

Antibacterial Activity of Ethanolic Root Extract of *Fibraurea tinctoria* Lour. Against Diabetic Wound Pathogenic Bacteria

Ayuni Syahira¹, I Gede Andika Sukarya^{2*}, Tiara Dini Harlita³, Askur⁴
Poltekkes Kemenkes Kaltim

Corresponding Author: I Gede Andika Sukarya dkha87@gmail.com

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ABSTRACT

This study examined the in vitro antibacterial activity of ethanolic root extract from *Fibraurea tinctoria* Lour. against two clinically relevant diabetic wound pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Using a Completely Randomized Design, the extract was tested at concentrations of 2%, 4%, 6%, 8%, and 10% via the disk diffusion method, and the resulting data were analyzed with one-way ANOVA followed by Duncan's Multiple Range Test. A concentration-dependent inhibition of *S. aureus* growth was observed ($p < 0.001$), reaching its peak at 10% (13.67 ± 0.82 mm), while *P. aeruginosa* showed no measurable susceptibility at any concentration tested. Taken together, these results point to a selective antibacterial effect of *F. tinctoria* root extract against Gram-positive bacteria, positioning it as a promising candidate for further exploration as a natural antibacterial agent in the management of diabetic wound infections.

INTRODUCTION

Diabetes mellitus remains one of the most widespread metabolic disorders globally, with its prevalence continuing to climb year after year. The chronic hyperglycemia that defines the disease drives a range of debilitating complications, among which diabetic foot ulcers stand out as particularly severe not simply because of how common they are, but because of what they tend to cost patients: extended hospital stays, long-term disability, and in many cases, lower-limb amputation. Poor peripheral circulation, neuropathy, and a weakened immune response together create ideal conditions for bacterial colonization, which in turn slows wound healing and raises the risk of infection becoming severe (Khan et al., 2020; Ong et al., 2023).

Bacterial infection, in fact, is widely regarded as one of the central drivers behind why diabetic wounds fail to heal properly. *Staphylococcus aureus* is consistently among the most frequently isolated Gram-positive organisms from diabetic foot ulcers, and its capacity to produce a range of virulence factors allows it to damage tissue and establish biofilms (Mamdoh et al., 2023). *Pseudomonas aeruginosa*, for its part, is an opportunistic Gram-negative pathogen notorious for its intrinsic resistance to antimicrobial treatment; combined with its biofilm-forming ability and various adaptive survival mechanisms, it tends to produce infections that are especially hard to clear (Tuon et al., 2022). The rising of antimicrobial-resistant bacteria has calls for the exploration of new antibacterial agents, especially from natural products (Elmaidomy et al., 2022).

Medicinal plants are of great interest as sources of antibacterial compounds due to the wide variety of bioactive secondary metabolite. The following material of this paper is an indigenous medicinal plant well distributed to Kalimantan, Indonesia: *Fibraurea tinctoria* Lour., called akar kuning in the local community. Of course, this plant has a long traditional use for infection and inflammation. There phytochemical studies have revealed that *F. tinctoria* contains alkaloids, flavonoids, tannins saponins, triterpenoids and phenolic compound especially berberine in its tissue with antibacterial activity by breaking down or osmotic lysis of the cell wall of bacteria, inhibition nucleic acid synthesis direct destruction on a bacterium membrane so it does not function to maintain normal cells within bacterial species even interference with metabolic catabolic processes (Fathiah et al., 2024; Zalizar et al., 2019).

The antibiotic activity of *F. tinctoria* against a spectrum of pathogenic bacteria is indicated by its positive results in several studies. Nevertheless, these studies mainly assessed stem extracts or infusions, addressed a single bacterial species and used disproportionately high extract concentrations in comparison to the present study. Therefore, there is little evidence of the antimicrobial activity of ethanolic root extract in relation to both *S. aureus* and *P. aeruginosa*, which are the most dominant bacteria found in diabetes-associated wounds. In addition, comparative data on the susceptibility of Gram-positive and Gram-negative diabetic wound pathogens to root-derived extracts is still missing. Filling this gap would be essential to further confirm whether the antibacterial activity of *F. tinctoria* root extract can differ according to characteristics of tested

bacteria; and provide scientific evidence on its potential as a natural antibacterial agent.

