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Novel Therapeutic Strategy for Multidrug-Resistant *Proteus mirabilis* in Diabetic Foot Ulcers: Exploring the Antibacterial Effects of *Sansevieria zeylanica* Extracts

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Abstract

Diabetic foot ulcers (DFUs) are a significant global health burden, exacerbated by the rise in multidrug-resistant (MDR) bacterial infections. This study evaluated the antibacterial effects of *Sansevieria zeylanica* (S. zeylanica) leaf and root extracts against MDR *Proteus mirabilis* isolated from diabetic patients with DFUs at a teaching hospital in Nigeria. Antimicrobial susceptibility testing of clinical isolates of MDR *P. mirabilis* was conducted using the Kirby-Bauer disc diffusion method. The antibacterial activity of *S. zeylanica* extracts was evaluated using the agar well diffusion method. Additionally, the synergistic/antagonistic effect of the extracts with Ofloxacin was assessed. Antimicrobial susceptibility testing revealed high resistance to conventional antibiotics, with 61.5% of isolates identified as MDR. Methanolic extracts of *S. zeylanica* demonstrated moderate antibacterial activity, with root extracts exhibiting greater inhibition zones (10-17 mm) compared to leaf extracts (8-13 mm). However, co-administration with Ofloxacin revealed an antagonistic effect. These findings suggest that *S. zeylanica* root extracts hold promise as an alternative therapeutic agent for DFU management. Further research is needed to optimize extract formulations and explore their clinical applications.

Keywords: Antibacterial activity, Antimicrobial resistance, Diabetes mellitus, Diabetic foot ulcers, Medicinal plants, Multidrug-resistant bacteria, *Proteus mirabilis*, *Sansevieria zeylanica*.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired glucose regulation. While the estimated global prevalence of DM in 2019 was 9.3% (463 million people), the prevalence is expected to rise to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Saeedi *et al.*, 2019). The World Health Organization (WHO) describes the impact of diabetes in Africa as one of the region's "New Silent Killers" (WHO, 2024). Sub-Saharan Africa is witnessing an alarming surge in diabetes incidence, with estimates suggesting a doubling of cases by 2030 (Sun *et al.*, 2022). Apart from disrupting glucose metabolism, DM predisposes individuals to a myriad of complications, including heightened susceptibility to infections. Individuals with DM are at particular risk of wound infections of the lower limbs. A study reported infections in about 40% of foot ulcers in patients with diabetes mellitus (Jia *et al.*, 2017).

Among the complications associated with DM, diabetic foot ulcers (DFUs) constitute a significant global health burden (Gamboa-Antiñolo, 2023). Diabetic foot ulcers result from a combination of factors including neuropathy, peripheral vascular disease, and immunological compromise, leading to increased susceptibility to infections and delayed wound healing (Rodríguez-Rodríguez *et al.*, 2022). Management of DFUs in sub-Saharan Africa and globally is hindered by slow wound healing and increasing antibiotic resistance. Although the most commonly isolated pathogens in DFUs are *Staphylococcus aureus* and *Streptococcus agalactiae*, polymicrobial infections and colonization by multidrug-resistant (MDR) bacteria of DFUs are not uncommon. Multidrug-resistant bacteria including *Proteus mirabilis* present formidable challenges in the management of DFUs due to limited treatment options (Parmanik *et al.*, 2022) and huge healthcare costs (Tansarli *et al.*, 2013).

Conventional treatments, including systemic antibiotics and topical antiseptics, are becoming increasingly ineffective against DFUs (Parmanik *et al.*, 2022; Zafar *et al.*, 2019). The urgency to explore alternative antimicrobial therapies is underscored by the exponential rise in antibiotic resistance, highlighting the need for innovative approaches to address this pressing public health issue.

Medicinal plants containing compounds that may prevent bacterial infections and have clinical value in treating resistant strains offer potential alternatives to conventional antibiotics (Parmanik *et al.*, 2022; Imran *et al.*, 2017). Widely available *Sansevieria zeylanica*, commonly known as mother-in-law's tongue or snake plant, is one such medicinal plant traditionally used in Africa for the treatment and management of various microbial infections (Buyun *et al.*, 2018; Kingsley *et al.*, 2013). Recent studies have reported the antimicrobial activity of *S. zeylanica* against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *P. vulgaris* (Omoruyi *et al.*, 2022; Ugbomoiko *et al.*, 2022).

This study aimed to evaluate the antibacterial effects of *S. zeylanica leaf* and root extracts against MDR *P. mirabilis* isolated from diabetic foot ulcers and explore its potential as an alternative therapy for diabetic foot infections.

MATERIALS AND METHODS

Recruitment of Participants and Collection of Bacterial Strains

Eighty-nine (89) diabetic patients presenting with DFUs attending the General Outpatient Department of Igbinedion University Teaching Hospital (IUTH) between March 2021 and September 2024 were enrolled for the study. Demographic data of the patients were collected and recorded after receiving their written informed consent. **S**trains of *P. mirabilis* were isolated from 13 out of the 89 DFU patients. The isolates underwent identification through standard microbiological methods (Barrow & Feltham, 2003).

