Trop J Nat Prod Res, November 2022; 6(11):1802-1808

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Potential Role of Betel Leaf (*Piper betle* L.) Water Extract as Antibacterial *Escherichia coli* Through Inhibition of *β*-Ketoacyl-[Acyl Carrier Protein] Synthase I

Ayu T. Agustin[1]*, Eko Julianto[2], Julianus Julianus[2], Jepri Riranto[2]

[1]*Medical Laboratory Technology Program, Politeknik Yakpermas Banyumas, Indonesia*
[2]*Diploma III of Nursing, Politeknik Yakpermas Banyumas, Indonesia*

ARTICLE INFO

Article history:
Received 26 October 2022
Revised 16 November 2022
Accepted 17 November 2022
Published online 01 December 2022

ABSTRACT

The exploration of bioactive compounds from herbal plants as inhibitors of fatty acid synthesis without adverse side effects is still the focus of the discovery and development of antibacterial drugs, especially to fight infections in chronic wounds. This study aimed to investigate the biological function of the bioactive compound of *Piper betle* L. leaf extract in inhibiting the target protein of *Escherichia coli*. 3D structure of the bioactive compound and thiolactomycin (as a positive control) were downloaded from PubChem. The Protein Data Bank database was used to retrieve the 3D structure of *β*-Ketoacyl-[Acyl Carrier Protein] Synthase I (FabB). Prediction of the active site of FabB was predicted using the Molegro Virtual Docker 5.0 program. Molecular interactions of the compound and target protein were analyzed using Molegro virtual docking version 5, superimposed using PyMol version 2.2, and visualized using the Discovery Studio program version 21.1.1. The results showed that the five bioactive compounds of *Piper betle* L. (eugenol, catechin, caffeic acid, quercetin, and ascorbic acid) could bind to the active site of FabB. All compounds were found to bind to the residues ALA271, PRO272 and HIS298. The catechin-FabB complex showed the lowest binding energy. This finding indicates that the *Piper betle* L. leaf extract's bioactive compound may inhibit the target protein of *E. coli β* - Ketoacyl-[Acyl Carrier Protein] Synthase I (FabB), which leads to the control of bacterial infection in chronic wounds at the cellular level.

***Keywords*:** Chronic wound, Competitive inhibitor, FabB, Fatty acid, Herbal medicine.

Introduction

The wound may represent the skin disorders that occur due to an accident or from various medical conditions (such as surgical trauma).[1,2] Wounds have a major impact on health systems and economies around the world. Wounds are classified into acute and chronic wounds.[3] Epidemiological studies demonstrate that the incidence of acute and chronic wounds globally reaches one billion.[4] Acute wounds pass through a healing phase and usually show indications of healing within four weeks. In contrast, chronic wounds have not exhibited healing within four weeks.[2] Infection is a common deterrent in wound healing, especially chronic wounds.[5] Chronic wound healing may be delayed by infection, usually with increased pain, inflammation, and decreased quality of life (clinical implications).[6] Wound infections cause approximately 70-80% of deaths in surgical patients. Appropriate care can reduce the risk of morbidity, mortality, and healthcare costs.[7] Endotoxins and exotoxins secreted by bacterial cells can induce an immune response in which neutrophils and macrophages are involved in this mechanism. The presence of overexpressed neutrophils causes excessive inflammation, which may additionally trigger adverse consequences.[8] Tissue damage and delayed re-epithelialization may result from the recruitment of neutrophils and other immune cells to the wound base. Furthermore, proteases are overproduced, which triggers a reduction in the extracellular matrix and delays wound healing.[8–10]

*Corresponding author. E mail: ayu.augustin11@gmail.com
Tel: +6285396469246

Citation: Agustin AT, Julianto E, Julianus J., Riranto J. Potential Role of Betel Leaf (*Piper betle* L.) Water Extract as Antibacterial *Escherichia coli* Through Inhibition of *β*-Ketoacyl-[Acyl Carrier Protein] Synthase. Trop J Nat Prod Res. 2022; 6(11):1802-1808.
http://www.doi.org/10.26538/tjnpr/v6i11.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Escherichia coli is a gram-negative pathogenic bacteria that most frequently colonizes chronic wounds. *E. coli* show resistance to most antibiotics, such as ampicillin and amoxicillin. Thus, they can survive and reproduce in the presence of minimal nutrients.[8,11] Previous studies have investigated the presence of *Escherichia coli* and its treatment in necrotizing soft tissue infections (NSTI).[12] Wound tissue debridement and antibiotics are conventional approaches to treating wound infections. Antibiotic application is often closely associated with the risk of therapeutic resistance and the persistence of antibiotic-resistant infections. [13] The ideal antimicrobial alternative to treat chronic wound infections is still being developed. At least it has antibacterial properties and does not cause adverse side effects.[14]

