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Antibacterial, Antioxidant, and Cytotoxic Properties of *Eclipta prostrata* Extracts

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ABSTRACT

***Eclipta prostrata* is traditionally used in Asian medicine to treat skin diseases. Given the side effects and bacterial resistance of conventional treatments, especially in aging populations, alternative therapies are needed. This study assesses the antibacterial, antioxidant, and cytotoxic properties of *E. prostrata* extracts, focusing on optimizing extraction and fractionation methods for better efficacy. Dried whole plants were extracted with ethanol. Antibacterial activity was tested against *Staphylococcus aureus*, MRSA, *S. epidermidis*, and *Pseudomonas aeruginosa* using broth microdilution to determine minimum inhibitory concentrations (MIC). Antioxidant activity was assessed using a DPPH radical scavenging assay, and cytotoxicity was tested in Vero cells using an MTT assay. The MIC values for crude ethanol extracts ranged from 1.56 to 3.12 mg/ml, and from 0.78 to 1.56 mg/ml for fractionated compounds, indicating increased antibacterial efficacy in fractionated compounds. Antioxidant assays showed an IC_{50} of 0.666 mg/ml for crude extracts and 0.174 mg/ml for fractionated compounds, indicating higher antioxidant activity. Total phenolic content was 23.25±2.03 mg GAE/g for crude extracts and 95.56±1.09 mg GAE/g for fractionated compounds. Cytotoxicity assays showed IC_{50} values of 0.10±0.02 mg/ml for crude extracts and 0.06±0.01 mg/ml for fractionated compounds. This study advances extraction and fractionation methods, yielding extracts with significantly increased antibacterial and antioxidant properties. *E. prostrata* extracts, particularly fractionated compounds, show promising therapeutic potential, though high cytotoxicity in some fractions requires further investigation. These findings support the potential of *E. prostrata* in modern pharmaceutical formulations for treating skin conditions, especially those associated with aging.**

1. Introduction

The skin is the primary protective barrier of the human body and an important component of the innate immune system. However, it is susceptible to various diseases, ranging from mild allergic reactions to serious infections. The involvement of bacteria, particularly gram-positive strains such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, plays a crucial role in many skin conditions, leading to symptoms such as pus discharge and, in severe cases, infections such as methicillin-resistant *Staphylococcus aureus* (MRSA) (Kazmi *et al.* 2021). Conventional treatments such as steroids and antibiotics have several limitations, including the risk of side effects and the development of bacterial resistance, necessitating the investigation of alternative therapies.

The global population is aging rapidly, with estimates suggesting that the number of individuals aged ≥60 years will increase from 900 million in 2015 to 2 billion by 2050 (He *et al.* 2015). Skin conditions, such as xerosis cutis, characterized by dry, itchy, and

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flaky skin, are becoming common, particularly in settings such as long-term care facilities. Its increased prevalence in older populations is linked to changes in skin composition such as reduced lipid content and altered keratinization, leading to compromised skin barriers and increased susceptibility to infections. The synergistic antimicrobial and antioxidant properties of herbal extracts from *Coccinia grandis, Clerodendrum inerme*, and *Acanthus ebracteatus* have been proven to be effective options for the treatment of xerosis cutis treatment. These plants are similar to *Eclipta prostrata* in their traditional medicinal roles. *E. prostrata* is known for its potent antibacterial and antioxidant activity, which is crucial for treating infections and managing oxidative stress in skin conditions (Pratoomsoot *et al.* 2020). Its chemical composition and significant antibacterial properties have been well documented (Batch *et al.* 2022).

