In Silico Study of Basil Leaves Active Compound (Ocimum sanctum) Against Inflamation of acne Vulgaris

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Abstract. Acne Vulgaris is a skin disorder caused by hypersensitivity of the sebaceous glands. This condition is exacerbated by the bacterium Propionibacterium acnes and involves activation of the C-Jun N-Terminal Kinase receptor which triggers inflammation. Based on previous research, basil leaves (Ocimum sanctum) are reported to contain secondary metabolites such as alkaloids, flavonoids and essential oils which have antibacterial and anti-inflammatory properties. The aim of this research is to find compounds in basil leaves that have potential as new drug candidates in silico using molecular docking. Molecular docking stages are carried out by receptor preparation, method validation, docking of test compounds, and visualization of results. The results obtained are RMSD values, bond energy, inhibition constant (KI) and type of amino acid bond compared with the reference ligand. The research results show that the Ladene compound found in basil leaves has the potential to be used as a candidate for acne medication through the mechanism of inhibiting the c-Jun N-Terminal Kinase receptor with RMSD values, binding energy and inhibition constants of 2,042 Å, -6.74 kcal/mol, 11.39 uM. And this compound has the most amino acid similarities to the comparison ligand compared to other test compounds.

Keywords: Ocimum Sanctum, in silico, c-Jun N-Terminal Kinase, molecular docking, quercetagetin, Acne Vulgaris.

Introduction

Acne Vulgaris is a skin disorder caused by excessive sebum production (the skin's natural oil). This condition commonly occurs in areas of the body rich in oil glands, such as the face, neck, and back. Hormonal fluctuations, especially during adolescence, are often the primary trigger for acne (Lestari et al., 2023). Acne vulgaris affects approximately 9.38% of the world's population, with the highest prevalence occurring in adolescents aged 15-17 years (Heng, 2020; Bhate and Wiiliam, 2013). The prevalence of acne vulgaris in adolescents and adults varies widely across countries and ethnicities (Alanazi et al., 2018).

Acne occurs due to hypersensitivity of the sebaceous glands to normal circulating androgen levels, exacerbated by Propionibacterium acnes and sometimes causing inflammation. Causes of acne include the use of medications such as lithium, steroids, and anticonvulsants, excessive sun exposure, the use of occlusive clothing, endocrine disorders, and genetic factors (Motosko et al., 2019).

Propionibacterium acnes (P. acnes) is a Gram-positive anaerobic bacterium that naturally lives in the sebaceous glands. Sebaceous glands in acne-prone skin have a higher population of P. acnes, although this bacterial count does not always correlate with acne severity. P. acnesplays a role in the pathogenesis of acne by breaking down triglycerides in sebum into free fatty acids, which trigger inflammation and bacterial colonization. An immune response to P. acnes cell wall components can exacerbate inflammation through complement system activation (Ramdani et al., 2015).

Acne treatment involves correcting follicular abnormalities, reducing excess sebum production, reducing skin inflammation, and reducing the number of P. acne colonies (Afifi, 2018). The P. acne population

can be reduced with antibacterials such as clindamycin, erythromycin, and benzoyl peroxide. One way to reduce antibiotic resistance is the use of herbal plants containing antibacterial and anti-inflammatory compounds, such as basil leaves (Ocimum sanctum).

Basil leaves contain secondary metabolites such as alkaloids, flavonoids, and essential oils, which are reported to have antibacterial and anti-inflammatory properties (Pakadang et al., 2022). These compounds were analyzed in silico for their antibacterial and anti-inflammatory properties. The insilico study was conducted between the active ligand and the C-Jun N-terminal Kinase (JNK) receptor with PDB code 3V3V. Before conducting in silico studies, basil leaves were first tested for their physicochemical and pharmacokinetic profiles.

Methods

Equipment and Materials

The primary materials in this study are the 3D structures of thirty bioactive compounds from the basil leaves stored in PDB format, and the receptor structure (target protein) C-Jun N-terminal Kinase (JNK) with the PDB code 3V3V, stored in PDB format on their respective webserver databases. The hardware used in this study includes a computer and the software/web server used includes Pubchem, The Research Collaboratory for Structural Bioinformatics Protein Data Bank, Protox, pkCSM, Swiss Target Prediction, Lipinski rule of five, AutoDockTools, and Discovery Studio Visualizer 2021.

Ligand and Macromolecule Structure Preparation

The ligand structure was obtained from the PubChem web server, then its toxicity was tested using the Protox application. In addition to toxicity, the ligand structure was tested for its pharmacokinetic profile using pkCSM and the Lipinski rule of five application. The ligand structure was added with hydrogen ions using Discovery Studio 2021 and saved in PDB format, then optimized using the Autodocktools application, and continued with setting the number of torsional bonds then saved in PDBQT format.

The C-Jun N-teriminal Kinase macromolecular structure was downloaded from the Protein Data Bank (PDB) at https://www.rcsb.org using the PDB code 3V3V. The macromolecular structure was separated from the solvent and native ligand using UCSF Chimera, and the macromolecular (receptor) file was then saved in PDB format. The macromolecular structure was then optimized using Autodock Tools by adding hydrogen ions and Kollman charges, and saved in PDBQT format.