The objective of this study was to in vitro evaluate the antibacterial activity of Ethanolic root extract of *F. tinctoria* against *S. aureus* and *P. aeruginosa* with disk diffusion method and to find out which concentration of the extract has the antibacterial activity for both bacteria.

LITERATURE REVIEW

***Antibacterial Activity of Fibraurea tinctoria* Lour.**

Fibraurea tinctoria Lour. is a medicinal plant native to Kalimantan that has been used in traditional medicine for generations to treat infections and inflammatory conditions (Purwaningsih et al., 2023). Its phytochemical profile is notably rich, comprising alkaloids, flavonoids, tannins, saponins, triterpenoids, and polyphenols. Among these, berberine stands out as the principal alkaloid and is widely regarded as the main compound driving the plant's antibacterial properties. Through several approaches, including disruption of the cell wall and increasing cell permeability, inhibition of nucleic acid synthesis and blocking key enzyme or metabolism processes, these compounds inhibit bacterial growth (Zalizar et al., 2019).

Studies conducted in the past have substantiated the antibacterial activity of *F. tinctoria* against some pathogenic microorganisms. Santoso et al. (2020) likewise mention that ethanolic stem extract inhibited the growth of *Staphylococcus aureus*. Kholifa et al. (2021) observed that stem infusion showed antibacterial activity against *Pseudomonas aeruginosa* only at relatively higher concentrations. This result suggests that the antibacterial activity of *F. tinctoria* may be affected by plant part, extraction method, solvent type and concentration level and also by microorganism species assessed.

Bacterial Pathogens in Diabetic Wounds

Chronic hyperglycemia leaves diabetic foot ulcers particularly vulnerable to bacterial infection, largely because it dampens host immune responses and slows the wound-healing process (Darwitz et al., 2024). Among the bacteria most frequently implicated in these infections, *S. aureus* stands out as one of the dominant Gram-positive pathogens; it drives tissue destruction through a combination of toxin production, extracellular enzyme activity, and biofilm formation a trio that helps the bacterium persist and makes treatment considerably harder (Shettigar & Murali, 2020). *P. aeruginosa*, by contrast, is a Gram-negative opportunist known for its formidable intrinsic and acquired resistance to antimicrobial agents, stemming from low outer membrane permeability, multidrug efflux pumps, enzyme production, and extensive biofilm formation (Krūmiņa et al., 2026; Qin et al., 2022). Together, these traits make *P. aeruginosa* one of the most stubborn pathogens to eradicate from chronic diabetic wounds.

Research Gap

Previous research on the antibacterial activity of *F. tinctoria* has been widely reported but many essential aspects still unknown. Previous research mainly

focuses on stem extracts or aqueous infusions, only explored one single bacterial species and used high concentrations of extracts. In addition, there is little evidence that describes the antibacterial property of ethanolic root extract on *S. aureus* and *P. aeruginosa* which are two important infectious pathogens clinically isolated from diabetic wound infections. More research is therefore needed to evaluate the antibacterial potential of root-derived extracts at lower concentrations and against standard Gram-positive and Gram-negative bacteria. The present study addresses this gap in three ways. First, while earlier work by Santoso et al. (2020) and Kholifa et al. (2021) focused on stem extracts or aqueous infusions, this is, to our knowledge, the first study to characterize the antibacterial activity of the ethanolic *root* extract a plant part likely to carry a different phytochemical profile and berberine concentration than the stem. Second, rather than relying on the disproportionately high concentrations used in previous studies, this study works within a considerably lower range (2–10%), offering a dose–response profile that is more realistically translatable to clinical use. Third, whereas earlier investigations typically tested a single bacterial species in isolation, this study directly compares susceptibility between *S. aureus* and *P. aeruginosa* the two pathogens most clinically relevant to diabetic wound infections providing comparative evidence grounded specifically in that clinical context rather than in bacterial susceptibility in general.

