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Comparative Study On Anti-Diabetic and Anti-Microbial Effect Between Tinospora sinensis and Plumbago zyelenica

(Estudio comparativo sobre el efecto antidiabético y antimicrobiano entre Tinospora sinensis y Plumbago zyelenica)

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Abstract (english)

To evaluate and compare the antimicrobial and antidiabetic potential of Tinospora sinensis and Plumbago zeylanica (Chitrak Root Powder). Antimicrobial efficacy was assessed using Mueller-Hinton agar and nutrient broth against five bacterial strains (S. aureus, A. baumannii, K. pneumoniae, E. coli and P. aeruginosa). Activity was quantified via zones of inhibition (ZOI). Antidiabetic potential was evaluated using an α-amylase inhibitory assay, with Metformin as the positive control. Plumbago zeylanica demonstrated superior antimicrobial activity, yielding a maximum ZOI of 23 mm compared to 22 mm for T. sinensis, with K. pneumoniae and E. faecalis showing the highest susceptibility. In antidiabetic assays, P. zeylanica exhibited stronger α-amylase inhibition (IC50 = 630.37 µg/mL) than T. sinensis (IC50 = 853.70 µg/mL). Both extracts possess significant therapeutic properties; however, Plumbago zeylanica consistently outperformed Tinospora sinensis. These results support further clinical investigation into these natural products.

Keywords(english)

Antidiabetic activity, Anti microbial efficacy, α-amylase inhibition assay, Zones of inhibition.

Resumen(español)

Evaluar y comparar el potencial antimicrobiano y antidiabético de Tinospora sinensis y Plumbago zeylanica (polvo de raíz de Chitrak). La eficacia antimicrobiana se evaluó utilizando agar Mueller-Hinton y caldo nutritivo contra cinco cepas bacterianas (S. aureus, A. baumannii, K. pneumoniae, E. coli y P. aeruginosa). La actividad se cuantificó mediante zonas de inhibición (ZOI). El potencial antidiabético se evaluó utilizando un ensayo de inhibición de α-amilasa, con metformina como control positivo. Plumbago zeylanica demostró una actividad antimicrobiana superior, con una ZOI máxima de 23 mm en comparación con 22 mm para T. sinensis, siendo K. pneumoniae y E. faecalis las que mostraron mayor susceptibilidad. En

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ensayos antidiabéticos, P. zeylanica mostró una mayor inhibición de la α-amilasa (CI50 = 630,37 µg/mL) que T. sinensis (CI50 = 853,70 µg/mL). Ambos extractos poseen importantes propiedades terapéuticas; sin embargo, Plumbago zeylanica superó consistentemente a Tinospora sinensis. Estos resultados justifican una mayor investigación clínica sobre estos productos naturales.

Palabras clave(español)

Actividad antidiabética, eficacia antimicrobiana, ensayo de inhibición de α-amilasa, zonas de inhibición.

Introduction

Diabetes mellitus is a rapidly increasing metabolic disorder and remains a major global health concern due to its chronic complications and associated infections (1). Type 2 diabetes mellitus constitutes the majority of cases and is frequently complicated by impaired wound healing, neuropathy and peripheral vascular disease, predisposing patients to diabetic foot ulcers (1,2). These ulcers often become infected, leading to prolonged hospitalisation, antimicrobial resistance and increased risk of limb amputation.

Effective management of diabetic foot infections requires dual therapeutic strategies that address both hyperglycemia and microbial burden. Conventional antidiabetic and antimicrobial drugs are effective but are often associated with adverse effects, long-term safety concerns and increasing antimicrobial resistance (3). This has prompted growing interest in plant-derived agents that possess multiple pharmacological activities and may serve as safer adjuncts or alternatives (4,5).

Medicinal plants have historically been used for the treatment of metabolic disorders and infections and many have demonstrated enzyme inhibitory and antimicrobial properties in experimental studies (5). Tinospora sinensis has been reported to exhibit antidiabetic, antioxidant and immunomodulatory activities, attributed to the presence of alkaloids, glycosides and diterpenoid compounds (6,7). Several in vitro studies have shown its ability to inhibit carbohydrate-hydrolyzing enzymes and modulate glucose metabolism (6).