Validation of Molecular Docking Parameters

Validation of the molecular docking method was carried out using Autodock Tools, through the stages re-docking method of the native ligand to the macromolecule (receptor). The parameter used was Root Mean Square Deviation (RMSD). The outcome of this process included the grid box parameters and RMSD values. The docking method is considered valid if it has an RMSD value < 2 Å, indicating that the protocol is accepted and docking can be performed (Nursamsiar et al., 2020).

Molecular Docking

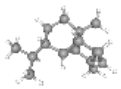
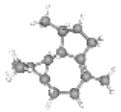
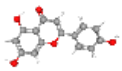
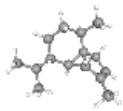
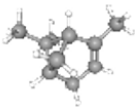
The molecular docking process was performed using PyRx software based on Auto Dock Tools. The optimized structures of the macromolecule and ligands were saved in a single folder. The docking process used the grid box and energy minimization parameters as per the validation results. Grid box parameters were set using the grid box coordinates determined based on the ligand coordinates from the receptor used in the docking validation process. Docking was then performed using PyRx software with the Auto Dock wizard feature. Docking data displayed included binding affinity values and amino acid residue interactions.

Result and Discussion

Toxicity analysis and pharmacokinetic profiles were carried out on 31 test ligands, only 5 test ligands passed the test, namely: α-copaene, ledene, apigenin, α-cubebene, and α-pinene. All test ligands that passed

the toxicity test showed a safe toxicity range, between 4 - 6. In addition, the five test ligands did not have carcinogenic, mutagenic or immunotoxic properties, as shown in the Table 1.

Tabel 1: Protox ligand test results

No	Ligand	3D structure	PredictdToxicity class	LD50 (mg/Kg)
1	*α-copaene*		5	3700
2	Ledene		5	5000
3	Apigenin		5	2500
4	α-cubebene		5	5000
5	α-pinene		5	3700

The receptor used in this study is C-Jun N-Terminal kinase which has a native ligand Quercetagetin. which is shown in the Figure 1.

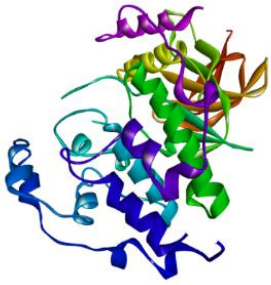


Figure 1. C-Jun N-Terminal Kinase (receptor)

In the preparation of the C-Jun N-Terminal kinase receptor structure, water molecules were first removed. This aims to maximize the interaction between the ligand and the receptor and improve the prediction of docking accuracy, because water molecules can occupy the same space as the ligand, thus preventing the ligand from binding to the receptor optimally (Fransiska et al., 2022). In addition to removing water molecules, polar hydrogens were added to the receptor structure to improve its structure and increase its stability. Meanwhile, for the preparation of the native ligand Quercetagetin, a Gastaiger charge was added to improve the accuracy of docking predictions and help identify ligands with high affinity and selectivity for the receptor (Akkoc et al., 2021).

Docking validation against the C-Jun N-terminal Kinase receptor was performed by re-docking with the native ligand quercetagetin. Validation results of the molecular docking method parameters by re-docking the native ligand (quercetagetin) into the active site of the receptor. The molecular docking method parameters were selected with 100 runs, with a grid box (40 x 40 x 40) of points and grid box coordinates (x, y, z) of 30.614 Å, 44.085 Å, and 4.212 Å, which produces an RMSD value of 0.787 Å as shown in the Figure 2.

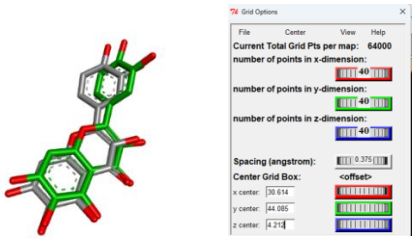


Figure 2. Molecular docking method validation results (Grey: Ligand, Green: Copy Ligand)

The docking validation results between Quercetagetin (native ligands) and the receptor formed four hydrogen bonds involving amino acid residues (MET A:111, GLU A:109, GLU A:73, LYS A:55), one carbon-hydrogen bond involving amino acid residues (ALA A:133). There are also hydrophobic Pi-Alkyl interactions involving amino acid residues (VAL A:158, VAL A:40, ILE A:32, ALA A:53, LEU) Pi-Sigma (A:168) Pi-Sulfur (MET A:108), and there is one unfavorable bond involving the ASN residue (A:144). Hydrogen bonds are interactions that stabilize the bond between the ligand and the receptor, while hydrophobic bonds are bonds that function to increase conformational stability (Nursamsiar et al, 2020), which is shown in the Figure 3.

