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In Silico *Test and* in Vitro *Test of* Asiatycoside Nanoemulsion Formula as Antibakteri *Propionibacterium Acnes*

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A B S T R A C T

Asiaticoside is a triterpenoid glycoside compound obtained from the pegagan plant (*Centella asiatica* L. Urban). This asiaticoside can be used to heal wounds and reduce reddish acne scars. Asiaticoside has poor absorption and excellent solubility. Nanoemulsion preparations will improve poor absorption of asiaticoside. Nanoemulsion can improve the properties of active compounds with poor absorption with very small particle sizes ranging from 1-100 nm, aiming to increase the permeability of active substances and improve absorption. This research aims to determine the in-silico test of the asiaticoside nanoemulsion formula against androgen receptor protein molecules in the pathogenesis of *Propionibacterium acnes* (PDB ID: 1VJY) and to determine the in vitro test of the asiaticoside nanoemulsion formula against the *Propionibacterium acnes* using the disc diffusion method. Based on the results of the in-silico test, it can be predicted that the asiaticoside compound has the potential as an antibacterial with a protein binding mechanism (1VJY), and the asiaticoside nanoemulsion formula has antibacterial activity against *Propionibacterium acnes.*

Keywords: asiaticoside, Nanoemulsion, pegagan, *Propionibacterium acnes*

Introduction

Asiaticoside is one of the triterpenoids contained in Centella asiatica. Asiaticoside has many pharmacological effects. One of the pharmacological effects that can be used is the anti-bacterial effect of asiaticoside (Norzaharaini et al., 2011; Singh et al., 2010). Triterpenoids can stimulate the formation of fats and proteins, essential for skin health. It can also convert alanine and proline into collagen, which functions to care for the skin. Another function is to speed up the healing of post-operative wounds, acne, and black spots on the skin (Sutardi, 2016).

Asiaticoside is widely used in the pharmaceutical industry, food, and cosmetics. However, asiaticoside has poor absorption and very good solubility (Ozdemir et al., 2016). So, nanoemulsion preparations will improve poor absorption of asiaticoside. Nanoemulsion applications are currently one of the most widely developed drug delivery systems. Nanoemulsions can improve the properties of active compounds that have poor absorption with very small particle sizes ranging from 1-100 nm, aiming to increase the permeability of active substances and improve absorption (Zhang et al., 2017). Acne occurs because fatty acids and skin oil are blocked. The drugs used for topical therapy mostly contain sulfur and other astringents. Meanwhile, for systemic therapy, tetracycline and enteromycin are used. Propionibacterium acnes plays a role in the pathogenesis of acne by producing lipase, which breaks down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when connected to the immune system and support the occurrence of acne. Nanoemulsion is a thermodynamically stable, transparent dispersion of oil and water stabilized by a film layer of surfactant and co-surfactant molecules and a droplet size of less than 100 nm (Shafiq et al., 2007).

Nanoemulsions are quite effective in increasing drug absorption into the systemic circulation because to have an effect, sufficient drug levels are required to be within the therapeutic window (Martien et al., 2012). The type of nanoemulsion depends on the composition used, namely the oil-in-water and water-in-oil nanoemulsion types. The oil-in-water type is in the form of oil droplets dispersed in the water phase, while the water-in-oil type is in the form of water droplets dispersed in the oil phase (Lina et al., 2017). The in silico test is a test that uses computerized assistance to determine the 3D structure of molecules and study the active sites that play a role in the molecule (Alfathan & Wathoni, 2018). This test is used to predict the ability of a compound with a large potential to cause biological effects computationally in the context of searching for new drugs, including compound design and its interaction with receptors (Bachtiar et al., 2021). In vitro testing is a technique for testing artificial media suitable in an environment with optimal conditions for bacterial growth. The purpose of the in vitro test is to determine the ability of antimicrobials to inhibit bacterial growth. (Lay, 1994).

Material and methods

The method used in the in-silico test is molecular docking, while the in vitro test is an antibacterial activity test using the disc diffusion method.

In Silico Test

In silico (computerized) test of the asiaticoside compound against the target protein Propionibacterium acnes (acne-forming bacteria) PDB ID (1VJY), which obtained the results of the docking score (binding energy), RSMD (Root Mean Square Deviation) value, 2D interaction image between ligand and protein (Wardaniati & Herli, 2018).

Optimal formula of asiaticoside nanoemulsion

This research began with weighing 3 mg of asiaticoside. Asiaticoside is dissolved first in water. Add 1% lecithin and tween 80 28.3%, then stir with a Thinky Mixer at 75 C with a speed of 1500 rpm for 15 minutes. Then, the oil phase, namely VCO, was added and stirred again with the Thinky Mixer. Distilled water was put into the mixture gradually until the volume was 100 ml, then remixed using the Thinky mixer at 1500 rpm for 15 minutes (Chairunisa et al., 2022).