H1: Ethanol extract of *Fibraurea tinctoria* Lour. Root exhibits antibacterial activity against *S. aureus*

H2: Ethanol extract of *Fibraurea tinctoria* Lour. root exhibits antibacterial activity against *P. aeruginosa*.

Conceptual Framework

The conceptual framework guiding this study rests on the assumption that the bioactive phytochemicals in *F. tinctoria* root extract — berberine, flavonoids, tannins, saponins, triterpenoids, and phenolic compounds — exert their antibacterial effects by disrupting bacterial cell structures and interfering with metabolic processes. As extract concentration increases, this effect was expected to become more pronounced, producing larger inhibition zones against *Staphylococcus aureus* and *Pseudomonas aeruginosa* as measured through the disk diffusion method.

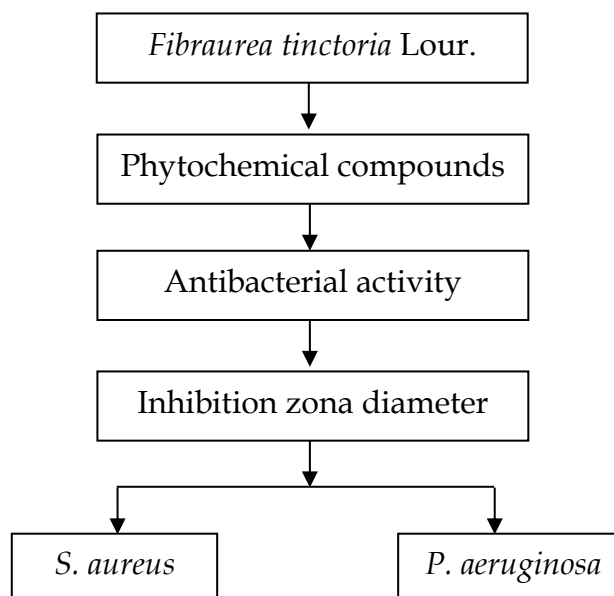


Figure 1. Conceptual Framework

METHODOLOGY

This study employed an in vitro laboratory experimental design using a Completely Randomized Design (CRD). The experiment was conducted from October to November 2025 at the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Kalimantan Timur, Indonesia. Botanical identification of *Fibraurea tinctoria* Lour. was performed at the Laboratory of Plant Anatomy and Systematics, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman.

Preparation of Plant Extract

Roots of *F. tinctoria* were collected from Central Kalimantan, Indonesia. The plant material was initially washed with distilled water, then air-dried and ground to powder. We macerated the powdered roots (1:10 w/v) with 96% ethanol as solvent. The filtrate was concentrated under reduced pressure using a rotary evaporator and diluted with sterile distilled water to achieve five different concentrations (2%, 4%, 6%, 8% and 10%).

Antibacterial Assay

The antibacterial activity of the extract was tested against *Staphylococcus aureus* ATCC 10145 and *Pseudomonas aeruginosa* ATCC 25923 by Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA). Prior to inoculation, bacterial suspensions were standardized to 0.5 McFarland. Sterile paper discs were infused with each concentration of extracts and placed on the inoculated agar plates. For positive control, Ciprofloxacin (5 µg) C was used, while sterile distilled water was taken as negative control. After incubation at 37°C for a duration of 24 hours, antibacterial activity was evaluated by measuring the diameter (mm) of the inhibition zone around each disc.

