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Antibacterial Activity of *Eugenia uniflora* L. Leaf Extract Against *Staphylococcus epidermidis*: An *In Vitro* and *In Silico* Study

Aktivitas Antibakteri Ekstrak Daun *Eugenia uniflora* L. terhadap *Staphylococcus epidermidis*: Studi *In Vitro* dan *In Silico*

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ABSTRACT

Acne is an inflammatory skin disorder commonly associated with *Staphylococcus epidermidis*. This study evaluated the antibacterial activity of 70% ethanolic extract of *Eugenia uniflora* L. leaves against *S. epidermidis*. The leaves were extracted by maceration using 70% ethanol and subjected to phytochemical screening. Antibacterial activity was tested using the disk diffusion method at concentrations of 30%, 40%, and 50%. In addition, molecular docking analysis was performed to investigate the potential interactions of literature-reported phytochemicals with the selected bacterial molecular target. Phytochemical screening revealed the presence of tannins, saponins, and steroids. The extract showed concentration-dependent antibacterial activity with inhibition zones of 10.88, 11.60, and 12.98 mm, respectively. Molecular docking suggested a possible interaction between 2,3-di-*O*-galloyl-D-glucose and tyrosyl-tRNA synthetase (TyrRS), with a binding affinity of −8.8 kcal/mol. These findings suggest that *E. uniflora* leaf extract has potential as a natural antibacterial agent against *S. epidermidis*.

ABSTRAK

Jerawat merupakan penyakit kulit inflamasi yang sering dikaitkan dengan *Staphylococcus epidermidis*. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri ekstrak etanol 70% daun *Eugenia uniflora* L. terhadap *S. epidermidis*. Daun diekstraksi dengan metode maserasi menggunakan etanol 70% dan dilakukan skrining fitokimia. Aktivitas antibakteri diuji menggunakan metode difusi cakram pada konsentrasi 30%, 40%, dan 50%. Selain itu, analisis penambatan molekuler dilakukan untuk mengkaji potensi interaksi senyawa fitokimia yang dilaporkan dalam literatur terhadap target molekuler bakteri terpilih. Hasil skrining fitokimia menunjukkan adanya tanin, saponin, dan steroid. Ekstrak menunjukkan aktivitas antibakteri yang meningkat seiring konsentrasi dengan diameter zona hambat masing-masing 10,88; 11,60; dan 12,98 mm. Hasil molecular docking menunjukkan kemungkinan interaksi antara 2,3-di-*O*-galloyl-D-glucose dengan tyrosyl-tRNA synthetase (TyrRS) dengan afinitas ikatan sebesar −8,8 kcal/mol. Hasil penelitian menunjukkan bahwa ekstrak daun *E. uniflora* berpotensi sebagai antibakteri alami terhadap *S. epidermidis*.

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1. INTRODUCTION

Acne vulgaris, one of the most prevalent inflammatory skin disorders, affects adolescents and young adults worldwide: more than 80% experience it at some point during adolescence or early adulthood (Tan et al., 2021), and among people aged 10–24 years the age-standardised prevalence was 9.8% in 2021, having risen steadily since 1990 (Layton et al., 2020; Zhu et al., 2024). Not only does the condition manifest as comedones, papules, and pustules, but it also significantly impacts patients' quality of life (Layton et al., 2025). The pathogenesis of acne is multifactorial, involving increased sebum production, follicular hyperkeratinization, inflammation, and microbial colonization (Cruz et al., 2023). Current therapeutic approaches for acne vulgaris, including topical antibiotics, retinoids, benzoyl peroxide, and systemic agents, have demonstrated clinical efficacy; however, their long-term use may be limited by adverse effects, skin irritation, antibiotic resistance, and treatment relapse (Aslan Kayıran et al., 2020). The increasing emergence of antimicrobial resistance associated with acne-related microorganisms highlights the need for alternative therapeutic strategies that are effective, safe, and capable of targeting microbial factors involved in acne pathogenesis (Ruchiatan et al., 2023). Among acne-associated microorganisms, *Staphylococcus epidermidis* has gained increasing attention due to its potential role in microbial dysbiosis, inflammation, and biofilm formation, making it a relevant target for the development of novel antibacterial strategies (Dreno et al., 2023; Lynn et al., 2016; Reynolds et al., 2024; Vasam et al., 2023).

S. epidermidis is a normal commensal of the skin microbiota; however, under specific conditions, including microbiota imbalance (dysbiosis) and enhanced biofilm formation, it may act as an opportunistic pathogen that contributes to acne-associated inflammatory processes (Severn & Horswill, 2022). Although it is not considered the sole cause or primary etiological agent of acne, increased abundance and biofilm-forming ability of *S. epidermidis* may promote microbial imbalance, follicular obstruction, and inflammatory responses (Hamann et al., 2025). Therefore, *S. epidermidis* remains an important microorganism for investigating antibacterial strategies aimed at supporting acne management (Fournière et al., 2020).