E. prostrata, also known as false daisy, has been extensively used in traditional Asian medicine to treat skin conditions. Several studies have highlighted its significant antibacterial effects. Bhandari *et al.* (2021) demonstrated that the methanolic extract of *E. prostrata* exhibits moderate to strong anti-biofilm activity against uropathogenic *Escherichia coli* strains. Kang *et al.* (2022) found that *E. prostrata* improves allergic skin inflammation and restores the skin barrier in a mouse model of atopic dermatitis, suggesting its potential as a therapeutic agent for this condition. Timalsina and Devkota (2021) found that *E. prostrata* shows several biological activities, including antimicrobial, anticancer, hepatoprotective, neuroprotective, and hair growth-promoting effects. Yang *et al.* (2023) found that *E. prostrata* extracts exhibit potent antioxidant activities and antiproliferative effects against cancer cells. In this study, the antibacterial activity of *E. prostrata* extracts was tested against relevant bacterial strains, including *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *S. epidermidis*, and *Pseudomonas aeruginosa*. These strains were selected due to their relevance in skin infections and their known resistance to conventional antibiotics, highlighting the need for alternative treatments. The cytotoxicity of the extracts was assessed using Vero cells, which are widely used to evaluate the safety of compounds due to their well-characterized nature and robustness in culture.

Research into the antibacterial, antioxidant, and cytotoxic properties of *E. prostrata* extracts aims to determine their efficacy and safety, thereby contributing to dermatology and pharmacognosy. The relationship between these activities is crucial because effective antibacterial agents must also be safe for human cells. The antioxidant activity further increases the therapeutic potential by protecting skin cells from oxidative damage. Cytotoxicity testing ensures that the extracts do not cause damage to normal cells, balancing effectiveness against pathogens with safety for therapeutic use. This study bridges the gap between traditional knowledge and contemporary scientific validation and provides insights into the potential applications of *E. prostrata* for the treatments of skin diseases. The findings highlight the value of incorporating traditional herbal medicines into current dermatological approaches.

2. Materials and Methods

2.1. Preparation of Extracts

Dried whole plants of *E. prostrata* were obtained from a certified herbal store in Chiangmai, Thailand. The Faculty of Science, Maejo University, Chiang Mai, Thailand, authenticated the plant (Specimen ID: MJU-62002). Maceration was performed by filling 50 g of dried whole plants of *E. prostrata* in a tightly closed 500 ml Duran glass bottle containing 400 ml ethanol (pro analysis (p.a.) grade), then soaking for three days at room temperature (30±5°C), with intermittent shaking (Simamora *et al.* 2023).

After extraction, the material was filtered twice with Whatman No.1 filter paper to remove particulate matter and contaminants. The filtrates from each round were combined to ensure homogeneity and consistency of the extract. The filtrates from each round were combined to ensure consistency. The extract was concentrated under reduced pressure using a rotary evaporator at 50°C (Wiggens Strike, 300. China), followed by freeze drying (Drawell DW-18ND, Germany) to obtain a crude extract. The crude extract was stored at 4°C in hermetically sealed containers to maintain its bioactive properties.

2.2. Bacterial Cultivation

Bacterial strains, including *Staphylococcus aureus* DMST 8840, Methicillin-resistant *S. aureus* (MRSA)

DMST 20651, *Staphylococcus epidermidis* DMST 3547, S. epidermidis DMST 4343, and *Pseudomonas aeruginosa* DMST 4739, were provided by the Department of Medical Sciences, Ministry of Public Health, Thailand. These strains were cultivated in Mueller-Hinton broth (MHB). Incubation was performed at 37°C for 24 h prior to use, as previously described (Sanguansermsri *et al.* 2024).

2.3. Antibacterial Activity Assay

The antibacterial efficacy of the extracts was evaluated using the broth microdilution method to determine the minimum inhibitory concentrations (MIC) (CLSI 2009). Extracts were prepared at a concentration of 50 mg/ml using 10% DMSO and a 2-fold dilution in a 96-well microtiter plate with MHB as the medium. A 10% DMSO solution served as a negative control, and tetracycline was used as a positive control. The tested bacterial strains were adjusted to a McFarland standard turbidity of 0.5, diluted to 1:100 in MHB, and 50 µL of this suspension was added to each well. After mixing by gentle tapping, the plates were incubated at 37°C for 24 h. The MIC was defined as the lowest extract concentration without visible bacterial growth. To ensure reliability, tests were performed in triplicate using tetracycline at the same concentration as the control antibiotic.