Statistical Analysis

Inhibition zone diameters were reported as mean $\pm$ standard deviation (SD). The data were first examined assuming normality and homogeneity of variance. Specifically, normality of the inhibition zone data was confirmed by the Shapiro-Wilk test (*S. aureus*: $W = 0.779$, $df = 5$, $p = 0.054$). Levene's test based on the mean indicated a significant difference in variances ($F(4,10) = 4.348$, $p = 0.027$); however, this result is likely attributable to the zero variance in the 2% concentration group, which can artificially inflate the mean-based statistic. The more robust median-based (Brown-Forsythe) modification of Levene's test, which is recommended in recent simulation studies for better Type I error control (Çelikten Demirel et al., 2025), showed no significant departure from homogeneity ($F(4,10) = 0.433$, $p = 0.782$), supporting the assumption of equal variances. Given the balanced design ($n = 3$ per group) and the demonstrated robustness of ANOVA to moderate heterogeneity of variance under balanced conditions (Blanca et al., 2018), One-Way ANOVA followed by Duncan's Multiple Range Test (DMRT) was considered statistically appropriate for this dataset. Following a significant One-Way ANOVA results, Duncan's Multiple Range Test (DMRT) was performed for pairwise comparisons. Differences among extract concentrations were assessed by one-way analysis of variance (One-Way ANOVA) and an arbitrary significance level at $P < 0.05$. For post hoc multiple comparisons, Duncan's Multiple Range Test (DMRT) was performed at an alpha level of 0.05. $P < 0.05$ was regarded as statistically significant. Statistical analysis, however, was not conducted for *P. aeruginosa* as no inhibition zone was observed at the concentrations tested.

RESEARCH RESULT

Inhibitory Zone Diameter of *Fibraurea tinctoria* Lour. Extract

The antibacterial activity of *Fibraurea tinctoria* Lour. root ethanol extract against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) was evaluated using the disk diffusion method at concentrations of 2%, 4%, 6%, 8%, and 10%. Ciprofloxacin (5 μ g) and sterile distilled water were used as the positive and negative controls, respectively. The antibacterial activity was determined by measuring the diameter of the inhibition zone formed around each paper disc after 24 hours of incubation.

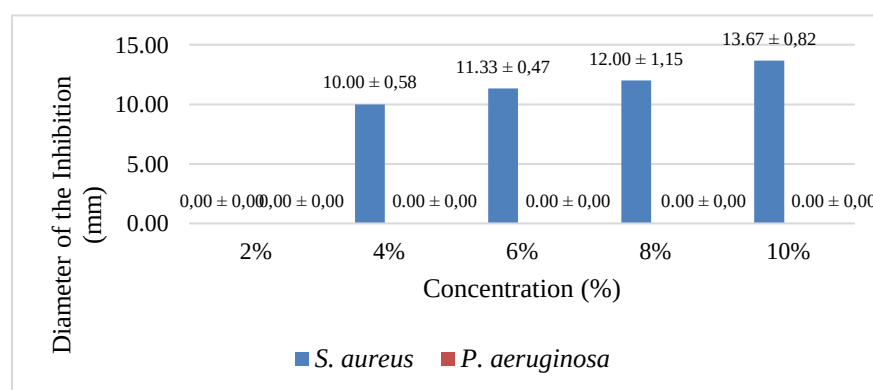


Figure 2. Mean Inhibition Zone Diameter of Ethanol Extracts from *Fibraurea tinctoria* Lour. Against Bacteria Causing Diabetic Wounds

Figure 2. presents the mean inhibition zone diameters produced by the ethanol extract at different concentrations. The inhibition zone against *S. aureus* increased as the extract concentration increased. No inhibition zone was observed at the 2% concentration, whereas measurable inhibition zones were formed at concentrations of 4%, 6%, 8%, and 10%. In contrast, no inhibition zone was observed against *P. aeruginosa* at any of the tested concentrations.

The Effect of F. tinctoria Extract on Bacteria That Cause Diabetic Wounds

To determine whether the differences among extract concentrations were statistically significant, the inhibition zone data for *S. aureus* were analyzed using one-way analysis of variance (One-Way ANOVA).