Plumbago zeylanica (Chitrak) is another medicinal plant traditionally used for treating infections and metabolic disorders (8). Its pharmacological effects are largely attributed to naphthoquinones, particularly plumbagin, which exhibit antimicrobial, anti-inflammatory and enzyme inhibitory activities (9,10). Experimental studies have demonstrated its efficacy against both Gram-positive and Gram-negative bacteria, as well as its ability to inhibit α-amylase activity (10,11).

Although both plants have been individually studied, direct comparative data evaluating their antimicrobial and antidiabetic potential under identical experimental conditions are limited. Such comparisons are essential to identify plant candidates with superior dual activity and greater translational relevance. Therefore, the present study was designed to comparatively evaluate the antimicrobial and α-amylase inhibitory activities of Tinospora sinensis and Plumbago zeylanica, with specific relevance to diabetes-associated infections.

Materials and Methods

The experiment began with various instruments and chemicals, including nutrient broth and Mueller-Hinton agar plates. To dissolve the plant extract, dimethyl sulfoxide (DMSO) was used as a solvent. The compound “sodium chloride (NaCl), sodium phosphate dibasic (Na2HPO4) and sodium phosphate monobasic (NaH2PO4)” were components of the buffer solution, which exhibited a pH of 6.9. An essential reagent for the assay was the α-amylase solution. Along with “sodium potassium tartrate tetrahydrate” and “3,5-dinitrosalicylic acid” solution were part of the DNSA reagent to halt the reaction and facilitate colorimetric measurement. As a positive control, metformin was used in the experiment.

Antibiotic susceptibility testing. For this experiment, we used a sterile straight wire to separate two or three colonies of the following bacteria from an agar plate: “S. aureus, A. baumannii, K. pneumoniae, E. coli and P. aeruginosa”. A nutrient broth was supplemented with these colonies and incubated the mixture at 37°C for 24 hours to promote bacterial growth (De Castro, 2004).We examined the results of the experiment after the incubation time had passed. Measuring the zones of inhibition surrounding the antibiotic disks allowed us to assess the efficacy of the plant extract against the different bacterial strains. The results of these tests provided important insight into the plant extract's antibacterial capabilities.

Anti-Diabetic Activity - α Amylase Inhibitory Assay. Wickramaratne et al. (2016) suggested the “3,5-

Table. 1. Zone of Inhibition of Microbial activity.

Culture	Sample		Meropenem 10- Standard Drug *(mm)*
	Chitrak Root Powder (mm)	*Tinospora sinesis (mm)*	
S.aureus	23	20	30
Acinetobacter baumannii	20	22	28
K.pneumoniae	18	13	28
E. faecalis	24	18	28
Pseudomonas aeruginosa	20	18	28

dinitrosalicylic acid" (DNSA) method for conducting the "α-amylase inhibition assay". The method involves dissolving the plant extract in a buffer containing "Na2HPO4/NaH2PO4 (0.02 M) and NaCl (0.006 M) at pH 6.9". A positive control was Metformin (500 mg) and followed the same protocol as before. One way to calculate the "α-amylase inhibitory activity" is using the following equation: The % "α-amylase" inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph.

$$\%\ of\ Inhibition = \frac{Abs\ of\ Control - Abs\ of\ Sample}{Abs\ of\ Control} X\ 100$$

Results

Antimicrobial Activity. The antimicrobial activity of Tinospora sinensis and Plumbago zeylanica against the selected bacterial strains is presented in Table 1. The zones of inhibition are expressed as mean ± standard deviation (n = 3).

Plumbago zeylanica demonstrated larger mean zones of inhibition against most tested organisms, with inhibition zones measuring 23.0 ± 0.0 mm against Staphylococcus aureus and 24.0 ± 0.0 mm against Enterococcus faecalis. In comparison, Tinospora sinensis produced smaller inhibition zones, measuring 20.0 ± 0.0 mm against Staphylococcus aureus and 18.0 ± 0.0 mm against Enterococcus faecalis.

Against Klebsiella pneumoniae, Plumbago zeylanica showed a mean inhibition zone of 13.0 ± 0.0 mm, whereas Tinospora sinensis exhibited a mean inhibition zone of 18.0 ± 0.0 mm. Meropenem, used as the standard drug, exhibited the highest antibacterial activity across all strains, with inhibition zones ranging from 28.0 to 30.0 mm, confirming assay validity (Table 1).

