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In vitro antibacterial activity of ethanolic *Eugenia uniflora* leaf extract and molecular docking study of myricetin as potential antibacterial compound

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ABSTRACT: *Bacillus cereus* is an opportunistic foodborne pathogen that frequently contaminates food products and causes diarrheal or emetic food poisoning, making it a continuing concern for food safety. The present study evaluated the antibacterial activity of a 70% ethanolic leaf extract of *Eugenia uniflora* against *B. cereus* while exploring its possible mechanism of action through molecular docking. Leaf extraction was carried out by maceration with 70% ethanol, followed by phytochemical screening to determine the major secondary metabolites. Antibacterial activity was assessed using the disk diffusion method with extract concentrations of 30%, 40%, and 50%. Phytochemical analysis identified flavonoids, tannins, saponins, steroids, and triterpenoids in the extract. Growth inhibition of *B. cereus* was observed at all tested concentrations, with inhibition zones ranging from 6.96±0.45 mm to 7.70±0.23 mm, while azithromycin 1% as the positive control showed a higher inhibition zone of 13.70±0.31 mm. Docking analysis further demonstrated that myricetin exhibited favorable binding toward phosphatidylinositol-specific phospholipase C (PI-PLC) from *B. cereus* (PDB ID: 1GYM), with a predicted binding affinity of −7.8 kcal/mol, suggesting a potential molecular basis for the observed antibacterial activity. Overall, these findings highlight the promise of *E. uniflora* leaves as a natural source of antibacterial compounds against *B. cereus*, although additional experimental studies are required to validate the proposed mechanism and confirm the activity of individual phytochemicals through in vitro and in vivo investigations.

KEYWORDS: Antibacterial activity; *Bacillus cereus*; *Eugenia uniflora*; molecular docking.

▪ INTRODUCTION

Foodborne diseases remain a major global public health challenge, with bacterial contamination contributing substantially to the worldwide disease burden [1]. Among the pathogens responsible for foodborne illness, *Bacillus cereus* is recognized as an opportunistic Gram-positive bacterium that can induce food poisoning through the production of enterotoxins and emetic toxins. The public health significance of *B. cereus* is further increased by its widespread occurrence in food products. The bacterium is frequently isolated from rice, dairy products, vegetables, and meat owing to its ability to form heat-resistant endospores [2]. Clinically, infections caused by *B. cereus* are generally associated with two distinct syndromes, namely diarrheal and emetic illness. In addition to toxin production, the pathogenicity and survival of *B. cereus* rely on several cellular mechanisms that facilitate colonization, persistence, and host infection, making this bacterium an important target for the development of novel antibacterial agents [3], [4].

The increasing prevalence of antimicrobial resistance, together with concerns regarding the prolonged use of synthetic antibacterial agents, has intensified the search for alternative antibacterial compounds from natural sources [5]. Medicinal plants have attracted considerable attention because they produce a wide variety of bioactive secondary metabolites with diverse pharmacological properties and generally favorable safety profiles [6],[7]. Although numerous plant species have demonstrated antibacterial activity, many remain insufficiently investigated against foodborne pathogens, particularly *B. cereus*. Therefore, further

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exploration of medicinal plants is necessary to identify promising natural antibacterial candidates with potential applications in food safety and public health.

One plant with considerable therapeutic potential is *Eugenia uniflora*, which is known to contain diverse secondary metabolites [8],[9]. Among these compounds, flavonoids have been extensively reported to exhibit antibacterial activity by disrupting bacterial membrane integrity, interfering with essential metabolic pathways, and inhibiting bacterial enzymes involved in virulence and survival [10]. Myricetin, one of the major flavonoids reported in *E. uniflora*, has attracted increasing interest because of its broad pharmacological activities, including promising antibacterial effects against several bacterial species [11], [12]. Nevertheless, information regarding the antibacterial activity of *E. uniflora* leaf extract against *B. cereus* remains limited, and the possible molecular interactions between its bioactive constituents and bacterial virulence-associated proteins have not been sufficiently explored.