Table 1. Results of the One-Way ANOVA Test for *S. aureus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	352.267	4	88.067	77.706	0.000
Within Groups	11.333	10	1.133		
Total	363.600	14			

The One-Way ANOVA results demonstrated a statistically significant effect of extract concentration on the inhibition of *S. aureus* growth ($p < 0.05$), indicating that antibacterial activity differed significantly among the tested concentrations. To complement the p -value and to strengthen the interpretation of this finding, the effect size was calculated as eta squared ($\eta^2 = SS_{\text{between}}/SS_{\text{total}}$), yielding $\eta^2 = 0.97$ ($352.267/363.600$), which indicates a very large effect according to Cohen's convention ($\eta^2 \geq 0.14$) and confirms that extract concentration accounted for approximately 97% of the total variance in inhibition zone diameter against *S. aureus*.

DMRT Test of Treatment Effects on S. aureus

Following the significant ANOVA result, Duncan's Multiple Range Test (DMRT) was performed to identify differences between individual treatment groups.

Table 2. DMRT Test of Treatment Effects on *S. aureus*

Concentration (%)	Mean Inhibition Zone (mm)	Notation
2%	0,00 $\pm$ 0,00	c
4%	10,00 $\pm$ 0,58	b
6%	11,33 $\pm$ 0,47	b
8%	12,00 $\pm$ 1,15	ab
10%	13,67 $\pm$ 0,82	a

The mean inhibition zone was shown to be greatest at the 10% extract concentration, as determined by DMRT. However, no significant difference was observed between the 8% and 10% extract concentrations, indicating comparable antibacterial activity. The difference between high concentration and lower concentrations were particularly pronounced for the 2% low dose which showed

no antibacterial activity against *S. aureus*. Based on the reported means and standard deviations ($n = 3$ per group), the corresponding 95% confidence intervals for the mean inhibition zone were approximately 8.56–11.44 mm at 4%, 10.16–12.50 mm at 6%, 9.14–14.86 mm at 8%, and 11.63–15.71 mm at 10%, while the 2% group showed no variability (0.00–0.00 mm). The relatively wide interval at 8% reflects the greater within-group variability at that concentration, whereas the overlapping intervals between the 8% and 10% groups are consistent with the non-significant DMRT comparison between these two concentrations, together providing a clearer picture of the precision and practical magnitude of the observed differences beyond the significance level alone.

Statistical analysis was not performed for *P. aeruginosa* as there was no inhibition zone at any extract concentration. Therefore, the results suggest that under the experimental conditions used in this study, *S. aureus* is the only strain inhibited by ethanol extract of *F. tinctoria* root.

In the present work we showed that ethanol extract of *F. tinctoria*. The higher antibacterial activities of root against *S. aureus* was with a concentration 10% of 13.67 mm a mean inhibition zone diameter (mean) and an antibacterial effectiveness in %. On the other hand no inhibition zone was seen against *P. aeruginosa* at any of the tested concentrations indicating 0% antibacterial effectiveness. Conclusions.

DISCUSSION

The present study showed that the ethanolic root extract of *Fibraurea tinctoria* Lour. showed concentration-dependent antibacterial activity against *Staphylococcus aureus* while no inhibitory effect on *Pseudomonas aeruginosa* was seen. Statistical analysis showed an increased inhibition to *Staphylococcus aureus* as the concentration of extracts rises with 10% exhibiting the highest antibacterial effect. Conclusions The above results suggest that the antibacterial activities of the *F. tinctoria* root extract are dependent on bacterial species and related to differences in specific characteristics of bacteria especially cell wall composition between Gram-positive and Gram-negative bacteria.

