMol 2.1 (by Schrodinger), Lipinski Rule of Five (<http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>), and PASS Online (<http://way2drug.com/passonline/>).

Materials

The materials used were 55 compounds contained in the ethanol extract of velvet beans based on the GC-MS analysis of the results of previous studies [8] and the NF- κ B receptor (1U36) downloaded via (<https://www.rcsb.org/>).

Ligand preparation

The tested ligands (chemical compounds of velvet bean leaf extract) were downloaded from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>) in 3D (SDF file format). Then open the BIOVIA Discovery Studio application > file > open (open the downloaded ligand) > file > save as (pdb file format).

Prediction of Biological Bioactivity of Chemical Compounds

Chemical compounds contained in the extract of the velvet beans were predicted for their bioactivity using PASS

Online (Prediction of Activity Spectra of Substances) in SMILE (Molecular Input Line Entry Systems) format [9].

Ligand Feasibility Test

Compounds that have anti-inflammatory properties are based on the PASS online test followed by the Lipinski Rule of Five Test to determine the feasibility of a ligand for docking. If the ligand fulfills 5 conditions, the Lipinski Rule of Five indicates that the ligand is suitable for docking. The conditions that must be met are the molecular weight of the compound < 500 Da; Log P value < 5; hydrogen bond acceptor < 10; and the number of hydrogen bond donors < 5. Ligands that meet these criteria are selected 10 ligands with the highest concentration (GC-MS) for docking [10].

Receptor Preparation

The receptor used was NF-B (1U36 Mus musculus) obtained from the Protein Data Bank website (<https://www.rcsb.org/>).

Ligand and Receptor Docking

The docking process uses the PyRx application so that the binding affinity and RMSD (Root Mean Square Deviation) values are obtained. The smaller the binding affinity value, the stronger the interaction formed between the ligand and the receptor. While the RMSD value describes the level of accuracy of the docking process carried out. The RMSD value < 2Å° indicates that the docking process is accurate [11].

Results and Discussion

Based on the online PASS test, the results showed that of the 55 chemical compounds contained in the extract of the velvet bean leaf based on the GC-MS analysis, 34 chemical compounds had anti-inflammatory properties with various bioactivities. Based on the results of the PASS online test which is presented in Table 1, there are several bioactivities of chemical compounds contained in the extract of the velvet bean leaf based on the PASS test, namely anti-eczematic, antiseborrheic, treatment of mucositis (inflammation of the mucous membranes), prostaglandin inhibitors, leukotriene inhibitors, JAK expression inhibitors (Janus kinases), membrane permeability inhibitors, and complement factor inhibitors.

Eczematic and seborrheic are types of skin diseases characterized by red, blistered, scaly, and thickened skin conditions [12]. Another compound bioactivity of velvet bean leaves is prostaglandin inhibitors. Prostaglandins are

Table 1. The results of the test of the biological activity of chemical compounds in the extract of the velvet bean leaf with PASS online