Therefore, this study aimed to assess the antibacterial activity of *E. uniflora* leaf extract against *B. cereus* as a preliminary step toward identifying natural antibacterial candidates for foodborne pathogen control. In addition, an *in silico* molecular docking analysis was conducted to evaluate the binding interaction between myricetin and phosphatidylinositol-specific phospholipase C (PI-PLC), a membrane-associated enzyme of *B. cereus*, to provide an initial mechanistic insight into the observed antibacterial effect.

▪ MATERIALS AND METHODS

Materials and instruments

The materials used in this study included leaves of *E. uniflora* L. and the bacterial strain *Bacillus cereus* FNCC 005, obtained from Universitas Pendidikan Nasional (Denpasar, Indonesia) and maintained under refrigerated storage conditions (4 °C) prior to use. Ethanol was purchased from Merck (Darmstadt, Germany), dimethyl sulfoxide (DMSO) from Rofa Laboratorium Centre (Bandung, Indonesia), nutrient agar from HiMedia Laboratories (Mumbai, India), 0.9% sodium chloride (NaCl) solution from Otsuka Pharmaceutical (Jakarta, Indonesia), sterile paper discs from Macherey-Nagel (Düren, Germany), and distilled water (aquadest) was used throughout the study. Azithromycin standard powder was used as the positive control.

The instruments used in this study included an incubator (Memmert, Schwabach, Germany), HVE-50 autoclave (HMC Hirayama, Japan), Pioneer series analytical balance (OHAUS, USA), vernier caliper for inhibition zone measurement, micropipettes (Nesco, Japan), rotary evaporator model R-300 (BÜCHI Labortechnik, Switzerland), and spectrophotometer (Thermo Scientific™ GENESYS™ 10S UV-Vis Spectrophotometer, USA).

Plant material authentication

Fresh leaves of *E. uniflora* were collected from Niti Mandala Renon, Denpasar, Indonesia. The plant material was identified and authenticated at the Science Laboratory Unit (Unit Laboratorium MIPA), UIN Siber Syekh Nurjati Cirebon, and the determination certificate was issued under registration number 76/In.08/LB.1.1/PP.009/9/2025.

Preparation and extraction of plant material

Freshly collected leaves were thoroughly cleaned and dried at room temperature, then pulverized using a mechanical grinder. Organoleptic evaluation of the simplicia, including appearance, color, odor, and taste, was performed [13]. The moisture content of the simplicia was also determined prior to extraction.

Extraction of the plant material was conducted by maceration using 70% ethanol as the extraction solvent. A total of 500 g of powdered leaves was immersed in 5,000 mL of 70% ethanol and macerated for 3 days with occasional stirring. The solvent was replaced every 24 h to optimize extraction efficiency. The obtained filtrate was then concentrated using a rotary evaporator at 70 °C under reduced pressure to obtain a viscous extract. The concentration process was performed under reduced pressure to facilitate ethanol evaporation at a lower boiling point and minimize potential thermal degradation of bioactive compounds.

Phytochemical screening

Qualitative phytochemical screening was performed to identify secondary metabolites in the extract following previously reported methods [14]. The screening procedures and interpretation criteria are summarized in Table 1.

Table 1. Qualitative phytochemical screening procedures.

Compound group	Method / reagent	Positive result
Alkaloids	Dragendorff's reagent	Orange to reddish-brown precipitate
Flavonoids	Magnesium–HCl	Red, yellow, or orange coloration
Steroids	Liebermann–Burchard reaction	Blue or green coloration
Triterpenoids	Liebermann–Burchard reaction	Purple or orange coloration
Tannins	Gelatin–NaCl test	White precipitate formation
Saponins	Foam test	Stable foam after acid addition

Antibacterial assay against *B. cereus*

The antibacterial activity was evaluated using the disk diffusion (paper disk) method. Bacterial inocula were prepared in sterile 0.9% NaCl solution and adjusted to the turbidity of the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL [14].