2.4. Chemical Analysis and Bioautography for Antibacterial Activity

Preliminary chemical profiling of the crude extracts was performed using thin-layer chromatography (TLC) (Wongkattiya *et al.* 2022). The fractions with antibacterial activity were identified using bioautography. The components of the extract were separated on silica gel 60 F254 plates using a solvent mixture of chloroform, methanol, and distilled water (8:2:0.2). Post-separation, the plates were overlaid with liquid Mueller Hinton Agar, inoculated with bacterial strains, and incubated at 37°C for 24 h. Antibacterial compounds in the extracts were indicated by the formation of clear zones that became visible after spraying the plates with 1% (w/v) thiazolyl blue tetrazolium bromide in distilled water. The fraction with the highest activity was further isolated and purified using column chromatography.

2.5. Partial Purification of Fractionated Compounds

The fraction exhibiting antibacterial activity was further purified from the crude extract using column chromatography for increased purification (Yu *et al.* 2020). Silica gel 60 F254 was used as a stationary phase. A step gradient elution technique, utilizing the first solvent system of hexane and ethyl acetate (100:0, 95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100), followed by the second solvent system of ethyl acetate and methanol (95:5, 90:10, 80:20, 60:40, 40:60, 20:80, and 0:100), facilitated separation (the volume of each elution was 500 ml). The eluted compounds were collected in fractions based on their coloration and analyzed by TLC. Fractions with similar chromatographic profiles were pooled. The solvents in the pooled fractions were evaporated using a rotary evaporator, and the residue was subsequently freeze-dried.

The fractionated compounds were tested for their antibacterial properties against *S. aureus* 8840 using TLC bioautography. A three-solvent system (hexane, ethyl acetate, and methanol) was used in thin-layer chromatography (TLC) analysis to assess the purity of the compounds. The TLC profiles showed the presence of multiple compounds, suggesting that further purification steps are required to isolate pure compounds. The current purification process aimed to enrich the active fractions significantly, but complete purification was not achieved.

2.6. Antioxidant Activity and Total Phenolic Content

The antioxidant capacity of the extracts was assessed using 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in 95% ethanol for a radical scavenging assay (Takahashi *et al.* 2018). This assay was performed in quintuplicate, employing 0.1 mg/ml ascorbic acid and 0.1 mg/ml tocopherol as positive controls. Briefly, 150 µL of a 0.13 mM DPPH solution in methanol was combined with 100 µL of the extract at various concentrations ranging from 0.02 to 10 mg/ml. The mixture was incubated for 30 min in the dark, and the absorbance was measured at 517 nm. Scavenging activity was quantified to determine IC50 values, which indicate the concentration required

to scavenge 50% of the DPPH radicals. The total phenolic content of the extracts was determined using the Folin-Ciocalteu method (Derakhshan *et al.* 2018). Gallic acid (0.002–1.0 mg/ml) was used as the standard for calibration. Briefly, 100 µL of a 10% (v/v) Folin-Ciocalteu solution in distilled water was combined with 20 µL of the extract at 1 mg/ml, followed by the addition of 80 µL of 7.5% (w/v) sodium carbonate solution. The mixture was incubated for 30 min in the dark, and the absorbance was measured at 765 nm. The phenolic content was quantified and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). All reagents and extracts were analyzed using a spectrophotometer (BMG Labtech, Spectrostar Nano, Germany).

2.7. Cytotoxicity Assessment

The cytotoxicity of the extracts was determined using the MTT assay in Vero cells (Mosmann 1983). Briefly, Vero cells were cultured in Dulbecco's modified Eagle medium (DMEM) (GibcoBRL, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin-streptomycin (DMEM, FBS, and penicillin/streptomycin; GibcoBRL Biochemicals, Grand Island, NY, USA) at 37°C in a humidified atmosphere containing 5% CO_2. The cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 h. The cells were then treated with crude extracts ranging from 0.002–2.485 mg/ml and 0.002–2.270 mg/ml of the fractionated compounds for 24 h. After treatment, the cells were incubated with 100 µL of MTT solution (5 mg/ml) for 4 h at 37°C. The formazan crystals were dissolved in 100 µL of DMSO per well, and the absorbance at 570 nm was measured using a microplate reader. The 50% cytotoxic concentration (CC50) was determined, which causes visible morphological changes in 50% of Vero cells.