Three of the identified compounds were known to exhibit antibacterial properties against *S. aureus*; this is probably due to the existence of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, saponins and triterpenoids, phenolic compounds in particular compound. Berberine was reported to show a wide range of biological activities including anticancer activity and has been recognized for years. Berberine has been shown to have anti-bacterial effects since it can cause the destruction of bacterial cell wall integrity, enhance membrane permeability, inhibit synthesis of nucleic acid and interfere with vital metabolic pathways. Flavonoids and phenolic compounds have also been reported to enhance oxidative stress (through production of reactive oxygen species) and the destruction of cytoplasmic membranes, while tannins precipitate cell proteins and inhibit microbial enzymes. The synergistic effect of these phytochemicals may explain the increase in inhibition against *S. aureus* with increasing concentration (El-Zahar et al., 2022). This mechanistic interpretation is further supported by prior phytochemical characterization of *F.*

tinctoria ethanolic extract, in which Fathiah et al. (2024) quantified substantial flavonoid and phenolic content alongside antioxidant activity, and Zalizar et al. (2019) confirmed the presence of berberine, tannins, saponins and triterpenoids with demonstrable antibacterial potency; taken together with the present inhibition data, this literature-based compositional evidence strengthens the inferred relationship between these bioactive compound classes and the observed antibacterial effect against *S. aureus*. Nevertheless, as this study did not itself perform phytochemical screening or quantitative analysis (e.g., HPLC or GC-MS quantification of berberine, flavonoids, phenolics, and tannins) on the specific root extract batch used here, this compositional link remains inferential; direct quantitative phytochemical profiling of this extract is therefore recommended as an essential next step to firmly establish the causal relationship between specific bioactive compounds and the antibacterial activity observed.

On the contrary, inhibition against *P. aeruginosa* was not observed at any of the extract concentrations tested. Their study results were consistent with the intrinsic resistance features of *P. aeruginosa* because its outer membrane, which contains all lipopolysaccharides, limits passive diffusion to many antibacterial agents. Furthermore, the combined effect of multidrug efflux pumps, low membrane permeability and the production of effective biofilms leads to reduced susceptibility of plant-derived antimicrobial agents among bacteria (Saxena et al., 2023). Such defense mechanisms may have limited the effective intracellular concentrations of phytochemicals present in the extract under the experimental conditions utilized in this study. Several interrelated factors likely account for this complete absence of activity rather than a mere reduction in susceptibility. First, the extract concentrations evaluated in this study (2–10%) may simply have been too low to reach the intracellular threshold required to overcome the strong permeability barrier of *P. aeruginosa*; since ethanolic root extract was already at its maximum antibacterial capacity against *S. aureus* at 10%, it remains unclear whether higher concentrations would have been sufficient to inhibit the Gram-negative strain, or whether the compounds would remain ineffective regardless of dose. Second, the diffusion behaviour of the bioactive constituents themselves is a plausible contributor: berberine and related alkaloids are relatively large, positively charged, and moderately hydrophilic molecules whose penetration through the disk-diffusion agar matrix and, more importantly, through the asymmetric lipopolysaccharide-rich outer membrane of *P. aeruginosa* is inherently restricted; a disk-diffusion assay may therefore underestimate any activity that depends on prolonged bacterial-compound contact rather than free diffusion. Third, the intrinsic resistance repertoire of *P. aeruginosa*, including constitutively expressed multidrug efflux pumps (e.g., MexAB-OprM), reduced porin-mediated influx, and rapid biofilm-associated matrix formation even under short-term culture conditions, may actively expel or sequester phytochemicals that do manage to cross the outer membrane, compounding the diffusion barrier described above. Finally, methodological limitations of the present assay should be acknowledged: the disk diffusion method is qualitative and comparatively insensitive relative to broth microdilution assays capable of determining the minimum inhibitory concentration (MIC) and minimum

bactericidal concentration (MBC); it is therefore possible that sub-inhibitory or bacteriostatic activity against *P. aeruginosa* went undetected by this method alone. Future work using microdilution-based MIC/MBC determination, combined with efflux-pump inhibitor co-treatment and biofilm-disruption assays, would help clarify whether the absence of inhibition reflects a true lack of antibacterial potential or a limitation of the concentration range and detection method used here.