Invitro Test

A total of 15 mL of Nutrient Agar (NA) was put into a sterile petri dish, and then 100 µl of bacterial suspension was added. Then, it was homogenized by shaking the petri dish containing the media, and the media was then allowed to solidify. The sterile disc is soaked in the asiaticoside nanoemulsion formulation, asiaticoside active substance, and nanoemulsion base, and then the disc is attached to the agar surface. DMSO was used as a negative control, and the antibiotic clindamycin disc was used as a positive control. This treatment was repeated three times. This petri dish was incubated in an incubator for 24 hours at 37ºC. The antibacterial activity was determined by measuring the diameter of the inhibition area formed using a caliper (Rusdi, 2010).

Characterization of Nanoemulsion Base

Characterization of the nanoemulsion base includes examination of globule size (PSA), zeta potential, and polydispersion index, which is carried out using a dispersing medium to disperse the sample. The required amount of formulation is dispersed in distilled water to obtain a homogeneous dispersion (Gurpreet & Singh, 2018).

Antibacterial Activity Test

A total of 15 mL of Nutrient Agar (NA) was put into a sterile petri dish, and then 100 µL of bacterial suspension was added. Then, it was homogenized by shaking the petri dish containing the media; the media was then allowed to solidify. The sterile disc is soaked in the Asiaticoside Nanoemulsion Formulation, Asiaticoside Active Substance, and nanoemulsion base, and then the disc is attached to the agar surface. As a negative control, DMSO was used, and as a positive control, the antibiotic clindamycin disc was used. This treatment was repeated three times. This petri dish was incubated in an incubator for 24 hours at 37ºC. The antibacterial activity was determined by measuring the diameter of the inhibition area formed using a caliper (Rusdi, 2010).

Results and Discussion

From the results of in-silico research, the asiaticoside compound with GDP ID (1VJY) obtained an RMSD) result of 0.2187 Å, a Grid Score value of -93.255 (table 1) and 2D visualization (figure 1). Asiaticoside has a strong affinity for the 1VJY protein, as shown by the binding energy value of the docking results, which is -93.255. The ligands in the docking results were drawn and analyzed for their physicochemical properties. The parameters looked at are the log partition coefficient (Log P) and molecular weight (BM). Good absorption and permeability, namely log P, is not greater than 5. Judging from the log P value, it shows that the log P value is <5, namely 0.1. (Sulastri et al., 2019).

Sulastri et al., 2019).

Table 1. Results of In Silico Docking of Asiaticoside Compounds with Protein (1VJY)

Grid score	-93,255
Root Mean Square Deviation (RMSD)	0,2187 Å
Grid Van Der Wals energy	-93,255
Log P	0,1
Bobot molekul (BM)	959,1

Visualization of docking results is carried out to see the amino acid residues that bind to the protein. Amino acid residues are the active site of the receptor that binds to the ligand. The active site of the receptor plays a role so that the ligand inhibits the receptor's action, which can be seen in Figure 7, showing the interaction between the protein and the ligand with two amino acid residues in hydrogen bonds. The interacting amino acid residues are HIS A:283 and ASP A:351 (conventional hydrogen bond category) (figure 1).

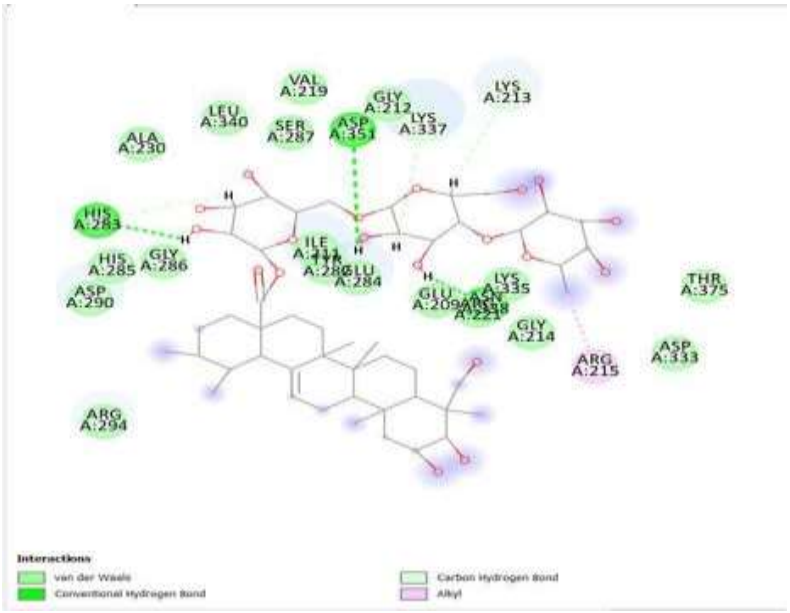

Figure 1. 2D visualization of ligand-protein interaction (1VJY)

From the results of in vitro antibacterial activity testing, the formulation of the active substance influences the antibacterial activity. Asiaticoside nanoemulsion formula at a concentration of 10% against Propionibacterium acnes bacteria produced an average inhibitory diameter of 15.35 mm, 19.2 mm, and 14.3 mm. The positive control results of the clindamycin disc were 21.3 mm (table 2).