Inclusion Criteria:

1. Adult patients aged 18 years and above diagnosed with DM and presenting with DFUs at the General Outpatient Department of Igbinedion University Teaching Hospital (IUTH) between March 2021 and September 2024.
2. Individuals willing to provide informed consent for participation in the study, including the collection of clinical and demographic data.
3. Patients who had not received antibiotic therapy or topical antimicrobial treatments in the preceding two weeks.

Exclusion Criteria:

1. Patients below 18 years of age.
2. Individuals with non-diabetic foot ulcers or wounds unrelated to DM.
3. Individuals undergoing immunosuppressive therapy or with a history of malignancy.
4. Patients unwilling or unable to provide informed consent.

Ethical Consideration

Approval was obtained from the institution's Research and Ethical Committee for the study.

Collection of Plant Samples

Fresh leaves and roots of *S. zeylanica* (Figure 1) were collected from Igbinedion University Okada (IUO) and authenticated at the Department of Biological Sciences of the same institution.

Figure 1: ***Sansevieria zeylanica***

Preparation of Extracts

Dried leaves (132.23g) and roots (54.81g) were pulverized and subjected to extraction using methanol as a solvent via a Soxhlet extractor apparatus. The resulting extracts were filtered using Whatman No 1 filter paper, followed by solvent removal using a rotary evaporator apparatus at 45°C to achieve a concentration of 1g/ml. The extracts were subsequently stored at 4°C until further analysis.

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, was employed to assess the antimicrobial susceptibility of the Proteus isolates against various antibiotics (CLSI, 2016).

Antibiotic Resistance Phenotypes

Multidrug-resistant (MDR) phenotypes were defined as resistance to at least one antibiotic from three or more classes of antibiotics active for a given species (Gajdács *et al*., 2020; Magiorakos *et al*., 2012).

Antibacterial Activity of the Plant Extract

The agar well diffusion method, described by White and Reeves (1987), was utilized to evaluate the antibacterial activity of the sample extracts against the bacterial isolate. The bacterial cultures were prepared on Muller-Hinton agar, and wells were created using a cork borer for extract application. A 200µl each of the different concentrations of the extracts prepared using 5% Dimethyl Sulfoxide (DMSO) was introduced into each well, allowed for 15 minutes at room temperature (for diffusion of extracts into the agar), and thereafter incubated at 37^0C for 24 hours. Zones of inhibition were measured and recorded after incubation. This procedure was repeated in triplicate.

Determination of the Synergistic/Antagonistic Effect of the Extracts with Ofloxacin

According to the method of Kingsley *et al*., (2013), Ofloxacin discs (5µg) were impregnated each with 500µg/ml of the leaf and root extracts, respectively. After 24 hours of incubation, the zone of inhibition of the Ofloxacin-extract combination was measured and compared with that of Ofloxacin alone. A synergistic relationship was indicated when the zone of inhibition produced by the Ofloxacin-extract combination is greater than that of the Ofloxacin or extract alone while an antagonistic relationship was indicated when the zone of inhibition produced by the Ofloxacin or extract alone is greater than that of the Ofloxacin-extract combination.

RESULTS AND DISCUSSIONS

Socio-demographic Characteristics of the Patients

The majority of patients were male (84.6%), while females accounted for 15.4%. Most were aged ≥70 years (76.9%), with 15.4% in the 50–69 age group, 7.7% in the 30–49 range, and none ≤29 years. Primary education was the most common (53.8%), followed by tertiary (30.8%) and secondary (15.4%) education. Farmers comprised the largest occupational group (30.8%), with artisans, civil servants, and others each representing 23.1%. Additionally, 61.5% of patients were smokers, while 38.5% were non-smokers (Table 1).

Table 1: Demographic Characteristics of the Diabetic Patients with *P. mirabilis* Positive Wound Culture

Variable	Frequency (%)
Gender	
Male	11 (84.6)
Female	2 (15.4)
Age (years)	
≤ 29	0 (0.0)
30-49	1 (7.7)
50-69	2 (15.4)
≥ 70	10 (76.9)
Level of Education	
Primary	7 (53.8)
Secondary	2 (15.24)
Tertiary	4 (30.8)
Occupation	
Farmer	4 (30.8)
Artisan	3 (23.1)
Civil Servant	3 (23.1)
Others	3 (23.1)
Smoker	
No	5 (38.5)
Yes	8 (61.5)

Majority of the patients in the present study are male and above 70 years old. Thabit *et al*. (2020) reported a higher rate (65%) of Proteus infections in diabetic wounds among male patients. In the same study, 45% of the patients were between 50 and 82 years (Thabit *et al*., 2020). The higher proportion of DFU among men may be because men participate in outdoor activities more frequently, which increases their risk of getting hurt and developing ulcers (Atlaw *et al*., 2022). The high prevalence among patients aged ≥70 years (76.9%) highlights the vulnerability of elderly diabetic patients due to factors like impaired immune responses, delayed wound healing, and the higher prevalence of chronic diseases (Gould *et al*., 2015).