Medicinal plants of *Piper betle* L.have become promising alternative treatments because of their potency in treating wound infections.[15] In vitro study showed that *Piper betle* L. has bioactivity as an antibacterial agent (inhibiting *Staphylococcus aureus*) in conjunctivitis patients.[16] *Piper betle* L. leaf extract was also reported to enhance the wound healing process in diabetes mellitus by declining oxidative stress markers and 11β HSD-1 expression.[17] Previous studies have shown that betel leaf extract (*Piper betle* L.) with a concentration of 10% can improve the wound-healing process in *Rattus novergicus*.[15] Methanol extract of betel leaf has been tested in vitro and showed an increase in NIH3T3 cell proliferation.[18] HPLC analysis showed that the aqueous extract of *Piper betle* L. contained several compounds, especially quercetin, ascorbic acid, eugenol, catechin, and caffeic acid.[19]

Bioactive compounds in *Piper betle* L. have been reported to inhibit bacterial colonization,[16] thereby accelerating wound healing.[9] Previous studies have revealed the potential of *Piper betle* L. leaf extract in inhibiting gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) *in vitro*.[20] The crude water extract of *Piper betle* L. leaves showed an inhibition zone of bacteria (*Escherichia coli*) of 4.54 ± 0.72.[21] Research related to the potential role of medicinal plants as antibacterial showed that the methanol extract of *Cayratia auriculata* was effective in inhibiting the growth of *Escherichia coli in vitro*.[22]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The in silico study on the potential role and cellular mechanism of the bioactive compound *Piper betle* L. as an inhibitor of *Escherichia coli* is still limited. Therefore, the study was carried out to predict the biological function of bioactive compounds from an aqueous extract of *Piper betle* L. in inhibiting the target protein of *E. coli β* - Ketoacyl-[Acyl Carrier Protein] Synthase I (FabB). *β*-Ketoacyl-[Acyl Carrier Protein] Synthase I (FabB) is a protein responsible for the synthesis of fatty acids in the peptidoglycan membrane of *E. coli* bacteria.[23] FabB dysfunction in *E. coli* bacteria can cause the inability to synthesise essential organic compounds (auxotrophy) required for growth.[24] Fatty acids (FA) play a crucial role as an energy and a carbon source for bacteria growth.[25] Fatty acids are precursors for synthesising various signaling molecules and secondary metabolites, such as phospholipids, sphingolipids, and sterols.[24] The acyl chains of glycerophospholipids produced from fatty acids act as major components of bacterial membranes.[26] FabB inhibitors of both natural and synthetic compounds have validated this enzyme as an antibacterial target.[26]

Materials and Methods

Materials

The compounds of quercetin (CID 5280343), ascorbic acid (CID 54670067), eugenol (CID 3314), catechin (CID 9064), and caffeic acid (CID 689043) were retrieved from the PubChem Database (https://pubchem.ncbi.nlm.nih. gov/). The thiolactomycin compound was used as a positive control. The thiolactomycin was retrieved from the PubChem database with CID 502. Thiolactomycin (TLM) is a natural molecule that exhibits potent antibacterial activity against gram-positive and gram-negative pathogenic bacteria.[27] Thiolactomycin belongs to antibiotics thiolactone produced from fermented broth strains of actinomycetes (species Nocardia) and is an inhibitor of the FabB protein.[26,27] *E. coli β* - Ketoacyl-[Acyl Carrier Protein] Synthase I (FabB) were downloaded from the database of the protein data bank (PDB ID 1FJ4).[28]

Protein Structure Modeling

The Molegro Virtual Docker 5.0 program software investigated the protein structure for its active site. The maximum Molecular surface van der Waals parameter was set at 5. The active site for the protein (FabB) was formed on the grid (X= 41.01), (Y = -3.01); and (Z=45.69) with a radius of 15.

Molecular Docking

Compounds interacted with target proteins using the Molegro virtual docker program with a specific grid [the active side grid (X= 41.01), (Y = -3.01), and (Z=45.69) Radius 15]. The docking parameters are score Function Moldock Score [Grid]; grid resolution 0.30; algorithm MolDock SE; Max iteration 1500; max population size 50; pose generation energy threshold 100, multiple poses number of poses 5; tries 10 – 30; Number of Runs 10, simplex evolution max steps 300; neighbour distance factor 1.00; and energy threshold 0.00; cluster similar poses RMSD threshold 1.