No	Compounds	Pubchem ID	Bioactivity	Pa Value (Probability Activity)
1	Hexadecanoic acid	985	Prostaglandin-A1 DELTA-isomerase inhibitor	0,921
2	Geranylgeraniol	5281365	TNF expression inhibitor	0,840
3	Geraniol	637566	TNF expression inhibitor	0,840
4	3-Aminobenzamide	1645	Membrane permeability inhibitor	0,742
5	Octadecanoic acid	5281	Antiinflammatory	0,727
6	4-Hydroxycinnamic acid	637542	Antiseborrheic	0,869
7	Benzyl Acetate	8785	Antiseborrheic	0,836
8	Carvacrol	10364	Membrane permeability inhibitor	0,832
9	Androstanolone	10635	Antiseborrheic	0,953
10	Pentalene	5460726	Complement factor D inhibitor Leukotriene-B4 20-monooxygenase inhibitor	0,919 0,710
11	Bicylo heptane	562714	Antiseborrheic	0,867
12	2-Acetyl-3-methylpyrazine	32093	Antiseborrheic	0,743
13	Cyclopropanecarboxylic acid	15655	Antieczematic	0,855
14	3-tert-Butyl-4-hydroxyanisole	8456	JAK2 expression inhibitor	0,854
15	Benzofuranone	68382	Complement factor D inhibitor	0,851
16	4-tert-Octylphenol	8814	Antiseborrheic	0,915
17	Isobornyl acrylate	639970	Antieczematic Membrane permeability inhibitor	0,820 0,794
18	(2Z)-3,7-Dimethylocta-2,6-dien-1-yl 3-oxobutanoate	54611954	TNF expression inhibitor	0,806
19	Morpholine	8083	Complement factor D inhibitor	0,745
20	Zedoarondiol	24834047	Antiseborrheic	0,812
21	Artemin	3678270	Antieczematic	0,856
22	(E)-2-Methyl-2-buten-1-ol O-beta-D- Glucopyranoside	10753054	Membrane permeability inhibitor	0,868
23	Prenyl glucoside	14239303	Membrane permeability inhibitor	0,890
24	Limonene	22311	Antieczematic	0,896
25	Beta-pinene	14896	Antieczematic	0,902
26	Cinene	22311	Antieczematic	0,896
27	Alpha-pinene	6654	Prostaglandin E1 antagonist	0,764
28	Cyclopropane	6351	JAK2 expression inhibitor	0,804
29	Isopropyl Myristate	8042	Mucositis treatment	0,751
30	1-Phenyl-1-nonyne	143501	Antieczematic	0,891
31	Sterculic Acid	12921	Prostaglandin-A1 DELTA-isomerase inhibitor	0,888
32	32-Hydroxy-24,25-dihydrolanosterol	15698821	Prostaglandin-E2 9-reductase inhibitor	0,913
33	2(1H)-Naphthalenone	11400841	Antiseborrheic	0,864
34	Heptadecanoic acid	10465	Leukotriene-B4 20-monooxygenase inhibitor	0,860

Table 2. Test results of chemical compound ligand justification on Velvet Bean Leaf Extract with Lipinski rule of five test

No	Compunds	Mass	Donor	Acceptor	LogP	Molar	Justification
1	Hexadecanoic acid	256	1	2	5,55	77,95	-
2	Geranylgeraniol	290	1	1	6,12	95,49	-
3	Geraniol	154	1	1	2,67	49,51	√
4	3-Aminobenzamide	136	4	3	0,37	39,37	-
5	Octadecanoic acid	284	1	2	4,94	99,19	√
6	4-Hydroxycinnamic acid	164	2	3	1,49	44,77	√
7	Benzyl Acetate	150	0	2	1,75	41,91	√
8	Carvacrol	150	1	1	2,82	46,93	√
9	Androstanolone	290	1	2	3,96	82,74	√
10	Pentalene	102	0	0	2,28	35,13	-
11	Bicyclo heptane	188	0	0	3,92	57,92	√
12	2-Acetyl-3-methylpyrazine	136	0	3	0,99	36,77	-
13	Cyclopropanecarboxylic acid	86	1	2	0,48	20,36	-
14	3-tert-Butyl-4-hydroxyanisole	180	1	2	2,69	53,36	√
15	Benzofuranone	134	0	2	1,15	35,78	-
16	4-tert-Octylphenol	206	1	1	4,11	65,2	√
17	Isobornyl acrylate	208	0	2	2,93	59,31	√
18	(2Z)-3,7-Dimethylocta-2,6-dien-1-yl 3-oxobutanoate	238	0	3	3,2	68,69	√
19	Morpholine	87	1	2	-0,39	23,69	-
20	Zedoarondiol	252	2	3	2,21	70,08	√
21	Artemin	266	1	4	3,07	75,88	√
22	(E)-2-Methyl-2-buten-1-ol O-beta-D-Glucopyranoside	248	4	6	-1,23	59,15	-
23	Prenyl glucoside	248	4	6	-1,23	59,15	-
24	Limonene	136	0	0	3,31	45,91	√
25	Beta-pinene	136	0	0	2,99	43,75	√
26	Cinane	136	0	0	3,31	45,91	√
27	Alpha-pinene	136	0	0	2,99	43,75	√
28	Cyclopropane	42	0	0	1,17	13,85	-
29	Isopropyl Myristate	270	0	2	5,64	82,31	-
30	1-Phenyl-1-nonyne	200	0	0	4,39	66,69	√
31	Sterculic Acid	294	1	2	6,25	89,59	-
32	32-Hydroxy-24,25-dihydrolanosterol	444	2	2	7,53	134,32	-
33	2(1H)-Naphthalenone	144	0	1	1,83	44,19	√
34	Heptadecanoic acid	270	1	2	5,94	82,56	-