In recent years, the use of natural products in pharmaceutical and cosmetic industries has increased due to their bioactive properties and relatively lower risk of side effects (Liu, 2022). Although numerous medicinal plants have been extensively investigated for their antibacterial potential, some plant species remain insufficiently explored. One such underexplored plant is *Eugenia uniflora* L. (dewandaru), which contains bioactive compounds, including quercetin, myricetin, gallic acid, ellagic acid, and hydrolyzable tannins such as 2,3-di-*O*-galloyl-D-glucose, which have been reported to exhibit antimicrobial activity (Anwar et al., 2022; Fidelis et al., 2022; Tenório et al., 2024). Notably, tannins have been reported to exert antibacterial effects by disrupting bacterial cell membranes and inhibiting biofilm formation, thereby reducing bacterial survival and virulence (Kaz et al., 2025; Villanueva et al., 2023). Nevertheless, studies evaluating its activity against *S. epidermidis* are still limited.

Therefore, this study aims to evaluate the antibacterial activity of *E. uniflora* leaf extract against *S. epidermidis* as a preliminary step toward the development of natural-based alternatives for acne treatment. In addition, an *in silico* molecular docking study was conducted to explore the possible interaction of a reported tannin constituent with tyrosyl-tRNA synthetase (TyrRS). TyrRS was selected as a molecular target due to its essential role in bacterial protein synthesis through tRNA aminoacylation, where inhibition of this enzyme may interfere with bacterial growth and survival (Pang et al., 2014). To explore the possible molecular basis underlying the antibacterial activity of *E. uniflora*, the hydrolyzable tannin 2,3-di-*O*-galloyl-D-glucose was selected as a representative ligand. This compound was chosen based on its reported presence in *E. uniflora*, its classification as a hydrolyzable tannin, and the presence of multiple galloyl groups that may facilitate interactions with bacterial target proteins (Fortes et al., 2015). The docking analysis was subsequently performed as a preliminary approach to provide insight into the possible molecular interaction between 2,3-di-*O*-galloyl-D-glucose and TyrRS. Based on these approaches, we hypothesized that *E. uniflora* leaf extract exhibits antibacterial activity against *S. epidermidis*, while the docking analysis may provide preliminary molecular insights into the possible contribution of 2,3-di-*O*-galloyl-D-glucose to this activity.

2. METHODS

2.1. Materials and Instruments

The materials used in this study included leaves of *E. uniflora*. The bacterial strain used was *S. epidermidis* FNCC 0048, obtained from Universitas Pendidikan Nasional and maintained under refrigerated storage conditions (4 °C) prior to use. The bacteria were subcultured on nutrient agar and incubated aerobically at 37 °C for 24 h to obtain fresh cultures for inoculum preparation. All bacterial handling procedures were conducted under standard aseptic practices and Biosafety Level 2 (BSL-2) laboratory conditions.

For antibacterial testing, a fresh bacterial culture of 18–24 h was used to prepare the inoculum. Nutrient agar (HiMedia Laboratories, Mumbai, India) was used as the culture medium. Ethanol, analytical grade (Merck, Darmstadt, Germany), dimethyl sulfoxide (DMSO), analytical grade (Rofa Laboratorium Centre, Bandung, Indonesia), sodium chloride (NaCl) 0.9% (Otsuka Pharmaceutical, Tokyo, Japan), doxycycline (PT IFARS Pharmaceutical Laboratories, Surakarta, Indonesia) were used during the experiment. Sterile paper disks (Macherey-Nagel, Düren, Germany) and distilled water (aquadest) were also used.

The instruments used in this study included an incubator (Memmert, Schwabach, Germany), HVE-50 autoclave (HMC Hirayama, Tokyo, Japan), Pioneer series analytical balance

(OHAUS, Parsippany, NJ, USA), vernier caliper for inhibition zone measurement, micropipettes (Nesco, Tokyo, Japan), rotary evaporator model R-300 (BÜCHI Labortechnik, Flawil, Switzerland), AutoDock Tools version 1.5.7 (The Scripps Research Institute, La Jolla, CA, USA), PyMOL version 2.5.5 (Schrödinger LLC, New York, NY, USA), and Discovery Studio Visualizer version 21.1.0.20298 (Dassault Systèmes BIOVIA, San Diego, CA, USA).