Data Analysis

The molecular docking result was analyzed using Molegro virtual docking version 5 combined with protein (superimposed) using PyMol version 2.2 software. The 3D and 2D views and ligand-binding interactions were visualized with the Discovery Studio program version 21.1.1.[29]

Results and Discussion

The molecular docking approach revealed that eugenol, catechin, caffeic acid, quercetin, and ascorbic acid compounds bind to FabB in the inhibitor region (Figure 1A). The FabB region inhibitor consists of a catalytic triad with cysteine (Cys), histidine (His) and histidine (Asp) residue configurations. The FabB active site array includes CYS 163-HIS 298-HIS333 and CYS163-HIS 303-HIS 340.[30]

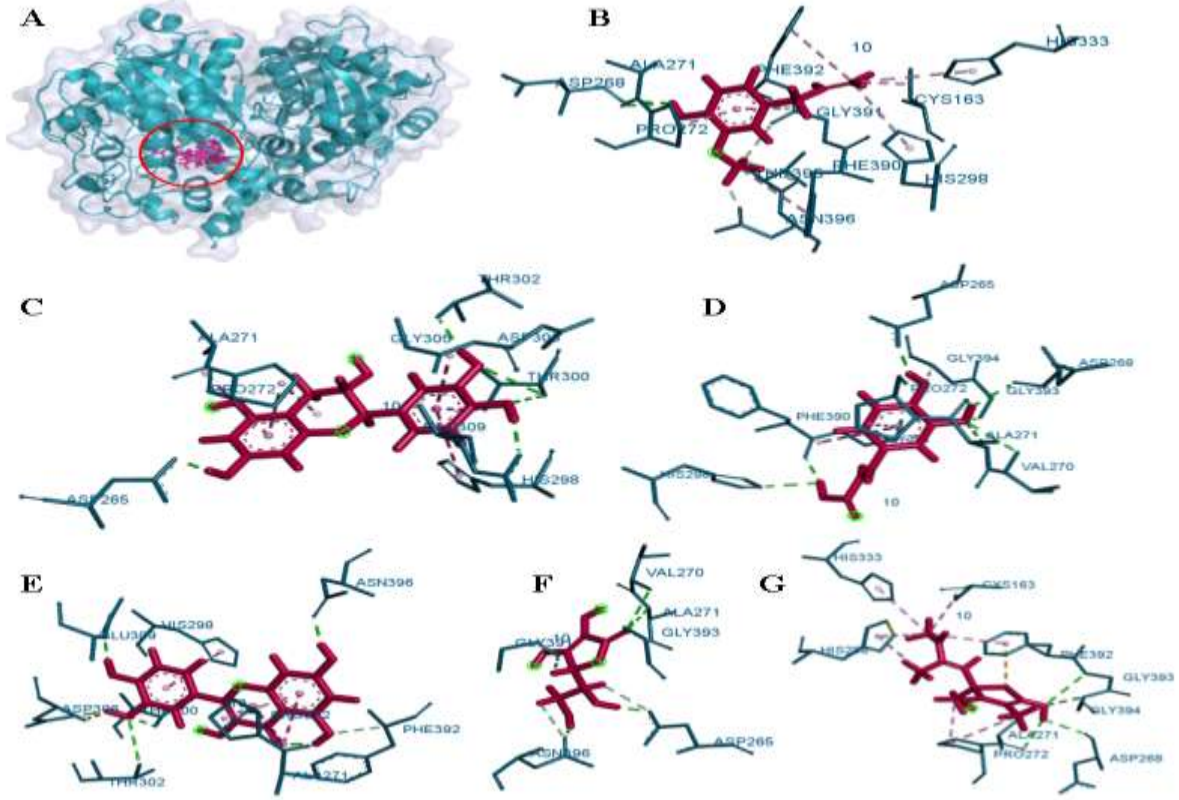

Figure 1: The 3D view of compound-complex with FabB protein, A. Superimposed ligands – FabB protein, B. Eugenol, C. Catechin, D. Caffeic acid, E. Quercetin, F. Ascorbic acid, G. Thiolactomycin. turquoise colour shows FabB protein, while the pink colour indicates the compound.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Inhibitors that bind to the active site of FabB may destroy fatty acid synthesis necessary for the survival of *E. coli* bacteria.[27]