Table 3. Results of docking of bioactive compounds in Velvet Bean Leaf Extract as NF-B antagonist

No	Compound	Binding Affinity Value (kcal/mol)	RMSD (Å)
1	Geraniol (C10H18O)	-4,4	0
2	Octadecanoic acid (C18H36O2)	-3,7	0
3	Benzyl Acetate (C9H19O2)	-4,5	0
4	Carvacrol (C10H14O)	-4,5	0
5	4-tert-Octylphenol (C14H22O)	-4,4	0
6	Zedoarondiol (C15H24O3)	-6,2	0
7	Artemin (C15H22O4)	-7,5	0
8	Alpha-pinene (C10H16)	-4	0
9	1-Phenyl-1-nonyne (C15H20)	-4,4	0
10	3-tert-Butyl-4-hydroxyanisole (C11H16O2)	-4,3	0
11	Ketoconazole (control) (C26H28Cl2N4O4)	-7,2	0

inflammatory mediators that cause pain and vasodilation and increase vascular permeability. When prostaglandin formation is inhibited, inflammatory symptoms such as pain, increased capillary permeability, and vasodilation can be overcome [13]. In addition, leukotrienes are also one of the mediators of inflammation. Whereas JAK is related to cellular signaling that regulates cytokine release [14].

In the online PASS test, the probability activity (Pa) value is obtained which describes the level of activity of a chemical compound to become active. A total of 34 chemical compounds that have anti-inflammatory properties in this velvet bean leaf extract have a value of Pa > 0.7. This indicates that these 34 compounds have high biological activity and may be analogs of commercial drugs.

Compounds that have anti-inflammatory properties were further tested using the Lipinski Rule of Five Test to determine the feasibility of a ligand. Table 2 shows that of the 34 compounds that have anti-inflammatory properties (based on the PASS test), 19 compounds qualify as ligands for the docking process. Of the 19 compounds, 10 compounds with the highest percentage were selected (based on the results of the GCMS test) for docking, namely geraniol, octadecanoic acid, benzyl acetate, carvacrol, 4-tert-octylphenol, zedoarondiol, artemin, alpha-pinene, 1-phenyl-1-nonyne, and 3-tert-butyl-4-hydroxyanisole.

Based on Table 3, the lowest binding affinity value was the result of docking ligands with NF-B protein, namely the artemin compound with a binding affinity value of -7.5 kcal/mol. The binding affinity value of the artemin

compound was even lower than the control ketoconazole (a commercial anti-inflammatory drug) which was -7.2 kcal/mol. This indicates that the ability of artemin compounds to inhibit the inflammatory pathway by blocking NF-B protein signaling is better than ketoconazole controls based on the value of binding affinity. The RMSD value of all docking results is 0 Å which indicates that all docking results are accurate because the RMSD value is < 2 Å.

The binding affinity value is a value that describes the strength of the bond between the ligand (chemical compound) and the receptor (protein). The value of binding affinity uses the principle of the third law of thermodynamics. If the value of binding affinity < 0 indicates that the reaction is spontaneous (producing products). If the value of binding affinity = 0 indicates that the reaction can be reversed, whereas if > 0 indicates that there is no reaction. The smaller the binding affinity value, the stronger and more stable the bond between the ligand and the receptor [15]. The binding affinity value describes the amount of energy released by the ligand compound to interact and form a bond with the receptor. Therefore, the lower (minus) the value of binding affinity, the more energy is used by the ligand to form a bond with the receptor so that the bond will be stronger [16].