2.2. Collection and Preparation of Plant Material

The plant material used in this study was *E. uniflora*, which was collected from Niti Mandala Renon, Denpasar, Indonesia, in October 2025. Plant identification and determination were conducted at the Science Laboratory Unit (Unit Laboratorium MIPA), UIN Siber Syekh Nurjati Cirebon. The determination certificate was issued under number 77/In.08/LB.1.1/PP.009/10/2025.

The collected leaves were washed with running water to remove adhering impurities and then dried under shade at room temperature until a constant weight was obtained. The dried leaves were subsequently pulverized using a mechanical grinder. The powdered simplicia was stored in an airtight container protected from light and moisture until extraction (Shukla et al., 2015; Sung, 2012).

2.3. Extraction Procedure

The extract of *E. uniflora* leaves was obtained by maceration using 70% ethanol as the extraction solvent. The use of 70% ethanol was selected based on its suitability for extracting a broad range of bioactive constituents from *E. uniflora*. Previous studies have also reported the use of 70% ethanol for *E. uniflora* extraction and identified bioactive constituents associated with biological activities (Kozhantayeva et al., 2024; Wenas et al., 2025). A total of 350 g of powdered leaves was macerated in 3500 mL of solvent at a ratio of 1:10 (w/v) and macerated for 3 days (3 × 24 h) with occasional stirring every 24 h. The resulting extract was filtered using Whatman No. 1 filter paper.

The filtrate was concentrated using a rotary evaporator at a water-bath temperature of 60 ˚C under reduced pressure to obtain a viscous extract. The concentrated extract was subsequently weighed, and the extraction yield was calculated based on the initial weight of the powdered simplicia, resulting in a yield of 14.14%. The extract was then stored in an airtight container at 4 ˚C until further use.

2.4. Phytochemical Screening

2.4.1. Flavonoid Test

Flavonoid detection was performed by extracting approximately 0.1 g of extract with 10 mL of hot distilled water, followed by boiling for 5 min and hot filtration. An aliquot of 5 mL filtrate was treated with 0.1 g magnesium powder, 1 mL concentrated hydrochloric acid, and 2 mL amyl alcohol. After thorough mixing and phase separation, the presence of flavonoids was indicated by the appearance of red, yellow, or orange coloration in the amyl alcohol layer (Yanti et al., 2025; Rahimah et al., 2019).

2.4.2. Steroid and Triterpenoid Test

Steroid and triterpenoid screening was conducted by dissolving 100 mg of *E. uniflora* extract in chloroform, followed by filtration. A 5 mL aliquot of the filtrate was evaporated to dryness, and the resulting residue was treated with two drops of acetic anhydride and one drop of concentrated sulfuric acid. The development of blue or green coloration indicated steroids, while purple or orange coloration suggested triterpenoids (Yanti et al., 2025; Rahimah et al., 2019).

2.4.3. Tannin Test

Tannin detection was performed by dissolving approximately 0.5 g of extract in a small volume of distilled water, followed by heating in a water bath. The solution was then treated with a 1:1 (v/v) mixture of 1% gelatin and 10% sodium chloride. The formation of a white precipitate indicated the presence of tannins (Yanti et al., 2025; Rahimah et al., 2019).

2.4.4. Saponin Test

Saponin screening was carried out by dissolving 0.2 g of viscous extract in 4 mL of an appropriate solvent, followed by vertical shaking for 10 s. The formation of stable foam (1–10 cm in height) that persisted for at least 10 min and remained after the addition of 2 N hydrochloric acid indicated the presence of saponins (Yanti et al., 2025; Rahimah et al., 2019)

2.5. Antibacterial Activity Assay Against *S. epidermidis*

The antibacterial activity was evaluated using the disk diffusion (paper disk) method. The 70% ethanol extract of *E. uniflora* leaves was dissolved in 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 30%, 40%, and 50% (w/v), equivalent to 300, 400, and 500 mg/mL, respectively. Sterile paper disks (6 mm diameter) were impregnated with 10 µL of each preparation, corresponding to extract amounts of 3, 4, and 5 mg/disk, respectively, and were then dried under sterile conditions until completely free of solvent before application to the agar surface.

The positive control consisted of doxycycline prepared as a 1% (w/v) solution in sterile distilled water (10 mg/mL), and 10 µL of the solution was applied per disk, corresponding to 100 µg/disk. Doxycycline was used as a reference antibiotic due to its known activity against Gram-positive bacteria, including *S. epidermidis*, and its clinical use as a systemic therapeutic option in inflammatory acne management (Legiawati et al., 2023; Reynolds et al., 2024). The negative control consisted of 10 µL of 10% dimethyl sulfoxide (DMSO) applied to sterile disks.

