Baktiar et al | Article 8553
J Pure Appl Microbiol. 2023;17(3):1791-1799. doi: 10.22207/JPAM.17.3.44
Received: 05 March 2023 | Accepted: 08 August 2023
Published Online: 03 September 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Antibiofilm Activity of Tapak Kuda *Ipomoea pes-caprae* against *Pseudomonas aeruginosa* ATCC 27853 and Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300: *In-Vitro* and *In-Silico* Evaluation

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Abstract

***Ipomoea pes-caprae* is one of the herbal plants that can treat various health problems such as skin infections, burns, boils, and various diseases caused by microbial infections. This study aims to identify ethanol extract compounds of *I. pes-caprae* leaf and evaluate their antibiofilm activity through in-vitro and in-silico assays. This study used two test bacteria, *Pseudomonas aeruginosa* and Methicilin Resistant *Staphylococcus aureus*. Antibacterial activity is carried out using the agar diffusion method and antibiofilm using a microplate reader. The biological activity was also evaluated through a computational approach using molecular docking. The results of preliminary test demonstrated the antibacterial activity. At a concentration of 100 mg/mL, *I. pes-caprae* extract produced a substantial inhibitory zone of 13.9 mm for *P. aeruginosa* and a moderate 8.5 mm zone for MRSA. The extract also showed high antibiofilm activity. It achieved impressive biofilm inhibition rates of 82.58% and 78.29%, respectively. Molecular docking shows the interaction between extract compounds and macromolecules that play a role in biofilm formation, namely SrtA and associated protein biofilms. 1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid has the lowest binding energy of -7.5 Kcal/Mol and -5.9 Kcal/Mol at each target receptor. This study demonstrated the antibiofilm potential of *I. pes-caprae* extract, which was clarified through molecular docking studies.**

Keywords: *Ipomoea pes-caprae*, Biofilm, Antibacterial, *In-silico*

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Citation: Baktiar NH, Wardhani R, Husain DR, Haedar N, Gani F. Antibiofilm Activity of Tapak Kuda *Ipomoea pes caprae* against *Pseudomonas aeruginosa* ATCC 27853 and Methicilin Resistant *Staphylococcus aureus* (MRSA)ATCC 43300: *In-Vitro* and *In-Silico* Evaluation. *J Pure Appl Microbiol.* 2023;17(3):1791-1799. doi: 10.22207/JPAM.17.3.44

INTRODUCTION

The public has widely used herbal medicine to overcome various health problems since it has bioactive compounds with high therapeutic advantages and efficacy.[1] One of the traditional medicines often used was the leaf of *I. pes-caprae*. *I. pes-caprae* has been used by Australian Aboriginals to treat wounds, skin infections, and inflamed wounds. This plant has also been used in India and Brazil to treat indigestion, inflammation, and pain.[2] Several rural areas in Indonesia have used this plant to treat jellyfish stings, ulcers, and wounds in people with diabetes.[3] *I. pes-caprae* grows along sandy coastlines and can potentially treat various diseases.[4] The antibacterial activity of *I. pes-caprae* leaf extract against *Staphylococcus aureus* was also investigated in several studies.[5] Furthermore, this plant can inhibit the growth of *S. aureus* and *P. aeruginosa*. These two bacteria are biofilm-forming agents.[6]

Biofilm is a complex colony of microorganisms that attach to surfaces and form a protective matrix. Biofilm production is characterized by the presence of an amorphous structure surrounding the bacteria and clumps of bacterial cells immersed in a thick matrix. These characteristics play an essential role in the pathogenicity of certain bacteria and can lead to increased resistance to antibiotics.[7] *P. aeruginosa* and *S. aureus* are two bacterial species commonly associated with biofilm formation. These bacteria have been found to produce intricate biofilm structures that provide them with enhanced survival abilities and resistance against antibiotics.[8] Diseases that are caused by biofilm-forming bacteria, such as *P. aeruginosa* and *S. aureus*, can have significant implications for human health. These bacteria have been found to be commonly associated with wound infections and are known to cause chronic infectious states in wounds. The biofilms formed by *P. aeruginosa* and *S. aureus* stimulate the production of pro-inflammatory cytokines and prolong the inflammatory phase of the wound, leading to delayed healing and potential complications.[9]