The 3D structure of the ligand-FabB complex shows that all five compounds have active binding sites in the same region as Thiolactomycin (Figure 1). Eugenol was able to bind 12 amino acid residues (ASP268, ALA271, GLY391, THR395, ASN396, GLY391, CYS163, HIS298, HIS333, PHE390, PHE392, PRO272) of FabB (Figure 1B). The eugenol-FabB complex is possessed by hydrogen bonding and hydrophobic interactions. The amino acid residues THR300, SP265, THR302, GLU309, HIS298, ALA271, PRO272, GLY305, and ASP306 were involved in the catechin-FabB interaction (Figure 1C). Hydrophobic and hydrogen bonds maintain this interaction (catechin-FabB complex). There were 10 amino acid residues of FabB VAL270, ALA271, GLY393, ASP268, ASP265, HIS298, GLY394, PHE390, GLY391, and RO272 found bound to caffeic acid (Figure 1D). These interactions (VAL270, ALA271, GLY393, ASP268, ASP265, HIS298, GLY394, PHE390, GLY391, and RO272) are stabilized by hydrophobic and hydrogen bonds. Molecular docking results showed that quercetin could bind FabB at residues THR300, THR302, ASN396, ASP306, GLU309, PHE392, HIS298, ALA271, PRO272, and ASP306 (Figure 1E). Hydrophobic interactions and hydrogen bonds stabilize the quercetin-FabB complex. Ascorbic acid could bind to FabB target proteins involving residues VAL270, ALA271, GLY393, ASP265, ASN396, and GLY391 and maintained by hydrogen bonding (Figure 1F).

ASP268 residue is essential for acid catalysis in bacteria.[31] The HIS333 and HIS298 residues are active sites of FabB responsible for stabilizing the protein-inhibitor complex.[32] The molecular docking results showed that the five bioactive compounds from the aqueous extract of *Piper betle* L. leaves (eugenol, catechin, caffeic acid, quercetin, and ascorbic acid) competed with thiolactomycin for binding to FabB. The binding interaction of the eugenol-FabB and caffeic acid-FabB complexes involves the ASP268 residue. ASP268 residue was also found in the thiolactomycin-FabB interaction. The same amino acid residue bound by thiolactomycin is GLY393, owned by caffeic acid and ascorbic acid. PHE392 was bound by quercetin, eugenol, and thiolactomycin. CYS163 and HIS333 were also found to be bound by eugenol and thiolactomycin. Eugenol, catechin, caffeic acid, quercetin, ascorbic acid, and thiolactomycin were found to bind to the ALA271 residue of FabB. The PRO272 and HIS298 residues of FabB are involved in the binding interactions of eugenol, catechin, caffeic acid, quercetin, and thiolactomycin with FabB.

Eugenol showed antibacterial activity of *Escherichia coli* 06 strain with a minimum inhibitory concentration (MIC) of 1,024 µg/mL.[33] Previous molecular docking revealed that flavonoid compounds bind to active sites (HIS333 and HIS298) of *β*-ketoacyl acyl carrier protein synthase I (Kas I) of *Escherichia coli* with a low binding energy (135.76 kcal/mol)[32] The inhibitors are molecules that can reduce or completely inhibit the catalytic activity of enzymes. The binding of the enzyme's active site non-covalently and competing with the substrate is a competitive inhibitory process.[34] The results of this study revealed that the binding interaction of FabB target proteins by bioactive compounds from aqueous extracts of *Piper betle* L. leaves demonstrated the discovery of FabB inhibitor as an alternative treatment to fight bacterial infections. The 2D structure of the FabB protein complex showed the types of bonds formed, including hydrogen bonds, hydrophobic interactions and van der Waals forces (Figure 2). This type of bond (hydrogen bonds, hydrophobic bond, and van der Waals forces) contributes to the bond energy of all complexes.[35] The bond energy formed ranges from -353.8 to -258 kJ/mol. Low binding energy indicates that the compound binds to the target protein more strongly. Addditionally, high energy means a weak interaction between the compound and the FabB protein.[36,37] Interestingly, the lowest binding energy of the catechin-FabB complex was -353.8 kJ/mol, more promising than thiolactomycin (-282 kJ/mol) in inhibiting FabB.