Bacterial inocula were prepared in sterile 0.9% NaCl and adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The bacterial suspension was uniformly swabbed onto the surface of NA plates with an agar depth of approximately 4 mm under aseptic conditions. The inoculated plates were used within 15 min to ensure standardized bacterial growth conditions.

The impregnated disks were placed onto the inoculated agar surface with adequate spacing to prevent overlapping inhibition

zones. The plates were then incubated aerobically at 37 °C for 24 h. All experiments were performed in triplicate using independent agar plates for each treatment group. Following incubation, inhibition zone diameters were measured in millimeters using a vernier caliper. Each inhibition zone was measured in two perpendicular directions, and the mean value was recorded as the final result.

2.6. *In Silico* Identification of Potential Targets in *S. epidermidis*

Tyrosyl-tRNA synthetase (TyrRS) was selected as a potential antibacterial target because this enzyme plays an essential role in protein biosynthesis by catalyzing the attachment of tyrosine to its cognate tRNA (Pang et al., 2014; Xiao et al., 2015). Inhibition of TyrRS may disrupt bacterial protein synthesis and consequently suppress bacterial growth. As no experimentally determined crystal structure of *S. epidermidis* TyrRS was available in the Protein Data Bank, the TyrRS structure from *Geobacillus stearothermophilus* (PDB ID: 1TYD) was employed as a homologous bacterial model for docking analysis. Chain A of the protein structure was used for molecular docking analysis. The selected crystal structure was determined by X-ray crystallography and reported with a resolution of 2.3 Å (Brick et al., 1989). Although TyrRS from the closer homolog *Staphylococcus aureus* has been reported, the 1TYD structure was selected because it contains a co-crystallized ligand within the active site, enabling redocking validation and providing a clearly defined ligand-binding pocket for docking analysis.

The protein structure was prepared using AutoDockTools 1.5.7 by removing co-crystallized ligands and water molecules, followed by the addition of polar hydrogen atoms and assignment of Kollman charges to obtain a docking-ready structure. The final structure was saved in PDB format.

The native co-crystallized ligand was extracted from the protein structure and prepared using AutoDockTools 1.5.7. The ligand structure was optimized by adding all hydrogen atoms and assigning Gasteiger partial charges. The prepared ligand was saved in PDB format. Docking protocol validation was performed through redocking of the native co-crystallized ligand to evaluate the reliability of the docking parameters. The root-mean-square deviation (RMSD) between the redocked pose and the experimental binding pose was used as the validation criterion, where values $\leq$ 2.0 Å were considered acceptable, indicating good reproducibility of the docking protocol.

Ligand selection was based on previously reported constituents of *E. uniflora* (Fortes et al., 2015). These compounds were considered representative candidate ligands for molecular docking analysis and were not experimentally identified in the extract used in the present study. Among the reported constituents, 2,3-di-*O*-galloyl-D-glucose was selected for docking analysis. Its chemical structure was retrieved from the PubChem database (CID: 21146989) in 3D SDF format. The ligand structure was converted using Open Babel and subsequently prepared and energy-minimized using AutoDockTools 1.5.7 prior to docking analysis, and saved in PDB format.

Molecular docking was performed using AutoDock Vina. A targeted docking approach was employed, with the grid box centered on the binding site occupied by the native co-crystallized ligand (TYR). The grid box was defined with center coordinates of x = −11.7028, y = 14.8293, and z = −4.4420, with dimensions of 10.8453 × 9.8597 × 25.0000 Å. Docking simulations were performed using an exhaustiveness value of 8. The binding affinity values obtained from the docking simulations were used to evaluate ligand–protein interactions. Visualization and interaction analysis were performed using Discovery Studio Visualizer, while superimposition of the native and redocked ligand conformations was performed using PyMOL 2.5.5.

2.7. Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD) from three independent replicates. Statistical analysis was performed using IBM SPSS Statistics version 28. Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was determined at a threshold of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield of *E. uniflora* Leaves

The extraction of *E. uniflora* leaves using 70% ethanol by maceration yielded a viscous extract. A total of 49.49 g viscous extract was obtained, corresponding to an extraction yield of 14.14%. The extraction yield was calculated as the ratio of the weight of the concentrated extract to the initial weight of simplicia, multiplied by 100. This result was comparable with previous reports on *E. uniflora* leaf extraction using ethanol-based solvents. An extraction yield of approximately 18% was reported for ethanolic extraction of *E. uniflora* leaves (Garmus et al., 2019). The slightly lower yield obtained in the present study may be attributed to differences in extraction techniques, solvent composition, extraction duration, and operational conditions. Nevertheless, the obtained yield indicates that the selected extraction method and solvent system were effective in recovering a considerable amount of phytochemical constituents from the plant material. The relatively high yield obtained in this study may be attributed to the use of 70% ethanol, a semi-polar solvent capable of extracting a wide range of bioactive compounds. These compounds are known to have good solubility in hydroethanolic solvents due to their intermediate polarity (Lee et al., 2024).