The resistance of these two biofilm-forming microbes has become a serious problem amid the difficulty of finding effective antibiotics. The use of traditional herbs is quite promising and needs further exploration. Therefore, this study was conducted to determine the anti-biofilm potential of *I. pes-caprae* leaf ethanol through in vitro supported by in-silico assays using molecular docking studies.[10]

MATERIALS AND METHODS

The leaf of *I. pes-caprae* was obtained from the Tamalanrea district, Makassar, Indonesia. The sample is washed using distilled water, dredged, and then smoothed to obtain a simple powder. Maceration extraction was done using 96% ethanol and evaporated to obtain the extract. A viscous extract of *I. pes-caprae* leaf was analyzed using Thermoquest–Finnigan Trace GC–MS equipped with a methylpolysiloxane column DB-5 (5% phenyl) (60 m x 0.25 mm, film thickness 0.25 m). Helium was used as a carrier gas at 1 mL/min, and 1 L was injected for later analysis.[11] A total of 1 µl of the sample was injected into a GCMS device with a residence time of 40 minutes and heated at a temperature of 110 to 320°C, constant pressure at 15 kPa, scan mode, time of 60 minutes, and flow rate of 0.6 ml/min. Molecular weight scanners range from 45-600 m/z.[12] The compounds were identified using the NIST and Wiley libraries. The results of GC-MS can be seen in Table 1.

Antibacterial Test

Antibacterial tests were carried out using the disc diffusion method. The diffusion method was done by mixing 0.15 mL of *P. aeruginosa* suspension, and MRSA with a turbidity equivalent to MC Farland 0.5 inoculated on 15 ml of MHA medium. Blank disks that have been soaked with extract and then placed on the surface of the media. The test medium was then incubated for 24 hours at 37°C.[13]

Antibiofilm Test

P. aeruginosa and MRSA bacterial suspensions of 100 µL and *I. pes-caprae* leaf extract of 100 µL with concentration variations of 6.25mg/mL, 12.5mg/mL, 25mg/mL, 50mg/mL, 100mg /mL were inserted into the microplate. The incubation period was 72 hours at 37°C. The microplate was washed three times with PBS, dried, and added 0.1% violet crystal by

200 µL and then incubated for 15 minutes at room temperature. The microplate was washed with PBS three times and dried, then 96% ethanol of 200 µL was added and incubated for 15 minutes at room temperature. Antibiofilm activity was measured using a microplate reader at a wavelength of 595 nm with the following formula.[14]

$$\%PPB2=\frac{ODcn - ODse}{ODcn} \times 100$$

Annotation: PPB2 = Growth Inhibition Biofilm
ODcn = OD negative control
ODse =OD sample experiment

Computational Evaluation

The structure and information related to the identified compounds (ligands) are obtained from the link https://pubchem.ncbi.nlm.nih.gov/. The results of the compounds are downloaded in the PDB file, which will then be docked in the PyRX application. Macromolecules are downloaded on the UniProt database. The 3D structure is then visualized first in the PyMol application. It is then mounted on the PyRx application via the vina wizard to find the lowest affinity binding.[15] Interactions between ligands and macromolecules were visualized in the Discovery Studio 2019 application. SrtA (Sortase A) (PDB ID:2AKID) and Biofilm associated protein (PDB ID:7DM0) were chosen as a target in our study. The protein's crystal structure was taken from Protein Data Bank (https://www.rcsb.org/).

RESULTS

Antibacterial Activity

Antibacterial activity testing aims to determine the ability of leaf ethanol extract *I. pes-caprae* in inhibiting the growth of bacteria. It can be seen from the formation of inhibition zones around the disc paper.[16] The results can be seen at Figure 1 and Table 1.