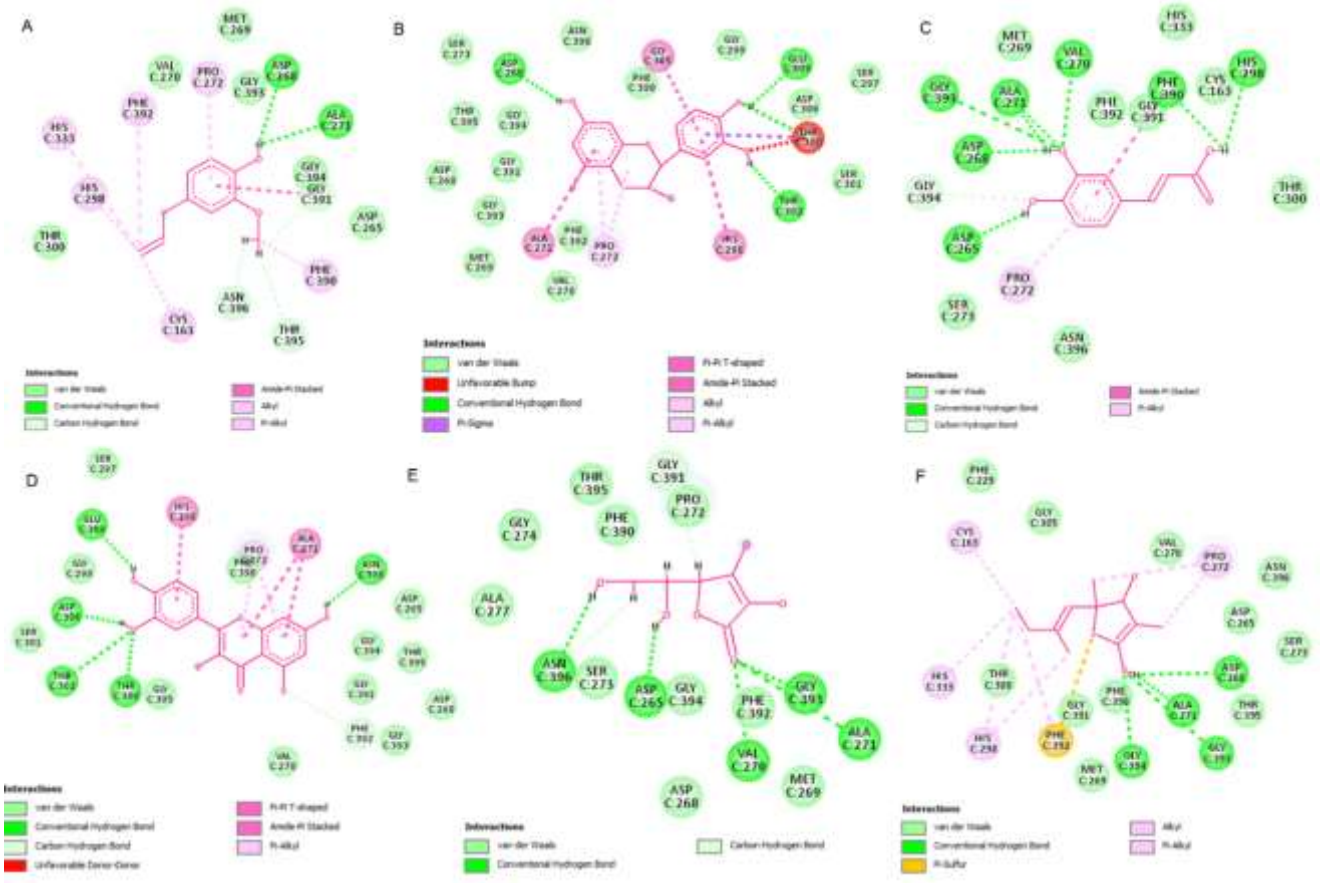

Figure 2: The 2D view of compound-complex with FabB protein, A. Eugenol, B. Catechin, C. Caffeic acid, D. Quercetin, E. Ascorbic acid, F. Thiolactomycin.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: Interaction between compounds (eugenol, catechin, caffeic acid, quercetin, and ascorbic acid) with FabB