Table 2. In vitro antibacterial activity test results

Sample	**Asiaticoside 10% nanoemulsion formula**	**Asiaticoside 3%**	**Control positive**	**Control negative**
Asiaticoside formula	15.35 mm	13.42 mm	21.3 mm	-
	19.2 mm	15.02 mm	27 mm	-
	14.3 mm	8.1 mm	26.5 mm	-
Average ±SD	16.26±2.57	12.18±3.62	24.93±3.15	-

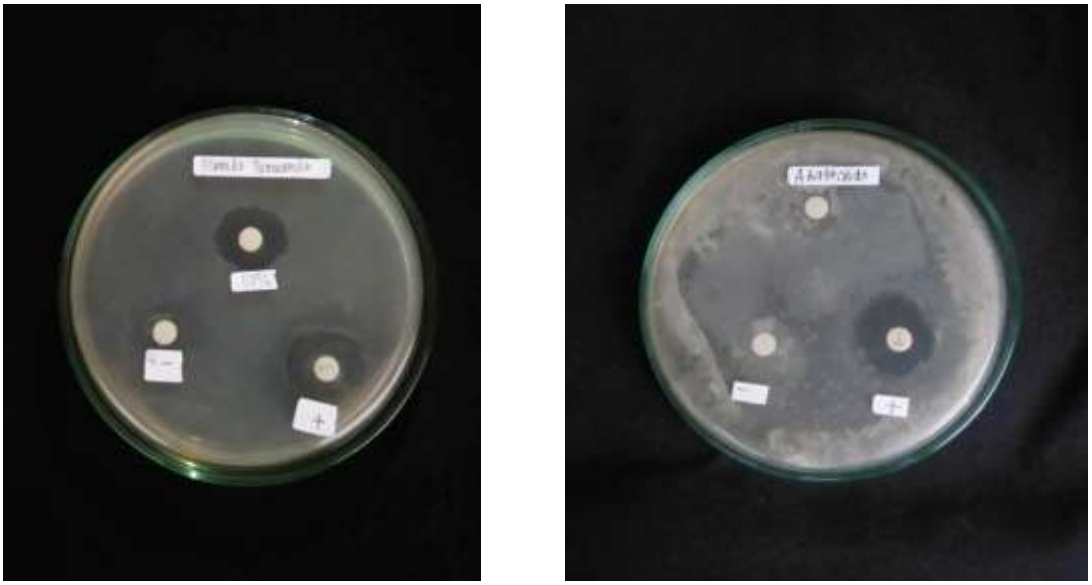

Figure 2. Antibacterial activity test results against Propionibacterium acnes bacteria: (a) asiaticoside nanoemulsion formula; (b) the active substance asiaticoside

The parameter tested in the in silico test is the RMSD value, also known as the Root Mean Square Deviation, which is the value used to determine whether the bond mode prediction is successful and is important for validating the docking program (Brooijmans, 2009). The docking score value is used to

predict the binding affinity between two molecules after docking; apart from that, the docking score is also used to predict the strength of the interaction between two molecules. The lower the free energy (Docking score), the more stable the binding interaction between the ligand and receptor. Visualization of docking results is carried out to see the amino acid residues that bind to the protein. Amino acid residues are the active site of the receptor that binds to the ligand.

The concentration used in the antibacterial activity test of the nanoemulsion formulation was 10%. The active substance asiaticoside is 3% and the nanoemulsion base is 10%. Then the antibacterial activity test was determined by measuring the diameter of the inhibition area formed using a caliper. The results of a good inhibition zone in the nanoemulsion dosage form are because the resulting nano-sized particles can penetrate bacterial cell membranes. Nanoemulsions that have a large surface area can encourage substances to diffuse into cells, thereby influencing the electrostatic interaction between the positive charges on the nanoemulsion compound content and the negative charges on the bacterial cell walls (Prasetya, 2021).

The positive control used in testing the antibacterial activity of the nanoemulsion formulation was clindamycin. Clindamycin is an antibiotic derived from lincomycin, which works by inhibiting protein synthesis (Huda et al., 2019).

Meanwhile, the negative control used was DMSO. The reason DMSO is used as a negative control is because DMSO is a solvent that can dissolve almost all polar and non-polar compounds. (Reynolds, 1996).

Asiaticoside belongs to the triterpenoid group, which has broad antimicrobial activity against bacteria, yeast, and filamentous fungi. Triterpenoids are antimicrobial because they can damage cell membranes or damage membrane lipid synthesis, which results in membrane permeability, resulting in leakage of cell components (Haraguchi et al., 1998). The mechanism of triterpenoids as antibacterials is to react with porins (transmembrane proteins) on the outer membrane of bacterial cell walls, forming strong polymer bonds resulting in damage to the porins (Cowan, 1999).

Conclusion

The results of the in silico test can predict that the asiaticoside compound has the potential as an antibacterial with a protein binding mechanism (1VJY), as shown by the RMSD value, docking score, and 2D visualization. Meanwhile, the in vitro test results of the asiaticoside nanoemulsion formulation had antibacterial activity against Propionibacterium acnes. The average inhibitory diameter of the asiaticoside nanoemulsion formula is in the strong category.

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