3.2. Phytochemical Screening Results

Phytochemical screening of *E. uniflora* leaf extract revealed tannins (gelatin test), saponins (foam test) and steroids (Liebermann–Burchard test), whereas flavonoids (Shinoda test) and triterpenoids (Liebermann–Burchard test) were not detected under the conditions used.

The negative results for flavonoids and triterpenoids may be related to variations in extraction conditions and plant material characteristics, which can influence the composition of bioactive compounds (El-Saadony et al., 2025). Nevertheless, the detection of tannins, saponins, and steroids suggests that these phytochemical groups may contribute to the antibacterial potential of the extract. Among these phytochemicals, tannins have been widely reported for their antibacterial properties through protein precipitation, which may interfere with bacterial cell wall integrity and enzyme function (Adamczyk et al., 2017). In addition, tannins may inhibit bacterial adhesion and biofilm formation, which are important factors in the pathogenicity of *S. epidermidis* (Trentin et al., 2013).

Saponins exhibit antibacterial activity primarily by disrupting the permeability of the bacterial cell membrane, leading to leakage of intracellular components and eventual cell lysis (Khan et al., 2018). Meanwhile, steroids have been reported to interact with lipid components of cell membranes, potentially altering membrane stability and function (Atkovska et al., 2018). However, since the present phytochemical screening was qualitative in nature, these compounds cannot be confirmed as the specific active constituents responsible for the antibacterial activity of the extract. These findings therefore provide preliminary information to support further antibacterial and *in silico* analyses.

3.3. Antibacterial Activity Against *S. epidermidis*

The antibacterial activity of *E. uniflora* leaf extract against *S. epidermidis* was evaluated using the disk diffusion method. The results are presented in **Figure 1**.

The extract inhibited *S. epidermidis* at all tested concentrations. One-way ANOVA confirmed a significant effect of treatment on inhibition zone diameter ($F(4, 10) = 1245.47$, $p < 0.001$). The largest zone among the extract treatments was recorded at 50% (12.98 ± 0.55 mm) and the smallest at 30% (10.88 ± 0.33 mm) (**Figure 1**). Tukey HSD showed that the 50% extract differed significantly from both the 40% ($p = 0.007$) and the 30% extract ($p < 0.001$), whereas the 30% and 40% extracts did not differ from each other ($p = 0.193$). Antibacterial activity therefore increased with concentration across the range evaluated, although the two lower concentrations were statistically indistinguishable.

In comparison, the positive control (1% doxycycline) exhibited a significantly larger inhibition zone (20.95 ± 0.46 mm) than all extract-treated groups ($p < 0.05$), indicating stronger antibacterial activity against *S. epidermidis*. These findings also confirmed the validity of the assay and the susceptibility of the tested bacteria. Meanwhile, the negative control (10% DMSO) showed no inhibition zone, indicating that the solvent did not contribute to the observed antibacterial activity.