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
Eugenol	-258	:10:H10 - C:ASP268:O	2,57468	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H10 - C:ALA271:O	2,83695	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H8 - C:GLY391:O	2,80076	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H8 - C:THR395:O	2,87379	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H9 - C:ASN396:OD1	1,65508	Hydrogen Bond	Carbon Hydrogen Bond
		C:GLY391:C,O;PHE392:N - :10	3,6621	Hydrophobic	Amide-Pi Stacked
		:10:C10 - C:CYS163	3,34733	Hydrophobic	Alkyl
		C:HIS298 - :10:C10	4,68694	Hydrophobic	Pi-Alkyl
		C:HIS333 - :10:C10	4,84732	Hydrophobic	Pi-Alkyl
		C:PHE390 - :10:C9	5,15013	Hydrophobic	Pi-Alkyl
		C:PHE392 - :10:C10	4,93619	Hydrophobic	Pi-Alkyl
		:10 - C:PRO272	4,22071	Hydrophobic	Pi-Alkyl
Catechin	-353,8	C:THR300:N - :10:O5	3,12731	Hydrogen Bond	Conventional Hydrogen Bond
		C:THR300:N - :10:O6	3,32313	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H12 - C:ASP265:OD2	1,88827	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H13 - C:THR302:OG1	2,16617	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H14 - C:GLU309:OE1	2,07136	Hydrogen Bond	Conventional Hydrogen Bond
		C:THR300:CG2 - :10	3,99466	Hydrophobic	Pi-Sigma
		C:HIS298 - :10	4,75988	Hydrophobic	Pi-Pi T-shaped
		C:ALA271:C,O;PRO272:N - :10	4,24228	Hydrophobic	Amide-Pi Stacked
		C:GLY305:C,O;ASP306:N - :10	3,77349	Hydrophobic	Amide-Pi Stacked
		C:PRO272 - :10	3,67786	Hydrophobic	Alkyl
		:10 - C:PRO272	3,8923	Hydrophobic	Pi-Alkyl
		C:THR300:OG1 - :10:O5	2,10572	Unfavorable	Unfavorable Bump
Caffeic acid	-293,6	C:VAL270:N - :10:O1	2,83302	Hydrogen Bond	Conventional Hydrogen Bond
		C:ALA271:N - :10:O1	3,1224	Hydrogen Bond	Conventional Hydrogen Bond
		C:GLY393:N - :10:O1	3,2249	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H6 - C:ASP268:O	2,29005	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H6 - C:ALA271:O	3,03163	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H7 - C:ASP265:OD2	1,82241	Hydrogen Bond	Conventional Hydrogen Bond

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
		:10:H8 - C:HIS298:NE2	2,55877	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H8 - C:PHE390:O	2,0171	Hydrogen Bond	Conventional Hydrogen Bond
		C:GLY394:CA - :10:O2	2,6047	Hydrogen Bond	Carbon Hydrogen Bond
		C:PHE390:C,O;GLY391:N - :10	5,17973	Hydrophobic	Amide-Pi Stacked
		:10 - C:PRO272	4,15193	Hydrophobic	Pi-Alkyl
Quercetin	-345,8	C:THR300:OG1 - :10:O6	2,19017	Hydrogen Bond	Conventional Hydrogen Bond
		C:THR302:OG1 - :10:O6	3,16922	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H7 - :10:O4	1,79209	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H8 - C:ASN396:OD1	1,76142	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H9 - C:ASP306:OD1	1,76545	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H10 - C:GLU309:OE1	2,04252	Hydrogen Bond	Conventional Hydrogen Bond
		C:PHE392:CA - :10:O3	3,47275	Hydrogen Bond	Carbon Hydrogen Bond
		C:HIS298 - :10	4,8046	Hydrophobic	Pi-Pi T-shaped
		C:ALA271:C,O;PRO272:N - :10	4,94383	Hydrophobic	Amide-Pi Stacked
		C:ALA271:C,O;PRO272:N - :10	4,26904	Hydrophobic	Amide-Pi Stacked
		:10 - C:PRO272	3,48096	Hydrophobic	Pi-Alkyl
		:10 - C:PRO272	3,93918	Hydrophobic	Pi-Alkyl
		C:ASP306:N - :10:H9	2,56398	Unfavorable	Unfavorable Donor-Donor
Ascorbid acid	-261	C:VAL270:N - :10:O6	2,83083	Hydrogen Bond	Conventional Hydrogen Bond
		C:ALA271:N - :10:O6	3,21562	Hydrogen Bond	Conventional Hydrogen Bond
		C:GLY393:N - :10:O6	3,12238	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H5 - C:ASP265:OD2	1,65575	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H7 - C:ASN396:OD1	2,14552	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H1 - C:GLY391:O	2,47777	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H2 - C:ASP265:OD2	2,85462	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H3 - C:ASN396:OD1	2,84355	Hydrogen Bond	Carbon Hydrogen Bond
Thiolactomycin	-282	C:GLY393:N - :10:O2	3,22729	Hydrogen Bond	Conventional Hydrogen Bond
		C:GLY394:N - :10:O2	3,03644	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H16 - C:ASP268:O	2,49665	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H16 - C:ALA271:O	2,59074	Hydrogen Bond	Conventional Hydrogen Bond
		:10:S1 - C:PHE392	4,03854	Other	Pi-Sulfur

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Ligands	Binding Energy (kJ/mol)	Interaction	Distance (A)	Category	Types
		:10:C8 - C:CYS163	3,47805	Hydrophobic	Alkyl
		:10:C10 - C:PRO272	3,51372	Hydrophobic	Alkyl
		:10:C11 - C:PRO272	4,50103	Hydrophobic	Alkyl
		C:HIS298 - :10:C8	5,09725	Hydrophobic	Pi-Alkyl
		C:HIS298 - :10:C9	4,1194	Hydrophobic	Pi-Alkyl
		C:HIS333 - :10:C8	4,33867	Hydrophobic	Pi-Alkyl
		C:PHE392 - :10:C8	4,89409	Hydrophobic	Pi-Alkyl