Overall, the results are in line with previous reports describing antibacterial activity of *Fibraurea tinctoria*. Santoso et al. (2020) It was reported that ethanolic stem extract of Def. growth inhibition on *S. aureus* whereas Kholifa et al. (2021) that according to, *P. aeruginosa* was only susceptible to at higher concentrations and after different extraction preparations. Discrepancies in studies may be due to multiple factors such as variances in plant organs, solvent for extraction, phytochemical profiles of the extracts and bacterial strains and extract concentrations used. These factors significantly affect the fate and stages of active principles that are responsible for antibacterial activity.

The 10% extract produced higher inhibition zone compared with other tested concentrations against *S. aureus*. Duncan's Multiple Range Test indicated, however, that there was no significant difference between the 10% and 8% concentrations indicating further that increasing the concentration above 8% afforded only minimal additional antibacterial activity during the time of this study. Our finding might suggest that Antimicrobial action is reaching the plateau at high concentration, however to confirm this response more acquisition with a wider range of concentrations are needed.

The above results support that the *F. tinctoria* root extract indeed has a high potential as an antibacterial agent against Gram-positive bacteria, however, some limitations need to be acknowledged here. Two reference strains of bacteria were evaluated and the antibacterial activity was only confirmed by disk-diffusion method. MIC, MBC, phytochemical quantification and biofilm inhibition assay were not performed either. Additionally, this also excluded extract concentrations of over 10% as well as clinical multidrug-resistant isolates from diabetic wounds. These limitations should be considered in future studies to better evaluate the antibacterial efficacy and therapeutic potential of *F. tinctoria* root extract.

CONCLUSIONS

Introduction The ethanolic root extract of *Fibraurea tinctoria* Lour. demonstrated specificity inhibition of *Staphylococcus aureus* but not *Pseudomonas aeruginosa*. Higher extract concentration provided the higher antibacterial effect against *S. aureus*; therefore, 10% extract produced the maximum inhibition zone. The statistical analysis showed that extract concentration had a significant influence on the inhibition of *S. aureus* growth. In contrast, in the experimental conditions used here, no detectable antibacterial activity was observed against *P. aeruginosa*. These observations suggest that *F. tinctoria* root extract has great potential as a natural antibacterial source against diabetic wound infection-

associated Gram-positive bacteria and contributes to the scientific evidence for further pharmacological research of *F. tinctoria*.

RECOMMENDATIONS

Further studies should assess higher concentrations of extracts and evaluate MIC and MBC to characterize more precisely the antibacterial potency of *F. tinctoria* root extract. Also, Phytochemical characterization must be done to identify and quantify the bioactive compounds that are responsible for its antibacterial activity. Additionally, studies with multidrug-resistant clinical isolates, biofilm inhibition assay, cytotoxicity assay and evaluation in vitro wound-healing models should be performed to confirm the efficacy and safety of this medicinal plant as a possible therapeutic option prior to going into human applications. Furthermore, future research is recommended to employ in vivo diabetic wound models, such as diabetic rodent excisional or infected wound models, to evaluate the effectiveness of *F. tinctoria* root extract on wound healing rate, tissue regeneration and re-epithelialization, and infection control under physiological conditions that more closely reflect the hyperglycemic, immunocompromised, and biofilm-prone microenvironment of diabetic wounds in vivo.

ADVANCED RESEARCH

This study gives initial evidence for the possible antibacterial activity of ethanolic root extract of *Fibraurea tinctoria* Lour. against *Staphylococcus aureus*. However, there are a few limitations which should be recognized. Antibacterial activity was assessed by means of a disk diffusion test with two reference bacterial strains and only two concentrations (2%, 4%, 6%, 8% and 10%). MIC, MBC, antibiofilm activity, phytochemical quantification and toxicity assays were also not conducted. Investigations in the future that integrate these strategies along with multimicrobial clinical isolates and in vivo experimental design should yield more appropriate evidence of the efficacy and safety for employing *F. tinctoria* as an

antibacterial plant-based therapy against diabetic wound infections caused by multidrug-resistant pathogens.

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