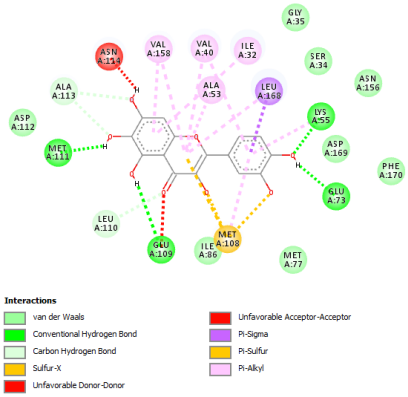


Figure 3. 2D Visualization of Quercetagetin Interactions with Amino Acids

The molecular docking simulation was performed 100 times for each compound, resulting in 100 ligand poses with varying free energies to achieve the best interaction. The results of the molecular docking simulation were then visualized using Discover Studio. The parameters analyzed were binding free energy (ΔG), hydrogen bonds, and other interaction patterns with amino acid residues in the receptor's active site. Observations of amino acid residue interactions aimed to identify interactions between the ligand and the receptor. Hydrogen bonds are interactions that can stabilize the ligand-receptor bond. Other interactions between ligands that can increase conformational stability are electrostatic interactions and van der Waals interactions (Wang et al., 2020).

Interactions through hydrogen bonds between the test ligand and the same amino acid residue as the natural ligand indicate similarity. This type of interaction reflects similar activity. All test compounds have negative binding free energy (ΔG) values. A more negative binding free energy value indicates a higher level of stability between the ligand and the target protein (receptor), resulting in a stronger bond (Suhadi et al., 2019). The visualization results of the docking between the test ligand and the receptor are shown in Table 2. The results of the docking of the test ligand to the receptor were also compared with the results of the docking

of the reference ligand (Isotretinoin), where this reference ligand is an active compound that is currently used as an acne medication.

Tabel 2: Docking results of test ligands with receptors

No	Ligand	RMSD value (Å)	Free binding energy (Kcal/mol)	Inhibition constant	Amino acid residue bonds
1	Quercetagetin	0.787	-9.26	161.71 nM	IHD: (MET A: 111; LEU A 110; GLU A: 109; GLU A: 73; LYS A: 55; ALA A: 113) IHF (MET A: 108; LEU A: 168; ALA A: 53; ILE A: 32; VAL A: 40; VAL A: 158)
2	Isotretinoin	3.25	-7.82	1.84 uM	IHD: (ASN A: 144) IHF: (MET A: 108; LEU A: 168; ILE A: 32; VAL A: 40; LEU A: 110; ALA: 53)
3	α-copaene	1.94	-6.42	19.66 uM	IHF: (ILE A: 32; VAL A: 158; LEU A: 110; ILE A: 86; ALA A: 53; LEU A: 168; MET A: 108)
4	Ledene	2.04	-6.74	11.39 uM	IHF: (ILE A: 32; VAL A: 40; ILE A: 86; LEU A: 168; MET A: 108; LEU A: 168; ALA A: 53; VAL A: 158; LEU A: 110)
5	Apigenin	8.87	-5.88	48.79 uM	IE: (ASP A: 112; LYS A: 160) IHD: (ASN A: 51; ARG A:50; MET A: 111) IHF: (LEU A: 110)
6	α-cubebene	2.79	-6.41	20.16 uM	IHD: (HIS A: 141)
7	α-pinene	2.13	-4.85	280.39 uM	IHF: (VAL A: 40; LEU A: 168; LEU A: 110; ILE A: 32; MET A: 111; VAL A: 158; MET A: 108; ALA A: 53)

Note: IHD (Hydrogen Bond), IHF (Hydrophobic Interaction), IE (Electrostatic Interaction)

Based on the docking results, Ledene is a potential acne drug candidate, with a RMSD value approaching 2 Å and a binding energy of -6.74 kcal/mol. It produces an inhibition constant of 11.39 μM, lower than the native ligand but higher than the reference ligand. Quercetagetin, as the native ligand, serves as a reference for the test ligands to determine their suitability as new drug candidates. The similar binding type and position of quercetagetin are thought to provide anti-inflammatory effects and inhibit receptor activity (Afladanti et al., 2022). The docking results between the ledene ligand and the receptor are then visualized as shown in Figure 4.

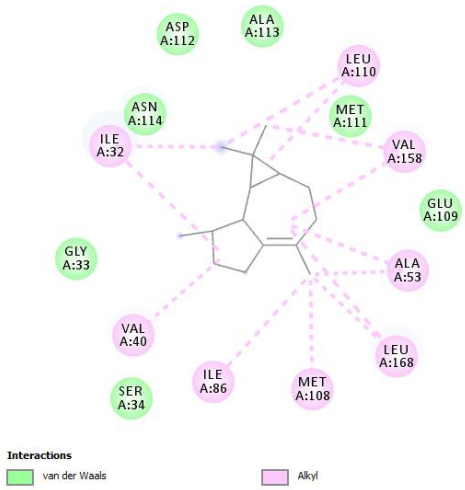


Figure 4. Visualization of the interaction of the amino acid ligand Ledene with the C-Jun N-Terminal Kinase receptor

Conclusion

Based on docking results compared to the native ligand (Quercetagetin), the active compound ledene has the potential to be a candidate acne drug due to its strong activity on the C jun-N Terminal Kinase receptor. The docking results obtained a binding energy of -6.74 Kcal/mol and an inhibition constant of 11.39 uM.

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