The results of the antibacterial test revealed the inhibitory potential of the extracts against both *P. aeruginosa* and MRSA at various concentrations. As the concentration of the extracts increased, the inhibitory zones exhibited a clear dose-dependent response. Specifically, at a concentration of 6.25 mg/mL, the extracts showed inhibitory zones 10.5 mm and 4.35 mm for *P. aeruginosa* and MRSA, respectively. When the concentration was raised to 12.5 mg/mL, the inhibitory zones reached 10.25 mm for *P. aeruginosa* and 5.5 mm for MRSA.The most potent inhibitory effect was observed at the highest concentration tested, 100 mg/mL, which produced precise zone diameters of 13.9 mm for *P. aeruginosa* and 8.5 mm for MRSA. In comparison, the positive control, ciprofloxacin, demonstrated

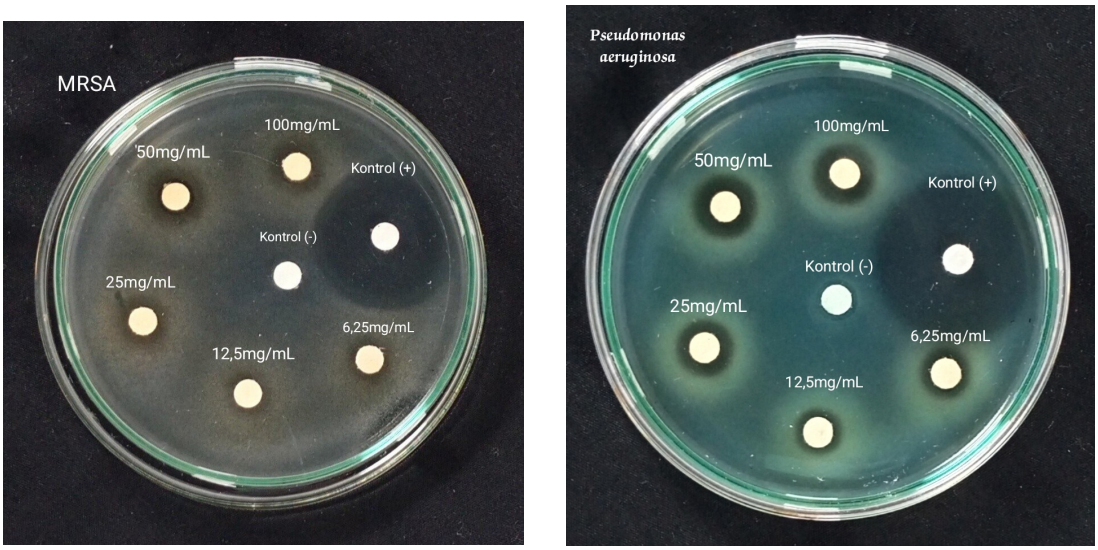


Figure 1. Antibaterial test result of *I. pes-caprae* leaf: (left) *P. aeruginosa* and (right) MRSA

antibacterial activity against both bacterial strains, exhibiting inhibitory zones greater than 20 mm.

Antibiofilm Activity

Figure 2 showed the percentage inhibition of *I. pes-caprae* leaf extract. The highest biofilm growth inhibition activity was seen in a concentration of 100mg/mL with a percentage of 82.58% in *P. aeruginosa* and 78.29% in MRSA. Meanwhile, the lowest biofilm inhibition activity was seen at a concentration of 6.25mg / mL with a percentage of 52.71% in *P. aeruginosa* and 52.79% in MRSA. These results clearly indicate a strong inhibitory activity as the concentration of the extract increases.

Molecular Docking Evaluation

The literature review results show that several identified *I. pes-caprae* extract compounds have biological activity, including 1,2,3,4,5-Tetrahidroxy-Cyclohexanecarboxylic Acid,[17] 2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5,5-Trimethyl-[18], Phenol, 2-Methoxy-3-(2-Propenyl)-,[19] 1,2,3,4 Butaneterol,[20] and Benzeneacetic acid.[21] These compounds have antibacterial and antibiofilm activity. The results

Table 1. The identified compound of *I. pes-caprae* leaf extract

No.	Compounds	Retention time	Area (%)
1.	1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid	13.923	18.09
2.	2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5,5-Trimethyl-, [R-[R	13.561	3.71
3.	Phenol, 2-Methoxy-3-(2-Propenyl)-	9.676	3.05
4.	1,2,3,4-Butanetetrol	9.968	3.19
5.	Benzeneacetic Acid	8.762	4.01