A total of 100 µL of the standardized bacterial suspension was evenly spread onto Nutrient Agar (NA) plates. The extract solutions were prepared by dissolving the concentrated *E. uniflora* leaf extract in 10% DMSO to obtain final concentrations of 30%, 40%, and 50% (w/v). Sterile paper discs (6 mm diameter) were then impregnated with 10 µL of each extract solution, corresponding to 3 mg, 4 mg, and 5 mg of extract per disc, respectively. The impregnated discs were dried under sterile conditions until complete solvent evaporation and subsequently placed aseptically onto the inoculated agar surface. Azithromycin (1%, w/v) was used as the positive control, with 10 µL applied per disc, while 10 µL of 10% DMSO was used as the negative control. The plates were incubated at 37 °C for 24 h, and the inhibition zones were measured in millimeters.

Selection of literature-reported compounds

Ligand selection was based on phytochemical compounds previously reported in *E. uniflora* through literature review[15], [16], [17], [18]. Phytochemical screening was performed in the present study to identify the presence of major metabolite groups; however, specific compound identification and quantification were not conducted. Therefore, compounds reported in previous studies were considered as representative candidate ligands for molecular docking analysis rather than confirmed constituents of the tested extract. Among these reported compounds, myricetin was selected as a representative ligand because it has been reported as a flavonoid constituent of *E. uniflora* leaves and has been associated with various biological activities [15], [16], [17], [18]. In addition, myricetin has been reported to exhibit antibacterial properties and contains multiple hydroxyl groups that may contribute to molecular interactions with protein targets, making it a relevant candidate for docking analysis [12], [19]. The chemical structure of myricetin was retrieved from the PubChem database (CID: 5281672). The downloaded structure was converted using Open Babel and subsequently prepared using AutoDockTools 1.5.7. Ligand preparation included the addition of hydrogen atoms, assignment of Gasteiger partial charges, and energy minimization prior to molecular docking analysis.

Target protein selection

PI-PLC was selected as the target protein because this enzyme is recognized as one of the virulence-associated enzymes produced by *B. cereus* [20], [21]. PI-PLC catalyzes the hydrolysis of phosphatidylinositol, a phospholipid component of host cell membranes, into diacylglycerol and inositol phosphate. Through its phospholipase activity, PI-PLC contributes to membrane alteration and facilitates bacterial interaction with host cells, which may support the colonization and pathogenicity of *B. cereus* [22], [23]. Therefore, PI-PLC was selected to evaluate the potential interaction of phytochemical compounds with a bacterial virulence-related target. The crystal structure of PI-PLC from *B. cereus* (PDB ID: 1GYM) was retrieved and employed as the receptor for molecular docking analysis. In addition, the presence of a co-crystallized native ligand within the active site facilitated identification of the ligand-binding pocket and enabled subsequent docking validation through redocking analysis. To maintain structural consistency and simplify the docking procedure, only Chain A of the protein structure was used for subsequent analysis.

Preparation of ligands and protein structures

Protein preparation was performed using AutoDockTools 1.5.7. The co-crystallized ligand and water molecules were removed from the receptor structure, followed by the addition of polar hydrogen atoms and assignment of Kollman charges to generate a docking-ready protein model.

The native ligand was extracted from the receptor structure and prepared separately by adding hydrogen atoms and assigning Gasteiger partial charges to obtain an optimized ligand structure. Docking protocol validation was evaluated based on the root-mean-square deviation (RMSD) between the redocked conformation and the crystallographic binding pose, where an RMSD value of ≤ 2.0 Å was considered acceptable, indicating adequate reproducibility of the docking protocol.

Molecular docking

Molecular docking was performed using AutoDock Vina implemented in PyRx. Docking outcomes were assessed according to binding affinity values (kcal/mol), where lower binding energies indicated more favorable predicted ligand–protein binding. Superimposition of the crystallographic native ligand and the redocked ligand conformation was carried out using PyMOL 2.5.5 to evaluate docking reproducibility.

Statistical analysis

Data were presented as mean ± standard deviation (SD) from three independent replicates. Differences in antibacterial activity among treatment groups were evaluated using one-way analysis of variance (ANOVA) at a 95% confidence level. Multiple comparisons were subsequently performed using Tukey's post hoc test.