3. Results

3.1. TLC Bioautography

The ethanol extraction of *E. prostrata* resulted in a yield of 0.58 grams from the initial 50 grams of dried plant material, corresponding to an extraction efficiency of 1.16%. TLC bioautography analysis of the ethanol extract of *E. prostrata* revealed distinct bands under both visible and ultraviolet light at a wavelength of 254 nm, indicating zones of inhibition against *S. aureus* DMST 8840, as shown by the arrows in Figure 1. These inhibition zones indicated the presence of active antibacterial compounds in the ethanol extract. Further fractionation of the crude ethanol extract yielded 43 difficult fractions, which were grouped into 13 pooled fractions. Notably, as shown in Figure 2, fraction 8 significantly inhibited *S. aureus* DMST 8840, indicating its potent antibacterial activity.

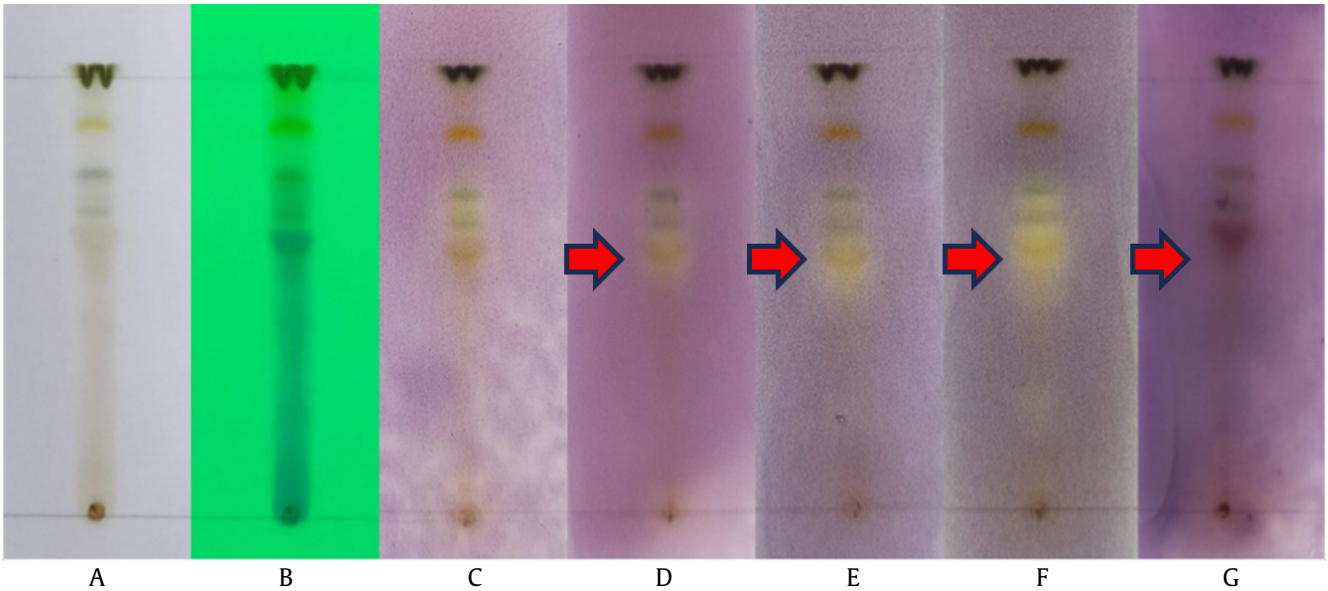

Figure 1. Thin-layer chromatography-bioautography of crude ethanol extract from *Eclipta prostrata*. (A) Visualization under white light and (B) ultraviolet light at 254 nm, (C) showing inhibition zones against *Staphylococcus aureus* DMST 8840, (D) Methicillin-resistant *S. aureus* DMST 20651, (E) *Staphylococcus epidermidis* DMST 3547, (F) *S. epidermidis* DMST 4343, and (G) *Pseudomonas aeruginosa* DMST 4739