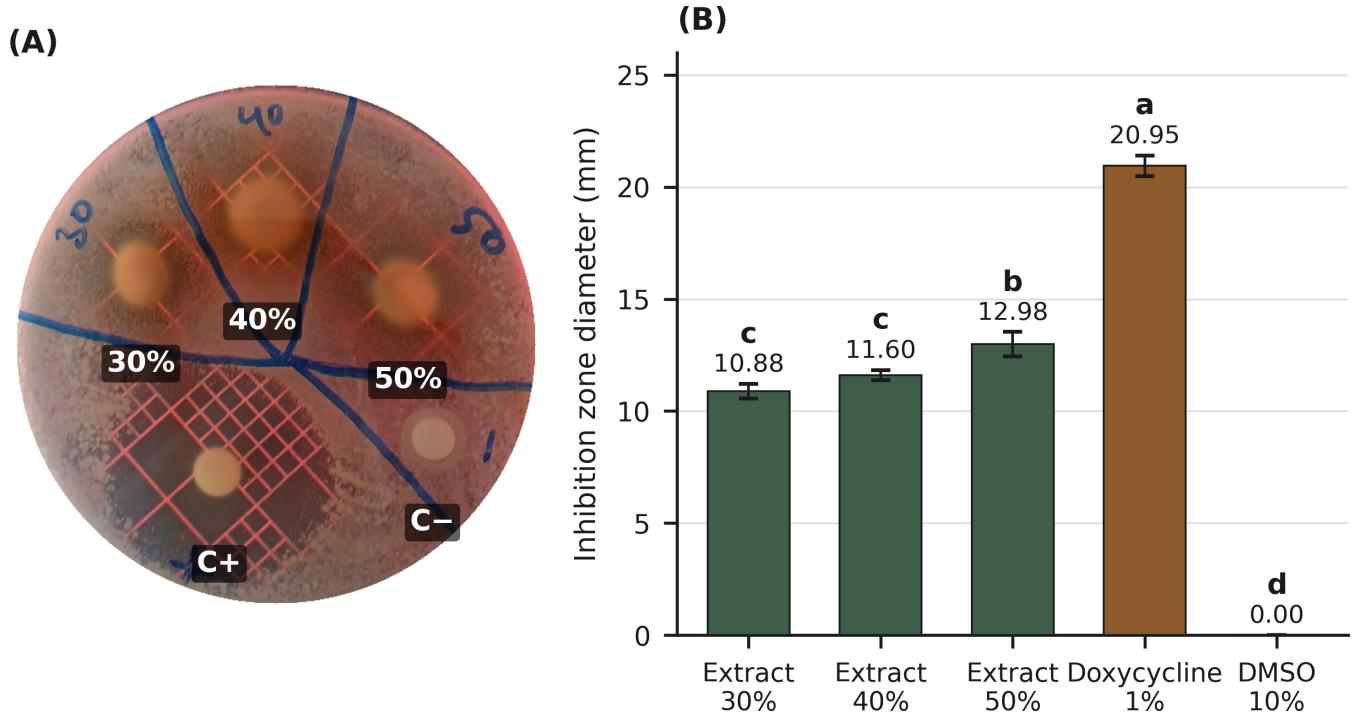

Figure 1. Antibacterial activity of the *E. uniflora* leaf extract against *S. epidermidis* determined by disk diffusion: (A) representative plate showing the extract at 30%, 40% and 50% (w/v), the positive control (C+, 1% doxycycline) and the negative control (C−, 10% DMSO). Only one representative plate from three independent replicates is shown; (B) inhibition zone diameters (mean ± SD, $n = 3$ independent plates). In panel (B), means marked with different lowercase letters differ significantly (one-way ANOVA, $F(4, 10) = 1245.47$, $p < 0.001$; Tukey HSD, $p < 0.05$). Sterile disks (6 mm diameter) were loaded with 10 µL of each preparation. The Petri dish diameter was 90 mm. DMSO = dimethyl sulfoxide

The extract demonstrated measurable antibacterial activity, with inhibition zones ranging from 10.88 to 12.98 mm. The inhibition zones were classified according to the interpretation scale, where inhibition zones of 10–20 mm are considered as strong antibacterial activity (Davis & Stout, 1971). Therefore, all tested extract concentrations (30%, 40%, and 50%) were categorized as

exhibiting strong antibacterial activity, with inhibition zones of 10.88, 11.60, and 12.98 mm, respectively. Although the activity was lower than that of doxycycline, the extract showed a consistent concentration-dependent inhibitory effect. These findings indicate that *E. uniflora* leaf extract possesses potential as a natural antibacterial agent, and further analysis through *in silico* studies was conducted to explore its possible mechanism of action.

3.4. *In Silico* Analysis of Tyrosyl-tRNA Synthetase (TyrRS)

Based on phytochemical screening, tannins were identified as one of the secondary metabolite groups present in the *E. uniflora* leaf extract. Previous studies have reported that this species contains hydrolyzable tannins, including 2,3-di-*O*-galloyl-D-glucose. Among the reported phytochemical constituents of *E. uniflora*, 2,3-di-*O*-galloyl-D-glucose was selected as a representative ligand rather than other phytochemical compounds due to its classification as a hydrolyzable tannin containing multiple galloyl moieties (Fortes et al., 2015). These structural characteristics may provide potential interaction sites with bacterial proteins, supporting its selection as a candidate ligand for docking analysis against TyrRS. Following ligand selection, the three-dimensional structure of 2,3-di-*O*-galloyl-D-glucose was retrieved from the PubChem database and subsequently optimized prior to molecular docking analysis.

The target protein selected for this study was TyrRS, an essential bacterial enzyme involved in protein synthesis through aminoacylation of tRNA. Due to its crucial role in bacterial survival, TyrRS is considered a well-established target for antibacterial drug development, where its inhibition may disrupt protein synthesis and suppress bacterial growth (Pang et al., 2014).