▪ RESULTS

Organoleptic properties and extraction yield of *E. uniflora* leaf extract

The organoleptic characteristics of *E. uniflora* leaf simplicia are presented in Table 2. The simplicia was obtained as a dark green powder with a characteristic odor and a bitter taste. The moisture content was 6.30%. Following quality evaluation, the powdered simplicia was extracted by maceration using 70% ethanol. Extraction of 500 g of powdered simplicia yielded 66.4 g of concentrated extract, corresponding to an extraction yield of 13.28%.

Table 2. Organoleptic characteristics and moisture content of *E. uniflora* leaf simplicia.

Sample	Appearance	Odor	Taste	Color
E. uniflora leaf simplicia	Powdered	Characteristic	Bitter	Dark green

Phytochemical profile of *E. uniflora* leaf extract

Qualitative phytochemical screening demonstrated the presence of flavonoids, tannins, saponins, steroids, and triterpenoids in the ethanolic extract of *E. uniflora* leaves, whereas alkaloids were not detected following treatment with Dragendorff's reagent (Table 3).

Table 3. Qualitative phytochemical screening of *E. uniflora* leaf extract.

Compound group	Method / reagent	Result
Alkaloids	Dragendorff's reagent	-
Flavonoids	Magnesium–HCl	+
Steroids	Liebermann–Burchard reaction	+
Triterpenoids	Liebermann–Burchard reaction	+
Tannins	Gelatin–NaCl test	+
Saponins	Foam test	+

(+ = detected; − = not detected)

Antibacterial activity of *E. uniflora* leaf extract against *B. cereus*

The antibacterial efficacy of *E. uniflora* leaf extract against *B. cereus* was assessed using the disk diffusion assay, and the findings are summarized in Table 4.

Table 4. Antibacterial activity of *E. uniflora* leaf extract against *B. Cereus.*

Treatment	Inhibition zone (mm)
30% extract	7.16±0.45[a]
40% extract	7.70±0.23[a]
50% extract	6.96±0.45[a]
Azithromycin 1%	13.70±0.31[b]
DMSO 10%	0.00±0.00[c]

Values are presented as mean ± SD (n = 3). Different superscript letters indicate significant differences at $p < 0.05$.

The extract inhibited the growth of *B. cereus* at all tested concentrations, with inhibition zones ranging from 6.96±0.45 mm to 7.70±0.23 mm (Figure 1). The largest inhibition zone was observed at the 40% extract concentration. Statistical analysis revealed no significant differences among the three extract concentrations. In contrast, azithromycin produced a significantly larger inhibition zone than all extract groups, whereas DMSO showed no inhibitory activity.

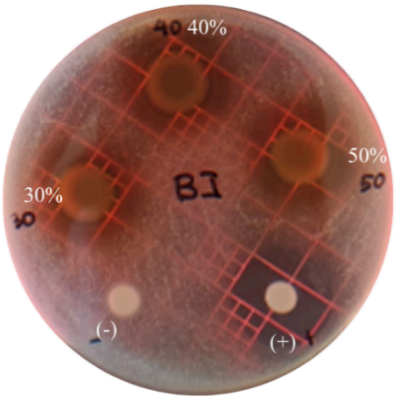

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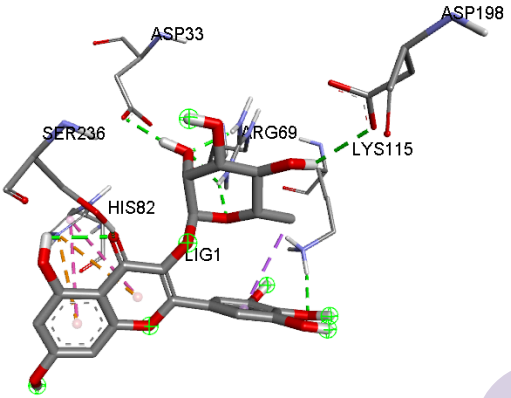
Figure 1. Antibacterial activity of *E. uniflora* leaf extract against *B. cereus* evaluated using the disk diffusion assay.