Based on the results of molecular docking in Figure 1, the interaction of artemin compounds with NF-κB protein. form van der Waals, hydrogen, pi-sigma, and alkyl bonds. A van der Waals bond in the artemin compound is formed at the amino acid residue CYS270, a hydrogen bond is formed at the amino acid residue ASP271, a pi-

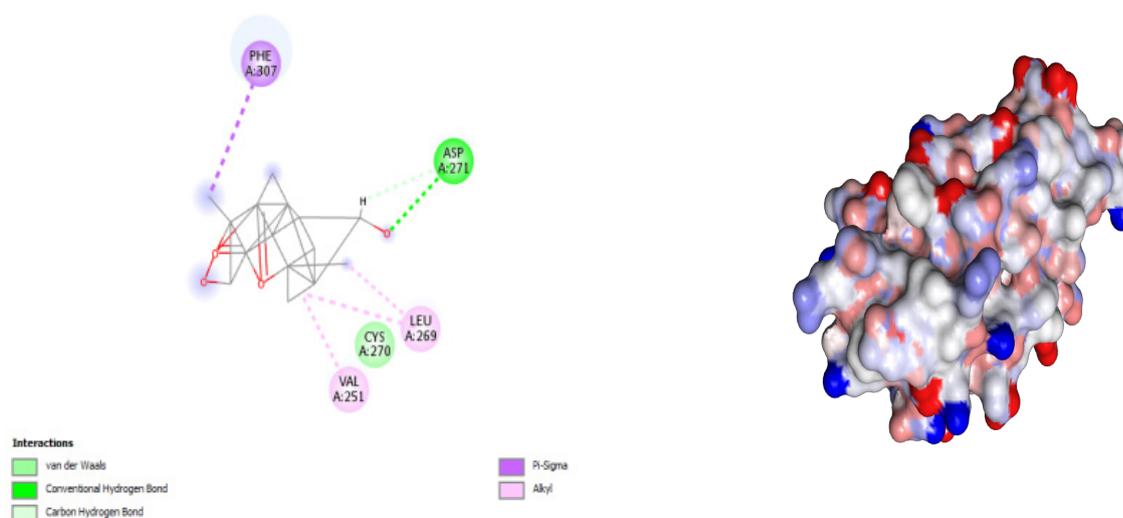


Figure 1. Results of docking artemin compounds with Artemin with NF-B receptors

sigma bond is formed at the amino acid residue PHE307, and an alkyl bond is formed at the amino acid residues VAL251 and LEU269.

The interaction between chemical compounds and proteins can form several types of bonds such as hydrogen bonds, van der Waal bonds, and hydrophobic bonds. A hydrogen bond is an interaction that is formed between a hydrogen atom and an atom that has a high electronegativity value. Hydrogen bonds are the strongest type of bond of all. The value of hydrogen bond donors and acceptors is related to the biological activity of drug molecules. Hydrogen bonding can affect the chemical-physical properties of compounds, such as boiling point, melting point, solubility in water, ability to form chelates, and acidity. Changes in these properties can affect the biological activity of compounds [17].

Van Der Waal bond is an attractive force between molecules or atoms that are not charged, and are located close together or $\pm 4-6$ apart. This bond occurs because of the polarization properties of molecules or atoms. The Van Der Waal bond strength is 0.5 kcal/mol [18]. The hydrophobic bond is one of the important forces in the process of combining the non-polar region of the drug molecule with the nonpolar region of the biological receptor. The non-polar region of the drug molecule that is insoluble in water and the surrounding water molecules will combine through hydrogen bonds to form a quasi-crystalline structure [19].

The ability of phytochemical compounds contained in the extract of velvet bean leaves to inhibit the activation

of NF- κ B protein in the inflammatory pathway can be clearly identified through molecular docking simulations. However, there is a limitation in this study, that is, only one type of protein was tested. In-vivo research should also be conducted to analyze the anti-inflammatory effect of velvet bean leaf extract using animal models.

Conclusion

Molecular docking simulations of 10 compounds found in velvet bean leaf extract with NF-B protein showed that artemin compounds were the most potent compounds in inhibiting NF-B activation in the inflammatory pathway which was characterized by the lowest binding affinity value of -7.5 kcal/ moles. The ability of artemin compounds in inhibiting the activation of NF-B is better than ketoconazole (a commercial drug).

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