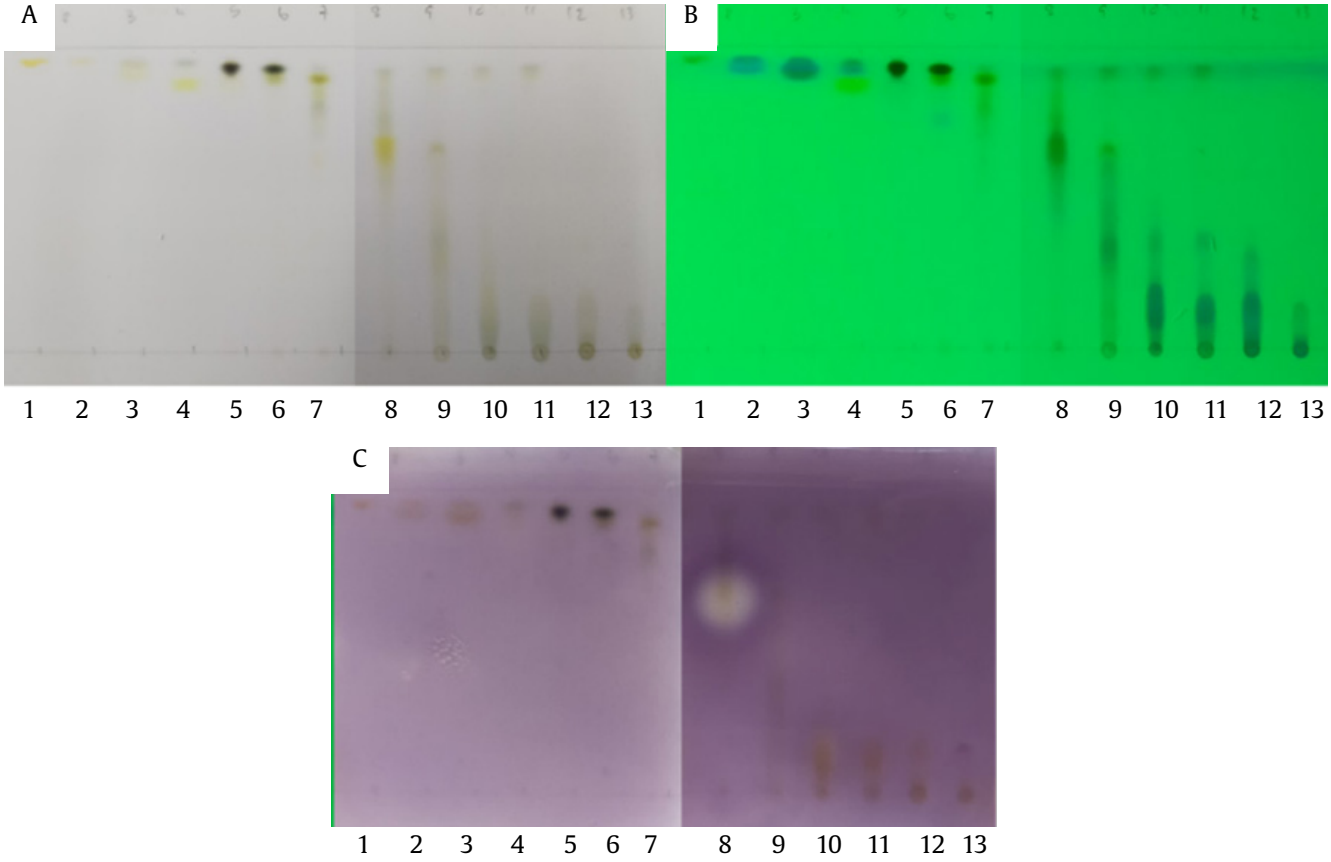

Figure 2. Thin-layer chromatography-bioautography of fractionated compounds (1–13) from column chromatography. (A) Visualization includes thin-layer chromatography under visible light, (B) thin-layer chromatography under ultraviolet light at 254 nm, and (C) thin-layer chromatography-bioautography showing inhibition against *Staphylococcus aureus* DMST 8840

3.2. Antibacterial Activity

The antibacterial properties of *E. prostrata* ethanolic extracts and fractionated compounds (fraction 8) were evaluated using the broth microdilution method to determine the MIC, as shown in Table 1.