Docking analysis and validation of myricetin on PI-PLC (*B. cereus*)

Based on phytochemical screening and previous phytochemical reports, myricetin was selected as the representative ligand for molecular docking analysis. The docking analysis revealed that myricetin exhibited a favorable predicted interaction with PI-PLC of *B. cereus*, with a binding affinity of −7.8 kcal/mol. The native ligand used for comparison showed a binding affinity of −6.4 kcal/mol, serving as a reference for evaluating the reliability of the docking protocol rather than as an indicator of relative antibacterial potency (Figure 2). Interaction analysis demonstrated that myricetin formed conventional hydrogen bonds with ARG69, LYS115, SER236, ASP198, and ASP33, along with π-cation and π–π T-shaped interactions involving HIS82, a catalytic residue of PI-PLC that may contribute to the disruption of enzyme function. Docking validation by redocking yielded an RMSD value of 0.899 Å, indicating acceptable reproducibility of the docking protocol (Figure 3).

(a)



(b)

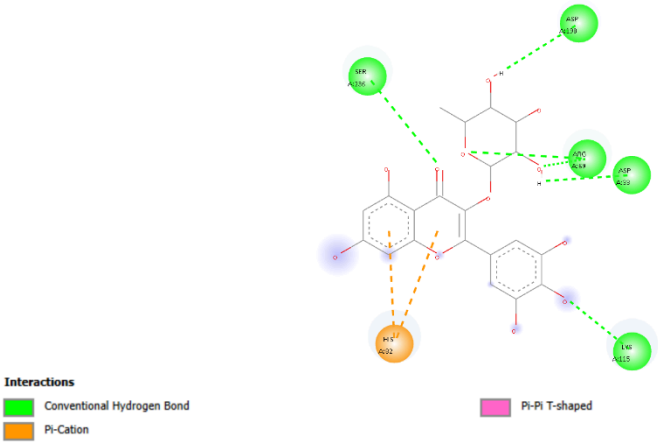


Figure 2. Three-dimensional **(a)** and two-dimensional **(b)** interaction of myricetin with PI-PLC from *B. cereus*.

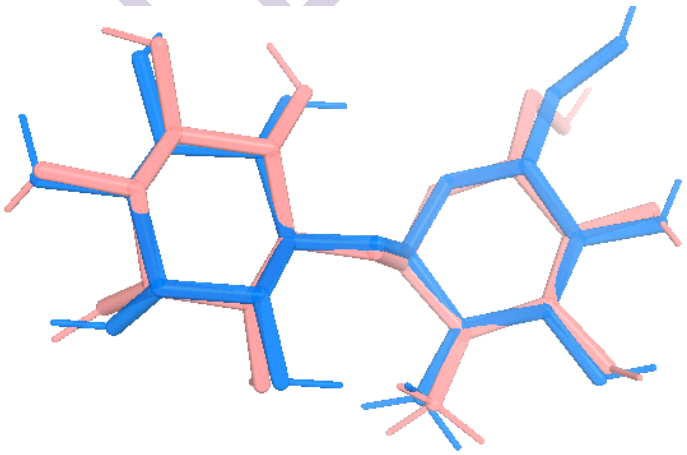


Figure 3. Superimposition of native ligand (blue) and redocked ligand (pink) in the binding pocket of protein 1GYM.

Overall, the docking results provide structural insight into the interaction between myricetin and the PI-PLC binding pocket, suggesting the involvement of key residues associated with ligand stabilization and potential interference with enzyme function. Nevertheless, these findings remain preliminary, as the inhibitory activity of myricetin against PI-PLC has not been experimentally validated and should therefore be interpreted as hypothesis-generating.

DISCUSSION

The quality of herbal raw materials is an important factor influencing the consistency, stability, and biological activity of plant-derived extracts. In the present study, the organoleptic characteristics of *Eugenia uniflora* leaf simplicia, including its dark green color, characteristic odor, and bitter taste, were consistent with those commonly reported for dried medicinal plant materials. These sensory attributes are associated with the presence of chlorophyll and secondary metabolites [17]. In addition, adequate moisture reduction is essential for maintaining the quality and stability of herbal raw materials during storage. Moisture content below the generally accepted limit for herbal simplicia minimizes microbial contamination, inhibits enzymatic degradation, and reduces the risk of hydrolytic reactions that may compromise the stability of bioactive constituents [14]. Therefore, the low moisture content observed in this study indicates that the prepared simplicia was suitable for extraction while preserving the stability of its phytochemical constituents. Furthermore, the extraction yield obtained using 70% ethanol demonstrates the suitability of a hydroethanolic solvent for recovering phytochemical constituents from *E. uniflora* leaves. The relatively high yield may be attributed to the intermediate polarity of 70% ethanol, which enables the extraction of a broad spectrum of phytochemicals compounds commonly present in *E. uniflora* leaves.