The comparative antibacterial activity of both plant extracts and the standard drug is illustrated in Figure 1, highlighting the relatively superior antimicrobial efficacy of Plumbago zeylanica.

Antidiabetic Activity – α-Amylase Inhibition. The α-amylase inhibitory activity of Tinospora sinensis

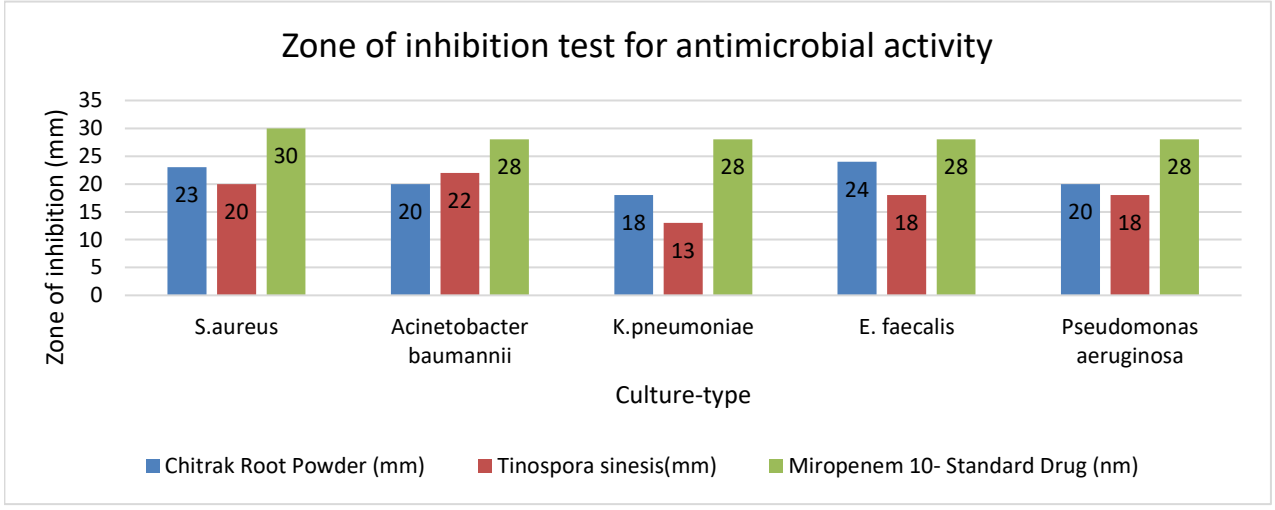


Figure 1. Zone of inhibition

Table. 2. % inhibition of α-amylase by Tinospora sinensis.

Concentration (µg/mL)	OD Value	% of Inhibition	
		Sample	Control
200	0.89	23.80952	12.54
400	0.75	28.57143	32.85
600	0.61	41.90476	53.17
800	0.58	44.7619	73.65
1000	0.42	60	84.36

at different concentrations is shown in Table 2 . Results are expressed as mean ± standard deviation (n = 3). A concentration-dependent increase in enzyme inhibition was observed, with percentage inhibition increasing from 12.54 ± 0.0% at 200 µg/mL to 84.36 ± 0.0% at 1000 µg/mL.

The dose-dependent inhibitory pattern of Tinospora sinensis is graphically represented in Figure 3.3, demonstrating a progressive increase in α-amylase inhibition with increasing concentration.

Similarly, the α-amylase inhibitory activity of Plumbago zeylanica is presented in Table 3. Plumbago zeylanica exhibited higher inhibition values at all tested concentrations, with percentage inhibition increasing from 12.54 ± 0.0% at 200 µg/mL to 72.38 ± 0.0% at 1000 µg/mL.

The concentration-dependent inhibitory response of Plumbago zeylanica is illustrated in Figure 3.4, showing a steeper increase in inhibition compared to Tinospora sinensis, particularly at higher concentrations.

IC_{50} Determination. The IC_{50} values for α-amylase inhibition by both plant extracts are presented in Table 3.4. Plumbago zeylanica exhibited a lower IC_{50} value of 630.37 ± 0.0 µg/mL, whereas Tinospora sinensis showed an IC_{50} value of 853.70 ± 0.0 µg/mL, indicating greater enzyme inhibitory potency of Plumbago zeylanica.