Table 2. Inhibition zone of *I. pes-caprae* leaf extract

Concen. (mg/ml)	Inhibition zone (mm)	
	P. aeruginosa	MRSA
6.25	10.5	4.35
12.5	10.25	5.5
25	11.25	6.95
50	13.1	7.9
100	13.9	8.5
Positive control	32.45	25.4

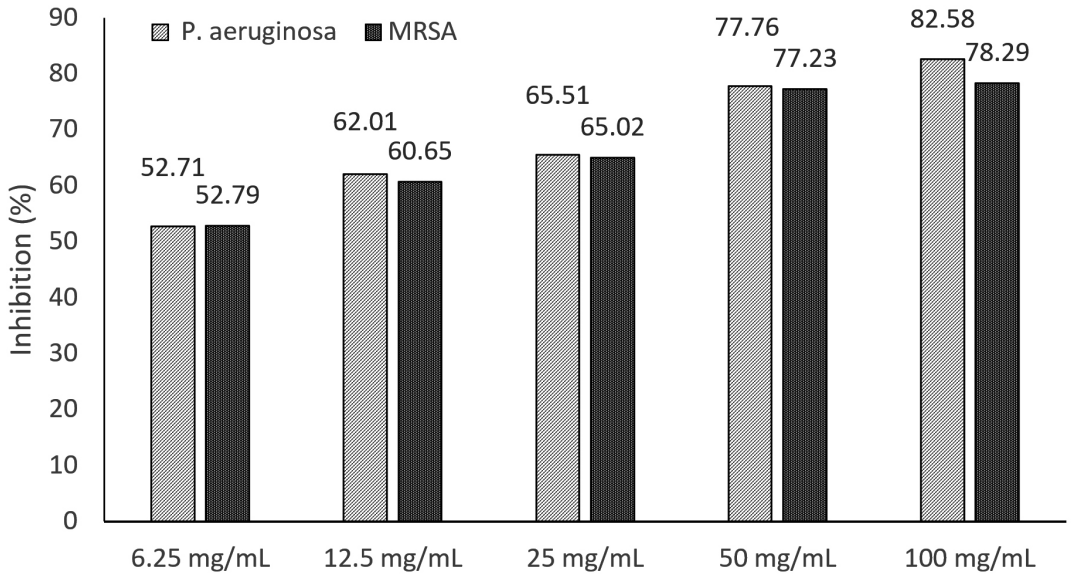


Figure 2. Biofilm Inhibition results

of molecular docking of *I. pes-caprae* leaf extract compounds against SrtA and associated protein biofilms can be seen in Table 2.

According to the molecular docking (Table 3), 1,2,3,4,5-Tetrahidroxy-Cyclohexane-carboxylic Acid has the lowest binding affinity value at both receptors, -7.5 Kcal/Mol on SrtA and -5.9 Kcal / Mol in associated protein biofilms. A low binding affinity value indicates a stronger interaction between receptors and ligands.[22] Figure 3 showed the interaction between *I. pes-caprae* compounds and receptor.

DISCUSSION

Biofilm growth will begin after the bacteria successfully attach to a surface. Bacteria attached to the surface will begin to conduct quorum sensing to coordinate growth and produce the EPS matrix. The presence of compounds in *I. pes-caprae* extract can inhibit the growth of bacterial biofilms. *I. pes-caprae* leaf extract contains antibacterial compounds such as phenolics, flavonoids, alkaloids, and tannins. Phenolic compounds, namely phenolic acids, can reduce EPS production and interfere with *quorum sensing*.[23] Tannins can interfere with EPS production from bacteria so that *matrix-deficient cell clusters* are formed. Tannins can also lower the surface area of the peptidoglycan layer and increase the penetration of the antibodies used.[24]

Other studies have evaluated ethyl acetate and acetone extract of *I. pes-caprae* leaf at 12.5 and 25 mg/mL concentrations may inhibit *S. aureus* and *P. aeruginosa*. In addition, the resulting quercetin compounds can help inhibit the inhibition of *S. aureus* and *P. aeruginosa*.[4] The similar results which reported that the extract of *I. pes-caprae* leaf with a concentration of 100% has antibacterial activity against *S. aureus* of 11.0 mm.[25] Another study stated that the leaf compound *I. pes-caprae* could inhibit MRSA at a concentration of 25mg/mL.[26]