The crude ethanolic extract exhibited moderate antibacterial activity, with MIC values ranging from 1.56 to 3.12 mg/ml. According to Andrews (2001), MIC values between 1 and 4 mg/ml may indicate moderate antibacterial activity. Notably, the fractionated compounds derived from the ethanol extract showed considerably high antibacterial activity. These compounds had MIC values ranging between 0.78 and 1.56 mg/ml, effectively doubling their inhibitory effect on bacterial growth compared to that of the crude extract. This increased activity suggests that fractionation concentrated the active antibacterial components by removing fewer active constituents. Compared to tetracycline (MIC 0.01-0.12 mg/ml), fraction 8 was less potent but still significant. This shows its potential as an alternative treatment, particularly against antibiotic-resistant strains. Further purification and identification of active compounds could increase their effectiveness to levels comparable to established antibiotics like tetracycline.

3.3. Antioxidant Activity and Total Phenolic Content Analysis

The antioxidant activity and total phenolic content of *E. prostrata* were analyzed using the DPPH radical scavenging assay and the Folin-Ciocalteu method (Table 2). Both the crude ethanol extract and the fraction 8 exhibited considerable antioxidant properties. The IC_{50} values were 0.666 mg/ml for the crude ethanol extract and 0.174 mg/ml for the fraction 8. The phenolic content was measured as 23.25±2.03 mg GAE/g extract in the crude extract and 95.56±1.09 mg GAE/g extract in the fraction 8. The results indicate that fractionation significantly increases the antioxidant activity and

Table 1. Minimum Inhibitory Concentrations (MIC) of *Eclipta prostrata* extracts and fractionated compounds against bacterial strains

Bacteria	MIC of the extracts and antibiotic (mg/ml)		
	Crude extract	Fraction 8	Tetracycline
S. aureus DMST 8840	3.12	0.78	0.01
MRSA DMST 20651	3.12	1.56	0.03
S. epidermidis DMST 3547	3.12	1.56	0.02
S. epidermidis DMST 4343	1.56	1.56	0.12
P. aeruginosa DMST 4739	1.56	0.78	0.06

Table 2. Antioxidant efficacy and phenolic content of *E. prostrata* extracts

Samples	DPPH assay IC_{50} (mg/ml)	Total phenolic (mg GAE/g extract)
Crude extract	0.666	23.25±2.03
Fraction 8	0.174	95.56±1.09
Ascorbic acid	0.013	ND
Tocopherol	0.031	ND

ND: not determined

phenolic content of *E. prostrata* extracts. The lower IC_{50} value for fraction 8 (0.174 mg/ml) compared to the crude extract (0.666 mg/ml) suggests a higher concentration of active antioxidant compounds in the fractions. This is further supported by the increased total phenolic content in the fraction 8 (95.56±1.09 mg GAE/g extract) compared to the crude extract (23.25±2.03 mg GAE/g extract). Phenolic compounds are known for their strong antioxidant properties, and their higher concentration in the fractionated compounds likely contributes to the observed increased antioxidant activity. The significant increase in antioxidant activity and phenolic content after fractionation suggests that the process effectively concentrates these bioactive compounds, enhancing the overall efficacy of the extract. Compared to the crude extract, the fraction 8 not only showed improved antioxidant properties but also had a higher phenolic content, indicating a more potent extract. This improvement in activity and content may be crucial for the development of more effective therapeutic agents derived from *E. prostrata*, particularly in diseases in which oxidative stress plays a key role.