The comparative IC_{50} values are graphically depicted in Figure 3.5, clearly demonstrating the superior antidiabetic activity of Plumbago zeylanica over Tinospora sinensis.

This difference in IC_{50} values is graphically represented in Figure 3.5, highlighting the superior antidiabetic activity of Plumbago zeylanica over Tinospora sinensis.

The combined antimicrobial and α-amylase inhibitory activities demonstrated in Tables 1–4 and Figures 1, 2, 3, 4 indicate that both plant extracts possess dual pharmacological potential. The consistently superior performance of Plumbago zeylanica across antimicrobial and antidiabetic assays underscores its relevance as a promising candidate for further investigation in the management of diabetes-associated infections.

Discussion

The present study demonstrates that both Tinospora sinensis and Plumbago zeylanica possess significant antimicrobial and antidiabetic activity in

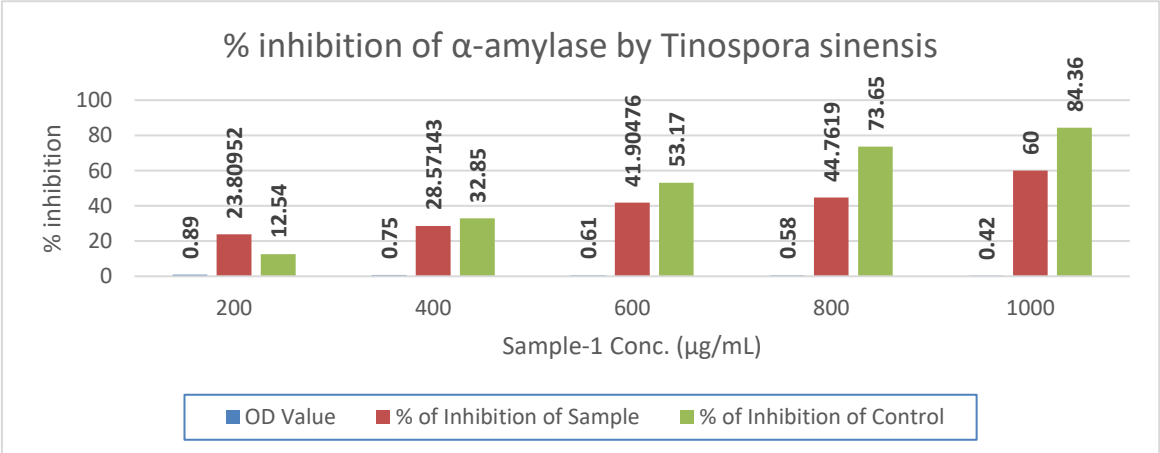


Figure 2. Bar graph showing % inhibition of α-amylase by Tinospora sinensis

Table. 3. % inhibition of α-amylase by Chitrak Root Powder.

Concentration (µg/mL)	OD Value	% of Inhibition	
		Sample	Control
200	0.8	23.80952	12.54
400	0.68	35.2381	32.85
600	0.51	51.42857	53.17
800	0.44	58.09524	73.65
1000	0.29	72.38095	84.36

vitro. However, Plumbago zeylanica consistently exhibited superior efficacy across both experimental models, indicating a greater dual pharmacological potential.

The enhanced antimicrobial activity of Plumbago zeylanica can be attributed to its phytochemical composition, particularly the presence of naphthoquinones such as plumbagin. These compounds exert direct bactericidal effects by disrupting bacterial cell membranes, impairing oxidative phosphorylation and inhibiting nucleic acid synthesis (9,12). Such mechanisms allow for rapid and effective bacterial growth inhibition, especially against Gram-negative organisms. Previous studies have reported pronounced antibacterial activity of Plumbago zeylanica against pathogens such as Klebsiella pneumoniae and Pseudomonas aeruginosa, which are

Table. 4. % inhibition of α-amylase by Chitrak Root Powder.

IC50 VALUE	Concentration (µg/ml)
(Sample-T) *Tinospora sinensis*	853.70
(Sample-C) Chitrak Root Powder	630.37

frequently isolated from diabetic foot infections (10,13). This mechanistic profile provides a plausible explanation for the larger zones of inhibition observed in the present study.

In contrast, the antimicrobial activity of Tinospora sinensis is primarily mediated through indirect mechanisms, including immunomodulatory and antioxidant effects (7,14). While these properties contribute to host defense and inflammation control, they may not translate into strong direct bactericidal activity in agar diffusion assays. Consequently, Tinospora sinensis demonstrated comparatively lower antimicrobial potency under the experimental conditions used, despite its established therapeutic value.