P. aeruginosa and *S. aureus* bacteria are bacteria that can infect skin tissue. Various disease disorders, such as urinary tract infections and gastrointestinal infections, are associated with the pathogenicity of *P. aeruginosa*.[27] MRSA is a strain of *S.aureus* resistant or resistant to the antibiotic *methicillin*.[28] *P.aeruginosa* and MRSA can be isolated from infected chronic wounds, cystic fibrosis, and chronic suppurative otitis medium. Both tend to form a biofilm to maintain their spread within the host cell.[6]

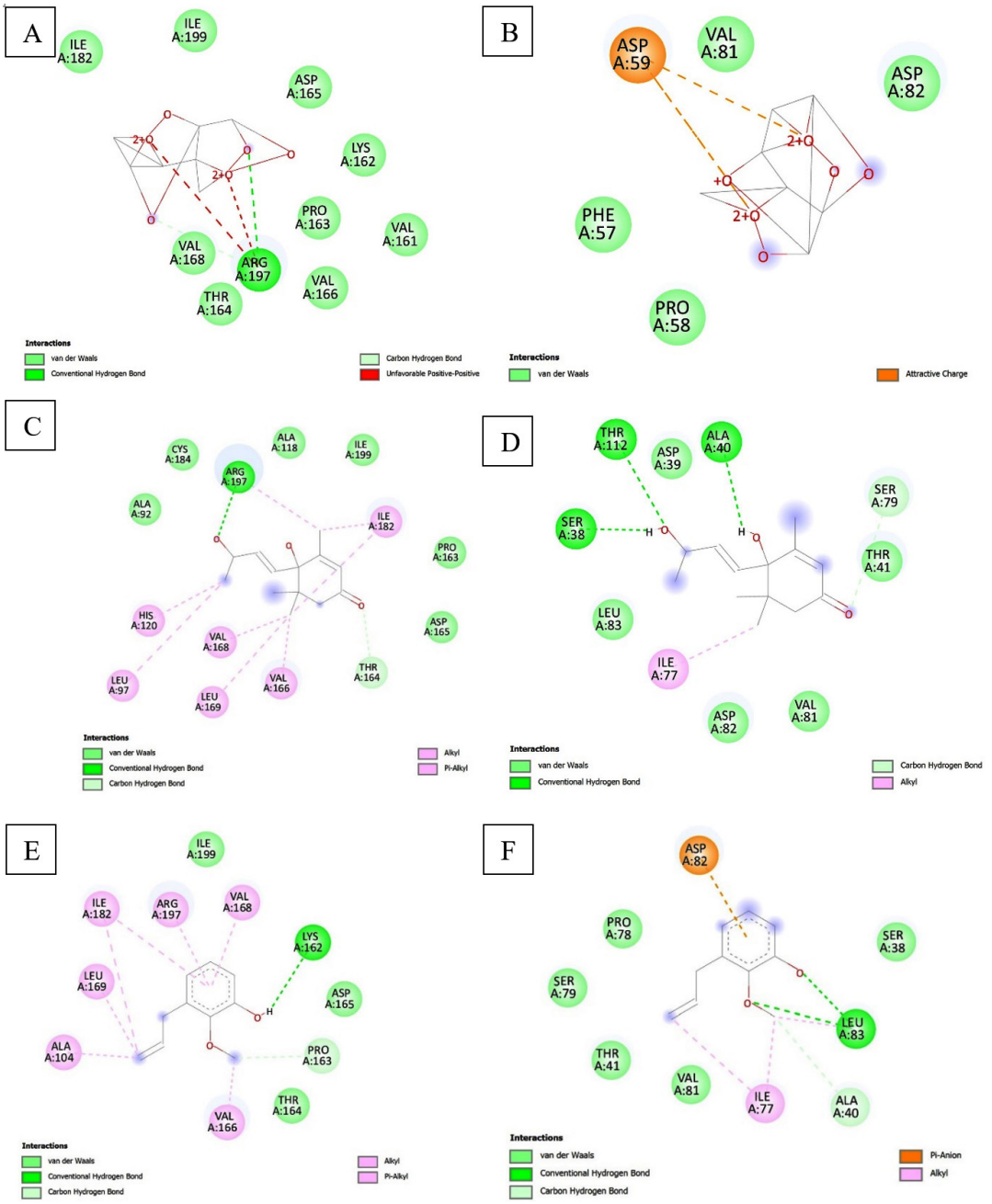
The molecular docking results showed the interaction between *I. pes-caprae* compound and protein targets. The purpose of evaluating the interaction of macromolecules and ligands is to assist in studying a drug, both its interaction and the compatibility of active sites on proteins, and calculating the interaction energy of the ligand to determine which ligand has the best effectiveness through the conformation formed.[29] The GC-MS test was carried out to determine the components of the ethanol extract compounds of *I. pes-caprae* leaf. The results of literature studies showed that five compounds identified from the GC-MS results had antibacterial and antibiofilm activity. The five compounds are then reacted with two target in the biofilm formation process, namely Sortase A (SrtA) and associated