Conclusion

The bioactive compounds from the aqueous extract of *Piper betle* L. leaves (eugenol, catechin, caffeic acid, quercetin, and ascorbic acid) were potent inhibitors of FabB protein. Inhibition of FabB from *Piper betle* L. compounds led to the discovery of new candidates for *E. coli* antibacterial therapy with a mechanism for inhibiting fatty acid synthesis. Thus, it may be worthwhile for healing chronic wounds.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was funded by the Matching Fund 2022 grant from the Kedaireka, Directorate General of Higher Education (No: 326/PKS/D.D4/ PPK.01.APTV/VII/2022).

References

1 Negut I, Grumezescu V, Grumezescu AM. Treatment strategies for infected wounds. Molecules 2018; 23:1–23.
2 Trinh XT, Long N Van, Van Anh LT, Nga PT, Giang NN, Chien PN. A Comprehensive Review of Natural Compounds for Wound Healing: Targeting Bioactivity Perspective. Int J Mol Sci. 2022; 23.
3 Raziyeva K, Kim Y, Zharkinbekov Z, Kassymbek K, Jimi S, Saparov A. Immunology of acute and chronic wound healing. Biomolecules. 2021; 11:1–25.
4 Garraud O, Hozzein WN, Badr G. Wound healing: Time to look for intelligent, 'natural' immunological approaches? BMC Immunol. 2017; 18:40.
5 Murphy PS and Evans GRD. Advances in Wound Healing: A Review of Current Wound Healing Products. Plast Surg Int. 2012; 2012:1–8.
6 Han G and Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. Adv Ther. 2017; 34:599–610.
7 Tom IM, Ibrahim MM, Umoru AM, Umar JB, Bukar MA, Haruna AB. Infection of Wounds by Potential Bacterial Pathogens and Their Resistogram. OALib. 2019; 06:1–13.
8 Ding X, Tang Q, Xu Z, Xu Y, Zhang H, Zheng D. Challenges and innovations in treating chronic and acute wound infections: from basic science to clinical practice. Burn Trauma 2022; 10.
9 Wilgus TA, Roy S, McDaniel JC. Neutrophils and Wound Repair: Positive Actions and Negative Reactions. Adv Wound Care. 2013; 2:379–388.
10 Liu YF, Ni PW, Huang Y, Xie T. Therapeutic strategies for chronic wound infection. Chinese J Traumatol - English Ed 2022; 25:11–16.
11 Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G. Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-years retrospective study. Antibiotics 2021; 10.
12 de Prost N, Lipman J, Mimoz O. Therapeutic targets in necrotizing soft tissue infections. Intensive Care Med 2017; 43:1717–1719.
13 De Francesco F, Riccio M, Jimi S. Contribution of Topical Agents such as Hyaluronic Acid and Silver Sulfadiazine to Wound Healing and Management of Bacterial Biofilm. Med. 2022; 58.
14 Mihai MM, Preda M, Lungu I, Gestal MC, Popa MI, Holban AM. Nanocoatings for chronic wound repair—modulation of microbial colonization and biofilm formation. Int J Mol Sci. 2018; 19.
15 Perdana I, Suharto S, Ramayanti ED, Yunalia M, Ulfa N. Betel Leaf Extract's Effect (*piper batle* Linn) on The Healing Process of Incision Wounds in Rats (Rattus Norvegicus). J Pendidik Keperawatan Indones 2021; 7:123–128.
16 Lubis RR, Marlisa, Wahyuni DD. Antibacterial activity of betle leaf (*piper betle* l.) extract on inhibiting Staphylococcus aureus in conjunctivitis patient. Am J Clin Exp Immunol 2020; 9:1–5.
17 Ghazali NA, Elmy A, Yuen LC, Sani NZ, Das S, Suhaimi F. *Piper betel* leaves induces wound healing activity via proliferation of fibroblasts and reducing 11β hydroxysteriod dehydrogenase-1 expression in diabetic rat. J Ayurveda Integr Med 2016; 7:198–208.
18 Lien LT, Tho NT, Ha DM, Hang PL, Nghia PT, Thang ND. Influence of phytochemicals in *piper betle* linn leaf extract on wound healing. Burn Trauma. 2015; 3:1–8.
19 Purba RAP and Paengkoum P. Bioanalytical HPLC method of Piper Betle L. for quantifying phenolic compound, water-soluble vitamin, and essential oil in five different solvent extracts. J Appl Pharm Sci. 2019; 9:33–39.