3.4. Cytotoxicity Assay

The cytotoxic effects of *E. prostrata* crude ethanol extract and the fraction 8 on Vero cells were analyzed using the MTT assay (Table 3). The IC_{50} values, indicating the concentration at which 50% of cells remain viable, were 0.10±0.02 mg/ml for the crude extract and 0.06±0.01 mg/ml for the fraction 8, suggesting that fractionation may enhance the cytotoxicity of the compounds in *E. prostrata*. IC_{50} values are classified as follows: <0.01 mg/ml (very strong), 0.01-0.1 mg/ml (strong), 0.1-0.5 mg/ml (moderate), and >0.5 mg/ml (weak). Thus, the crude extract shows strong cytotoxicity, while the fraction 8 exhibit very strong cytotoxicity. This increased cytotoxicity suggests that active components were concentrated by fractionation, which could benefit therapeutic development but requires careful dosage and safety assessments.

Table 3. Cytotoxicity of *E. prostrata* extracts

Samples	Cytotoxicity IC_{50} (mg/ml)
Crude extract	0.10±0.02
Fraction 8	0.06±0.01

4. Discussion

Our study on *E. prostrata* demonstrated significant findings regarding its antibacterial, antioxidant, and cytotoxic properties. The crude ethanolic extract showed moderate antibacterial activity, with MIC values ranging from 1.56 to 3.12 mg/ml, while the fractionated compounds showed higher activity, with MIC values between 0.78 and 1.56 mg/ml. This suggests that fractionation concentrates the active antibacterial components, thereby increasing the effectiveness of the extract. This improvement is crucial for the development of more effective antibacterial agents, especially given.

The antioxidant activity analysis revealed that fraction 8 had lower IC_{50} values (0.174 mg/ml) compared to the crude extract (0.666 mg/ml), indicating stronger antioxidant properties. This improvement correlates with the higher total phenolic content in fraction 8 (95.56±1.09 mg GAE/g extract) compared to the crude extract (23.25±2.03 mg GAE/g extract). Phenolic compounds are known for their antioxidant properties, and their higher concentration in the fractionated compounds likely contributes to the observed increased antioxidant activity.

In terms of cytotoxicity, the fractionated compounds demonstrated very strong cytotoxicity (IC_{50} 0.06±0.01 mg/ml), while the crude extract showed strong cytotoxicity (IC_{50} 0.10±0.02 mg/ml) according to Bakar *et al.* (2019). This suggests that

fractionation not only increases the effectiveness of antibacterial and antioxidant components but also concentrates cytotoxic compounds, which requires careful consideration for therapeutic use.

The phytochemical richness of *E. prostrata* is underscored by the presence of compounds such as coumestan derivatives, triterpenoids, and steroidal saponins, which contribute to its pharmacological efficacy (Phan *et al.* 2023). Flavonoid-rich extracts have been shown to play significant roles in various therapeutic applications (Kim *et al.* 2015). Bioactive terpenoids, including triterpenoids, diterpenoids, and sesquiterpenoids, exhibit α-glucosidase inhibitory, cytotoxic, and antibacterial activities (Yu *et al.* 2020). Specific bioactive triterpenoids such as 3-acetylaleuritolic acid and stigmasterol were identified in *E. prostrata*, confirming the diversity and possible pharmacological activity of the compounds in our extracts (Cherdtrakulkiat *et al.* 2015).

Comparing these findings with the literature, our results are consistent with previous studies, indicating that phenolic and flavonoid-rich extracts possess significant antioxidant and antibacterial activities. For example, Derakhshan *et al.* (2018) reported that higher phenolic content directly correlates with stronger antioxidant activity. Similarly, the presence of bioactive terpenoids and flavonoids, as identified in other studies (Yu *et al.* 2020; Kim *et al.* 2015), supports the pharmacological effects observed in our study.

In conclusion, the optimization of extraction and fractionation methods in our study significantly improved the antibacterial, antioxidant, and cytotoxic properties of *E. prostrata*. These results highlight the potential therapeutic applications of fractionated extracts, although their high cytotoxicity requires careful dosing and safety assessments. Future research should focus on further refining these methods to maximize therapeutic efficacy while minimizing potential side effects, paving the way for the incorporation of *E. prostrata* into modern pharmaceutical formulations.

Conflict of Interest

The authors declare no conflicts of interest.

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