The α-amylase inhibition results further support the superior antidiabetic activity of Plumbago zeylanica. The lower IC_{50} value observed indicates that a smaller concentration of the extract is required to achieve effective enzyme inhibition. Inhibition of α-amylase delays carbohydrate digestion and reduces postprandial glucose excursions, a key therapeutic strategy in the management of type 2 diabetes mellitus (3,15). The greater potency of Plumbago zeylanica may be attributed to the strong affinity of plumbagin and related phenolic compounds for the active site of α-

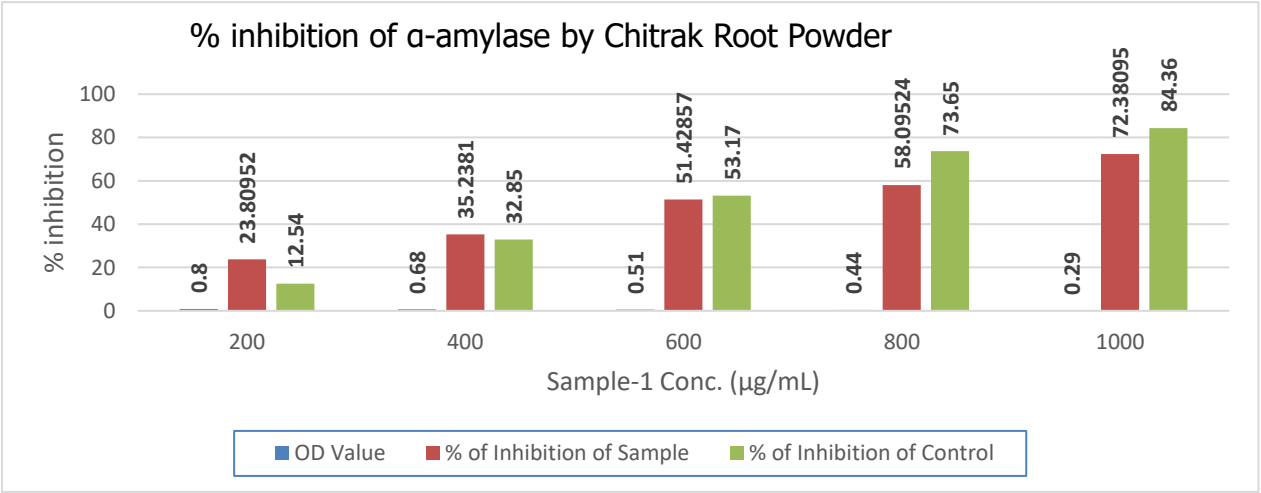
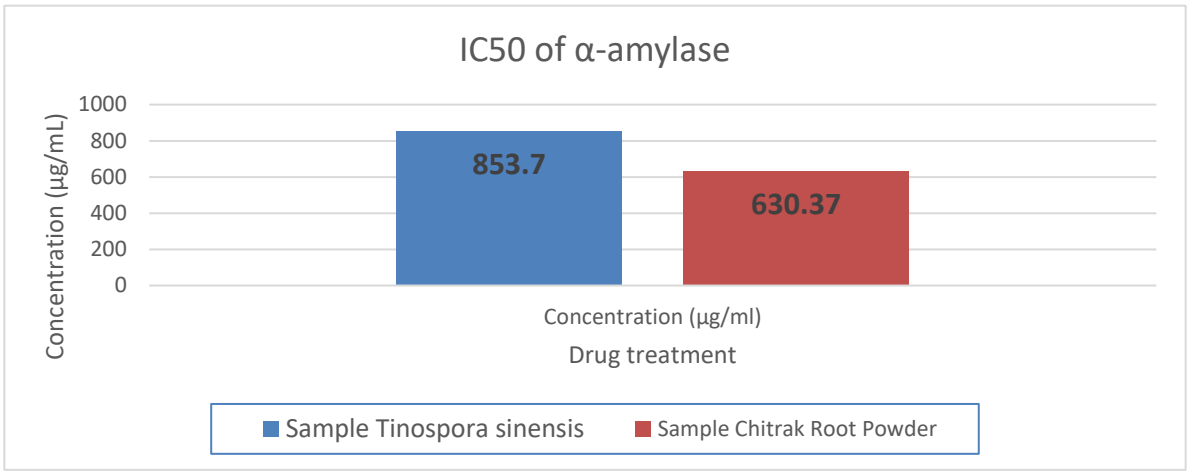
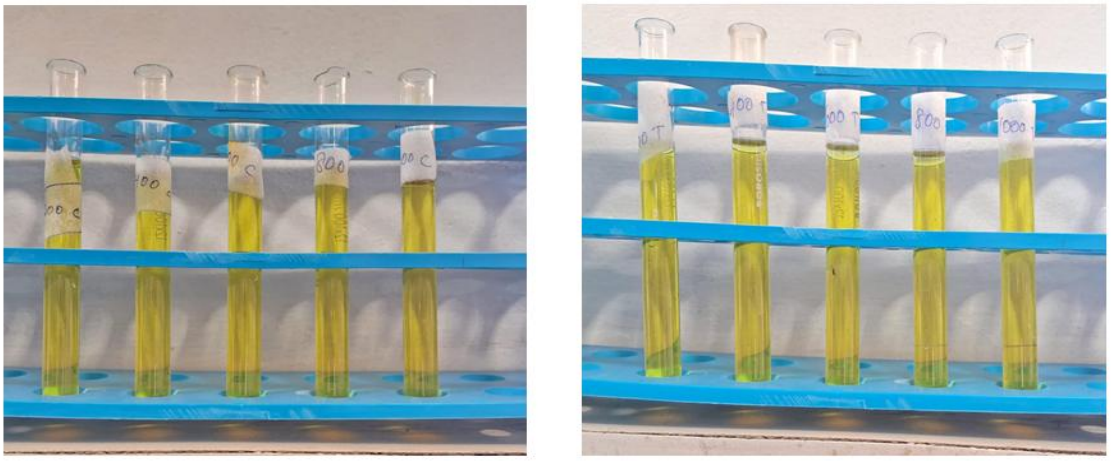