The molecular docking analysis demonstrated that 2,3-di-*O*-galloyl-D-glucose binds favorably to tyrosyl-tRNA synthetase (**Figure 2**). The best docking pose showed a binding affinity of −8.8 kcal/mol, which was more negative than that of the native co-crystallized ligand L-tyrosine (TYR) from TyrRS, which exhibited a binding affinity of −6.5 kcal/mol. These findings suggest that 2,3-di-*O*-galloyl-D-glucose may exhibit strong binding affinity toward the active site of TyrRS.

Interaction analysis revealed that the ligand formed several stabilizing interactions within the TyrRS binding pocket (**Table 1**). Conventional hydrogen bonds were identified with Asp38, Tyr169, Asp176, and Asp194, with bond distances ranging from 1.91 to 2.88 Å. These relatively short distances indicate close atomic contacts that may support the stability of ligand–target interactions. In addition, a carbon hydrogen bond involving His48 and a π-cation interaction involving Lys82 were observed, which may further enhance ligand accommodation and binding stability. The predominance of hydrogen bonding interactions indicates that polar contacts play an important role in maintaining ligand stability within the active site.

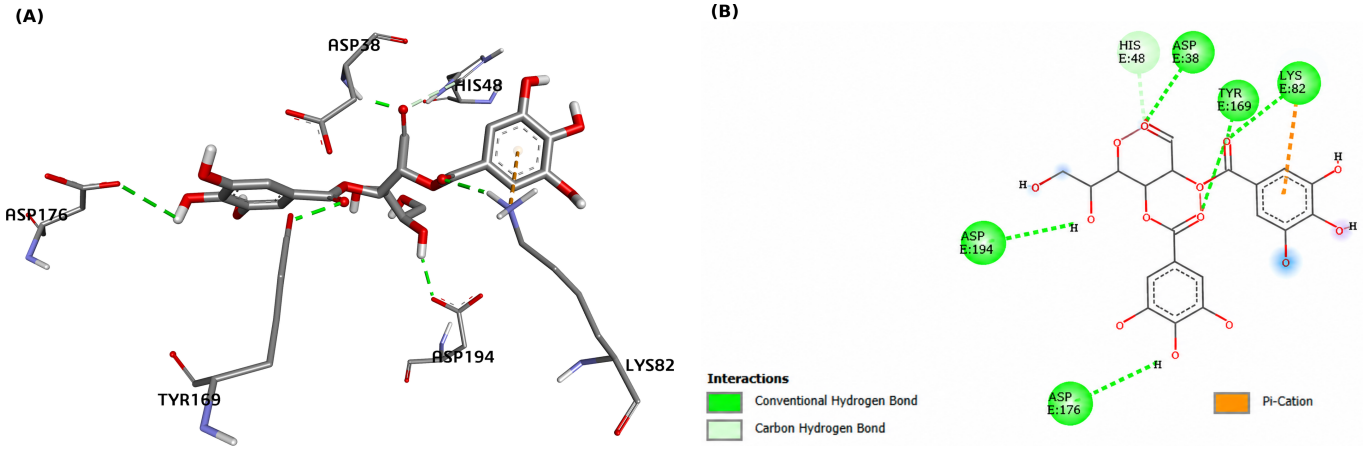

Figure 2. Predicted binding mode of 2,3-di-*O*-galloyl-D-glucose in the TyrRS active site (PDB ID: 1TYD): (A) three-dimensional and (B) two-dimensional representation of the interactions at a binding affinity of −8.8 kcal/mol

Table 1. Interactions of 2,3-di-*O*-galloyl-D-glucose within the TyrRS active site (PDB ID: 1TYD)

No.	Amino acid residue	Interaction type	Distance (Å)
1	Asp38	Conventional hydrogen bond	1.91
2	Tyr169	Conventional hydrogen bond	2.38
3	Asp176	Conventional hydrogen bond	2.88
4	Asp194	Conventional hydrogen bond	2.11
5	His48	Carbon hydrogen bond	3.51
6	Lys82	π-cation interaction	4.73

Note. Distances refer to the best-scoring docking pose. Å, ångström; TyrRS, tyrosyl-tRNA synthetase.

The involvement of key active-site residues suggests that 2,3-di-*O*-galloyl-D-glucose occupies the catalytic region of TyrRS and may potentially influence substrate recognition and aminoacylation activity. Docking protocol validation was performed through redocking of the native co-crystallized ligand, which yielded an RMSD value of 1.456 Å, indicating acceptable reproducibility of the docking method, as shown by the close overlap between the native and redocked ligand conformations (**Figure 3**). However, no known TyrRS inhibitor was included as a comparator ligand, which limits comparative evaluation of binding performance.