Table 3. Molecular docking results of Identified compound

Ligand	Binding Affinity (Kcal/Mol)	
	SrtA	Biofilm associated protein
1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid	-7,5	-5,9
2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5,5-Trimethyl-, [R-[R	-5,6	-4,8
Phenol, 2-Methoxy-3-(2-Propenyl)-	-5,4	-4,5
1,2,3,4-Butanetetrol	-3,6	-3,8
Benzeneacetic Acid	-5,2	-4,5

protein biofilms. These proteins play an important role in biofilm formation, inhibition of this enzyme prevent the colonization of microorganism for biofilm formation.

SrtA is an enzyme that is essential in the mechanism of signaling pathways and microbial adhesion to the host. It is also widely regarded as a generic therapeutic target for all Gram-positive bacteria. It is a membrane enzyme that plays a role in maintaining proteins on the cell wall's surface of gram-positive bacteria and enriching virulence and biofilm formation activities.[30] SrtA has three amino acid residues, namely His120, Cys184, Arg197, Val168, Leu169, Ala104, Ala118, and Leu97. The



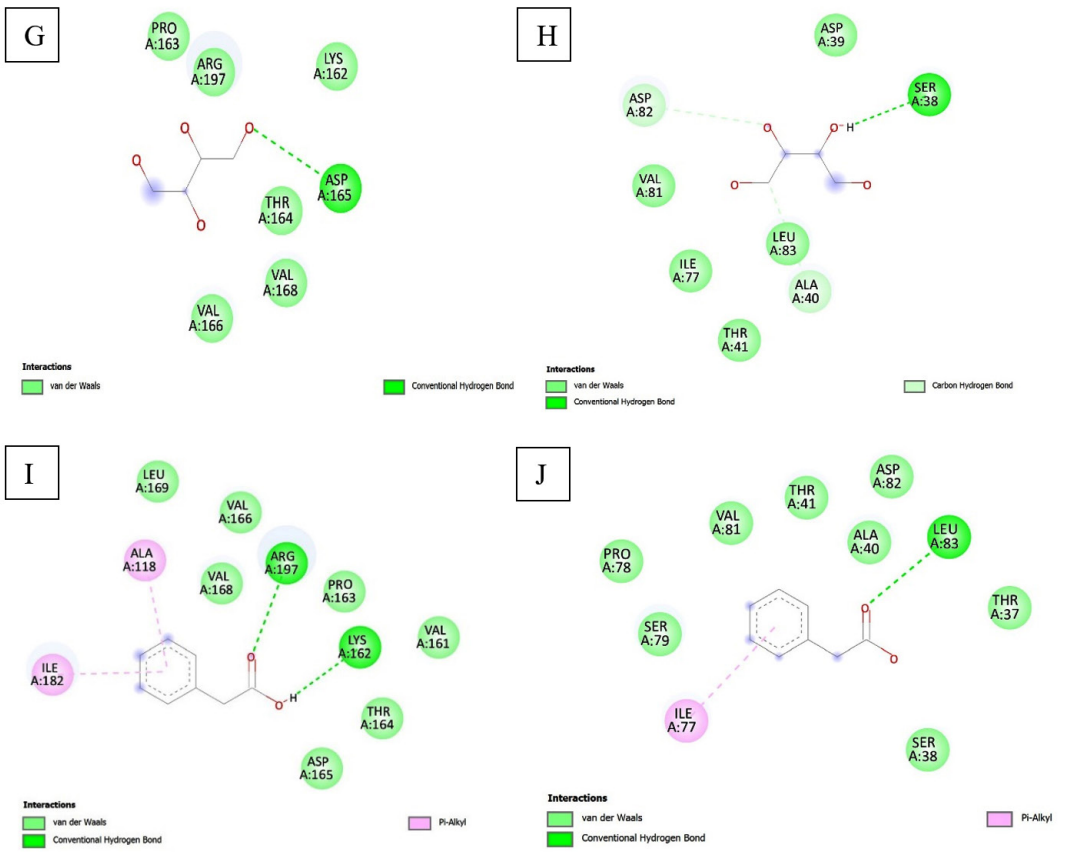


Figure 3. Interaction of (A) SrtA-1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid, (B) SrtA-2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5,5-Trimethyl, (C) SrtA-Phenol, 2-Methoxy-3-(2-Propenyl), (D) SrtA-1,2,3,4-Butanetetrol, (E) SrtA-Benzeneacetic Acid, (F) Biofilm associated protein-1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid, (G) Biofilm associated protein-2-Cyclohexen-1-One, 4-(3-Hydroxy-1-Butenyl)-3,5,5-Trimethyl, (H) Biofilm associated protein-Phenol, 2-Methoxy-3-(2-Propenyl), (I) Biofilm associated protein-1,2,3,4-Butanetetrol, (J) (+)-Biofilm associated protein-Benzeneacetic Acid

compound group will approach the active site of the aromatic ring SrtA that matches the lipophilic bag formed between Val166- Lys162, Val168-Leu169, and Leu181-Thr183 then the binding affinity of the compound to the active site of SrtA will be predicted. Nitrogen from the pyridine ring will have polar contact with the Glu105-Glu106 peptide bridge. Hydrophobic interactions with the binding site also involve Val166, Gly167, Val168, Leu169, Ile182, Ile117, Phe103, Gly90, and Ala104. For example two polar interactions: the nitrogen atom of the oxadiazole ring interacts with the peptide bond Ala104-Glu105, while the terminal acetyl group interacts with the Asn107- Glu108-Ser109 peptide bond.[31]