The phytochemical screening further supports the antibacterial potential of *E. uniflora* leaf extract through the presence of several bioactive secondary metabolites. Flavonoids have been reported to inhibit bacterial growth by disrupting cell membrane integrity. Similarly, tannins exert antibacterial effects by precipitating microbial proteins and inhibiting extracellular enzymes [24], [25]. Saponins may further contribute to antibacterial activity through their surfactant-like properties, which can increase membrane permeability, while steroids and triterpenoids are also known to interact with lipid components of microbial membranes, potentially leading to structural destabilization [26], [27].

The antibacterial activity demonstrated by *E. uniflora* leaf extract against *B. cereus* indicates that the extract possesses the ability to inhibit bacterial growth. This finding is consistent with previous studies reporting the antimicrobial potential of *E. uniflora* against both Gram-positive and Gram-negative bacteria [28]. However, despite exhibiting antibacterial activity at all tested concentrations, increasing the extract concentration did not proportionally enhance the inhibitory effect. This non-linear response may be explained by methodological and physicochemical limitations inherent to the disk diffusion assay. In this method, the diffusion of bioactive compounds through the agar medium is influenced not only by concentration but also by physicochemical properties such as molecular size, polarity, and viscosity of the extract. At higher concentrations, increased viscosity may reduce diffusion efficiency, thereby affecting the uniform distribution of active compounds within the agar matrix and resulting in a non-linear relationship between extract concentration and inhibition zone diameter [29]. Therefore, the absence of a concentration-dependent response should be interpreted cautiously, as it may reflect the inherent limitations of the disk diffusion method rather than the actual antibacterial potency of the extract.

To further explore the possible molecular basis underlying the antibacterial activity of *E. uniflora* leaf extract, molecular docking analysis was performed using myricetin as a representative flavonoid. Myricetin was selected because it has been previously identified as one of the major constituents of *E. uniflora* and has been reported to possess broad-spectrum antimicrobial activity [9], [30]. The docking results suggested that myricetin has a favorable binding affinity toward PI-PLC, an enzyme involved in phospholipid metabolism and bacterial virulence. Inhibition of this enzyme may interfere with membrane-related processes and contribute to bacterial growth suppression [22], [23].

However, these findings should be interpreted with caution due to several limitations. The presence and concentration of myricetin in the *E. uniflora* leaf extract were not quantitatively confirmed in this study, and its inhibitory activity against PI-PLC has not been experimentally validated. Therefore, the docking results should be considered as a computational prediction that requires further confirmation through enzyme inhibition assays and molecular dynamics simulations [31], [32]. The proposed interaction should consequently be regarded as a preliminary *in silico* hypothesis rather than direct evidence of its contribution to antibacterial activity. In addition, the experimental antibacterial evaluation was limited to the agar diffusion method without determination of minimum inhibitory concentration and minimum bactericidal concentration. Cytotoxicity and safety assessments were also not included. Therefore, further studies involving comprehensive phytochemical characterization, quantitative bioassays, and advanced

computational validation are necessary to confirm the proposed mechanism and evaluate the pharmacological relevance of *E. uniflora* leaf extract.

▪ CONCLUSION

The 70% ethanolic extract of *E. uniflora* leaves demonstrated antibacterial activity against *B. cereus*. Molecular docking analysis suggested that myricetin, a reported flavonoid constituent of *E. uniflora*, may interact favorably with PI-PLC, providing a preliminary hypothesis for its antibacterial mechanism. However, further phytochemical characterization, experimental validation, and additional computational validation are necessary to confirm these findings.

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