Figure 3. Bar graph showing % inhibition of α-amylase by Chitrak Root Powder





Sample C **Sample T**

Figure 4 Top. Bar graph representing the IC50 of α-amylase by different drugs. Bottom. Showing the photograph of α-amylase inhibition by test samples

amylase, leading to reduced catalytic efficiency and enhanced enzyme inhibition (11).

From a clinical perspective, diabetic foot infections present a dual challenge involving poor glycemic control and persistent microbial infection. Therapeutic agents capable of simultaneously addressing both metabolic dysregulation and microbial burden are therefore of significant clinical interest. The combined antimicrobial and antidiabetic activities demonstrated by Plumbago zeylanica suggest its potential utility as an adjunctive therapeutic agent in the management of diabetes-associated infections (4,16). Such dual-action agents may reduce the need for polypharmacy and improve treatment outcomes.

Nevertheless, the findings of the present study are limited to in vitro conditions. Factors such as bioavailability, toxicity, pharmacokinetics and optimal dosing remain unaddressed. Further in vivo studies and controlled clinical trials are essential to validate the safety and therapeutic applicability of Plumbago zeylanica before clinical translation.

In conclusion, the present study demonstrates that both Tinospora sinensis and Plumbago zeylanica possess significant antimicrobial and antidiabetic activity under in vitro conditions. However, Plumbago zeylanica consistently exhibited superior efficacy, as evidenced by larger zones of inhibition against clinically relevant bacterial pathogens and a lower IC_{50} value for α-amylase inhibition.

The dual activity of Plumbago zeylanica is of particular therapeutic relevance in the context of diabetes-associated infections, such as diabetic foot ulcers, where effective glycemic control and antimicrobial action are both required. The observed α-amylase inhibitory activity suggests its potential role in reducing postprandial hyperglycemia, while its

antimicrobial efficacy against Gram-negative organisms supports its possible application in managing infection-prone diabetic wounds.

Although the findings are limited to in vitro evaluation, they provide a strong pharmacological rationale for considering Plumbago zeylanica as a promising adjunctive therapeutic agent in diabetes management complicated by infection. Further in vivo studies, toxicity profiling and controlled clinical trials are necessary to establish its safety, optimal dosing and clinical efficacy before therapeutic translation.

Conflict of interest

None to declare

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