20 Nayaka NMDMW, Sasadara MMV, Sanjaya DA, Yuda PESK, Dewi NLKAA, Cahyaningsih E. Piper betle (L): Recent review of antibacterial and antifungal properties, safety profiles, and commercial applications. Molecules 2021; 26:1–21.
21 Surjowardojo P, Setyowati E, Ambarwati I. Antibacterial Effects of Green Betel (*Piper betle* Linn.) Leaf Against. AGRIVITA J Agric Sci. 2019; 41:569–574.
22 Niquehat Noor and Satapathy KB. Phytochemical Screening and Antibacterial Potential of Wild Leafy Vegetable- *Cayratia auriculata* (Roxb.) Gamble Against Selected Enteric Pathogens. Trop J Nat Prod Res. 2021; 5:621–625.
23 Kassab E, Fuchs M, Haack M, Mehlmer N, Brueck TB. Engineering Escherichia coli FAB system using synthetic plant genes for the production of long chain fatty acids. Microb Cell Fact. 2019; 18:1–10.
24 Janßen HJ and Steinbüchel A. Fatty acid synthesis in *Escherichia coli* and its applications towards the production of fatty acid based biofuels. Biotechnol Biofuels 2014; 7:1–26.
25 Pavoncello V, Barras F, Bouveret E. Degradation of Exogenous Fatty Acids in *Escherichia coli*. Biomolecules 2022; 12.
26 Yao J and Rock CO. Bacterial fatty acid metabolism in modern antibiotic discovery. Biochim Biophys Acta - Mol Cell Biol Lipids. 2017; 1862:1300–1309.
27 Nanson JD, Himiari Z, Swarbrick CMD, Forwood JK. Structural Characterisation of the Beta-Ketoacyl-Acyl Carrier Protein Synthases, FabF and FabH, of *Yersinia pestis*. Sci Rep 2015; 5:1–13.
28 Price AC, Choi KH, Heath RJ, Li Z, White SW, Rock CO. Inhibition of *β*-ketoacyl-acyl carrier protein synthases by thiolactomycin and cerulenin: Structure and mechanism. J Biol Chem 2001; 276:6551–6559.
29 Bitencourt-Ferreira G and de Azevedo WF. Docking Screens for Drug Discovery. Humana Press: Ria Grande do Sul, 2019.
30 Wang Y, Ma S. Recent advances in inhibitors of bacterial fatty acid synthesis type II (FASII) system enzymes as potential antibacterial agents. ChemMedChem. 2013; 8:1589–1608.
31 Hiseni A, Otten LG, Arends IWCE. Identification of catalytically important residues of the carotenoid 1,2-hydratases from Rubrivivax gelatinosus and Thiocapsa roseopersicina. Appl Microbiol Biotechnol. 2016; 100:1275–1284.
32 Sabbagh G and Berakdar N. Docking studies of flavonoid compounds as inhibitors of β-ketoacyl acyl carrier protein synthase I (Kas I) of Escherichia coli. J Mol Graph Model. 2015; 61: 214–223.
33 Silva JC, Silva Pereira RL, Sampaio de Freitas T, Rocha JE, Macedo NS, de Fatima Alves Nonato C. Evaluation of antibacterial and toxicological activities of essential oil of *Ocimum gratissimum* L. and its major constituent eugenol. Food Biosci 2022; 50.
34 Ouertani A, Neifar M, Ouertani R, Masmoudi AS, Cherif A. Effectiveness of enzyme inhibitors in biomedicine and pharmacotherapy. Adv Tissue Eng Regen Med Open Access. 2019; 5:85–90.
35 Primiani C, DRT S, GC K, P P, MA S. Anti-Inflammatory Potentials of *Elaeocarpus sphaericus* Schum Fruit Compounds by Molecular Docking Approach. Trop J Nat Prod Res 2022; 6:1663–1669.
36 Tribudi YA, Agustin AT, Setyaningtyas DE, Gusmalawati D. Bioactive Compound Profile and Biological Modeling Reveals the Potential Role of Purified Methanolic Extract of Sweet Flag (*Acorus calamus* L.) in Inhibiting the Dengue Virus (DENV) NS3 Protease-Helicase. Indones J Chem. 2022; 22:331–341.
37 Agustin AT, Safitri A, Fatchiyah F. An *in Silico* Approach Reveals the Potential Function of Cyanidin-3-o-glucoside of Red Rice in Inhibiting the Advanced Glycation End Products (AGES)-Receptor (RAGE) Signaling Pathway. Acta Inform Medica 2020; 28: 170–179.