Associated protein biofilms are the first proteins capable of inducing the development of biofilms by having a series of identical repetitions of 86 amino acids. Associated protein biofilms are found in gram-positive bacteria and gram-negative bacteria. It has the main function of helping maintain protein conformation that extends to the cell's surface.[32] Associated protein biofilms have amino acids with residues GLNA506, ARGA738, ARGA741, SERA363, LYSA415, LEUA364, LEUA364, LEUA364, LYSA362, LYSA415 ALAA726, THRA662. The ligand that has the strongest affinity will interact with the two proteins and hydrogen bonds Thr520 and Gln574 and alkyl bonds in Leu472 and Pro660.[33]

CONCLUSION

The leaf of *I. pes-caprae* has antibacterial and antibiofilm potential proven in the inhibitory zone and percentage inhibition, respectively. Antibiofilm testing showed high inhibition percentages in *P.aeruginosa* and MRSA of 82.58% and 78.29%. The molecular docking results showed an interaction between the five compounds of *I. pes-caprae* with the lowest affinity binding values reaching -7.5 Kcal/Mol on SrtA and -5.9 Kcal/Mol on biofilms associated with proteins with 1,3,4,5-Tetrahydroxy-Cyclohexanecarboxylic Acid. These results show the potential of *I. pes-caprae* leaf as therapeutic agents, especially in dealing with various health problems related to biofilms. Further studies using in-vivo methods must be evaluated for a comprehensive study.

ACKNOWLEDGMENTS

The author would like to thank Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University for providing facilities and technical support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DRH and RW conceptualized. NH, FG, and RW implemented the research. NH, RW, and DRH wrote the manuscript. NH and DRH gave advice to improve the research. NHB and RW revised the paper. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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