The docking results provide structural insight into the interaction between 2,3-di-*O*-galloyl-D-glucose and the TyrRS binding pocket, suggesting possible involvement of residues associated with catalytic function and potential inhibition of bacterial protein synthesis. Nevertheless, these findings remain preliminary, as TyrRS inhibitory activity was not experimentally validated and should therefore be interpreted as hypothesis-generating.

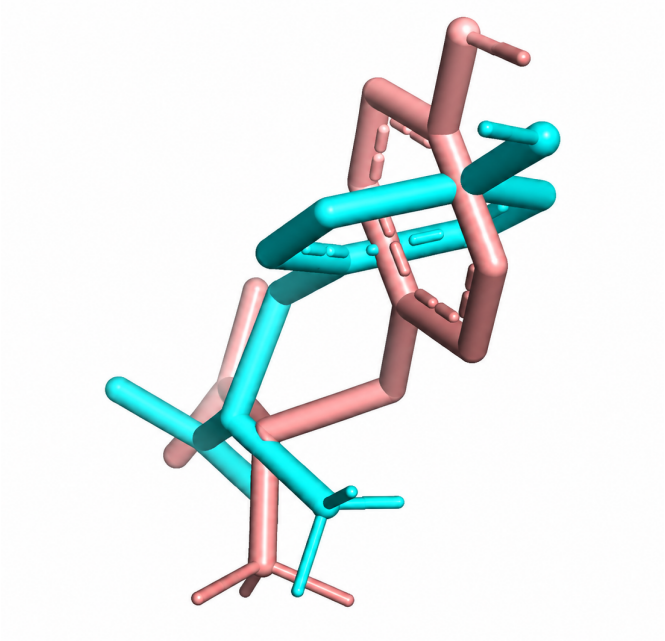

Figure 3. Superimposition of the native (cyan) and redocked (pink) ligand conformations in the TyrRS binding site

3.5. Integration of the *In Vitro* and *In Silico* Findings

The antibacterial activity observed *in vitro* may be associated with the presence of tannins identified through phytochemical screening. Molecular docking analysis showed that the reported tannin compound, 2,3-di-*O*-galloyl-D-glucose, exhibits favorable binding affinity toward TyrRS, suggesting a potential interaction with the enzyme's active site.

However, this finding should be interpreted cautiously, as the presence of 2,3-di-*O*-galloyl-D-glucose in the extract has not been experimentally confirmed, and TyrRS inhibitory activity has not been evaluated. Accordingly, the molecular docking results represent a computational prediction that has not been validated by enzyme inhibition assays or molecular dynamics simulations. Therefore, the docking results provide a preliminary computational hypothesis that a reported tannin constituent may interact with TyrRS, rather than serving as direct evidence of its contribution to the observed antibacterial activity.

In addition to the limitations associated with the *in silico* analysis, several limitations of the present experimental study should also be considered. The antibacterial evaluation was limited to the disk diffusion method without determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill kinetics, or antibiofilm activity. Furthermore, compound profiling and active-compound isolation were not performed, making it difficult to identify the specific constituents responsible for the observed antibacterial effect. Cytotoxicity and safety evaluations were also not conducted. Therefore, further studies are necessary to confirm the antibacterial activity, validate the proposed mechanism, and evaluate the safety and biological relevance of the extract.

4. CONCLUSION

The 70% ethanolic extract of *E. uniflora* leaves showed preliminary *in vitro* antibacterial activity against *S. epidermidis* in a concentration-dependent manner. However, further studies involving minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), biofilm inhibition assays, compound profiling, and in-depth mechanism-of-action investigations are necessary to validate these findings. These investigations may provide deeper insights into how the phytochemical constituents of *E. uniflora* contribute to antibacterial activity through potential interactions with bacterial targets.

AUTHOR CONTRIBUTIONS

Conceptualization, P.R.A. and A.A.S.; methodology, P.R.A. and A.A.S.; software, P.R.A.; validation, P.R.A. and N.L.K.M.Y.; formal analysis, P.R.A. and A.A.S.; investigation, A.A.S. and N.L.K.M.Y.; data curation, N.L.K.M.Y.; writing—original draft preparation, A.A.S.; writing—review and editing, P.R.A. and N.L.K.M.Y.; visualization, N.L.K.M.Y.; supervision, P.R.A.; project administration, P.R.A. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants or animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study, including raw inhibition-zone data, phytochemical screening records, docking input/output, and related images, are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, or readability of the text. After using this tool, the author(s) thoroughly reviewed, edited, and verified the entire content to ensure it accurately represents their own ideas and interpretations. AI was not used to generate data, perform docking, analyze results, or draw conclusions. The authors take full responsibility for the integrity and originality of the published work.

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