The predominance of patients with primary education (53.8%) underscores the impact of limited health literacy on effective wound management and prevention (Bouquettaya *et al*., 2023; Margolis *et al*., 2015). Enhancing education and awareness about diabetic foot care is crucial, particularly for those with lower educational attainment. Additionally, the high percentage of smokers (61.5%) aligns with evidence linking smoking to impaired wound healing, microvascular complications, and increased infection risk (McDaniel & Browning, 2014). Implementing smoking cessation programs could play a vital role in reducing these risks.

Antimicrobial Susceptibility Testing

Table 2 shows the antibiotic susceptibility profile of *P. mirabilis* isolated from diabetic foot ulcers. Tetracycline was completely ineffective, with 100% of isolates showing resistance. Amoxicillin-Clavulanic Acid demonstrated limited efficacy, with only 23.1% susceptibility and 76.9% resistance. Among the cephalosporins tested (Cefotaxime, Cefixime, Cefuroxime, and Ceftriaxone-Sulbactam), resistance rates were consistently high, ranging from 61.5% to 69.2%. Gentamicin showed a similar resistance rate of 69.2%, highlighting its limited effectiveness.

Fluoroquinolones exhibited slightly better activity, with Ofloxacin having a higher susceptibility rate (61.5%) compared to Ciprofloxacin (53.9%). Imipenem, a carbapenem, also demonstrated moderate activity, with a susceptibility rate of 53.9% and resistance in 46.1% of isolates.

Table 2: Antibiotic susceptibility profile of *Proteus mirabilis* isolated from diabetic foot ulcers

S/N	Antibiotics	Antimicrobial Class	S	R
1.	Tetracycline	Tetracyclines	0 (0.0%)	13 (100.0%)
2.	Amoxicillin-Clavulanic acid	Penicillin	3 (23.1%)	10 (76.9%)
3.	Cefotaxime	Cephalosporin	4 (30.8%)	9 (69.2%)
4.	Cefexime	Cephalosporin	4 (30.8%)	9 (69.2%)
5.	Cefotaxime	Cephalosporin	4 (30.8%)	9 (69.2%)
6.	Ceftriaxone-Sulbactam	Cephalosporin	5 (38.5%)	8 (61.5%)
7.	Gentamicin	Aminoglycosides	4 (30.8%)	9 (69.2%)
8.	Ciprofloxacin	Fluoroquinolone	7 (53.9%)	6 (46.1%)
9.	Ofloxacin	Fluoroquinolone	8 (61.5%)	5 (38.5%)
10.	Imipenem	Carbapenem	7 (53.9%)	6 (46.1%)

The high resistance to Tetracycline (100%), Cephalosporins (61.5%-69.2%), and Amoxicillin-Clavulanic Acid (76.9%) reflects *Proteus mirabilis* capability to resist commonly prescribed antibiotics. These results are in contrast with the reports of Atef *et al*., (2019) who reported 100% sensitivity to Cephalosporins for the strain of *P. mirabilis* isolated from wound samples, but in tandem with the results of Thabit *et al*., (2020) who reported high resistance to Amoxicillin-Clavulanate. This resistance may result from the widespread misuse or overuse of these antibiotics in clinical practice.

In underdeveloped nations, where the majority of patients arrive at medical institutions later than expected with severe illnesses, Gram-negative bacteria (GNB) are more frequently linked to infected diabetic ulcers (Zubair, 2020). Several studies (Thabit *et al*., 2020; Heravi *et al*., 2019; Mathew & Suchithra, 2014; Ramakant, 2011; Raja, 2007; Oguachuba, 1985) have reported Proteus species as one of the commonest of these pathogens. Patients with DFU have a heavy burden of ESBL-producing GNB, particularly in low-resource nations where incidence rates have been estimated to vary from 23% to 49% in Africa and Asia (Saltoglu *et al*., 2018; Chaudhry *et al*., 2016; Dwedar *et al*., 2015; Anjli *et al*., 2013; Varaiya *et al*., 2008). The CTX-M ESBL gene was shown to be the most common variant globally, according to reports (Shaikh *et al*., 2015). Resistance to carbapenems, which are used as last resort medications to treat resistant GNB infections, varies but is on the rise (Pitocco *et al*., 2019). The resistance of *P. mirabilis* to Amoxicillin-Clavulanate and Cephalosporins in this study could be due to the ability of the pathogen to produce inducible β-lactamase that is capable of hydrolyzing primary and extended-spectrum Penicillins and Cephalosporins (Thabit *et al*., 2020). Since *P. mirabilis* lacks intrinsic β-lactamase genes on its chromosomes, it must acquire distinct β-lactamase genes to show a β-lactamase-mediated resistance phenotype (Thabit *et al*., 2020). According to Feglo (2010), the isolate multidrug resistance and a high degree of β-lactamase synthesis are signs of a growing resistance threat (Feglo, 2010).

The slightly higher susceptibility to Ofloxacin (61.5%) and Imipenem (53.9%) highlights their potential utility in treating MDR *Proteus mirabilis* (Sheu *et al*., 2019). However, resistance rates still suggest cautious use and a need for combination therapies.

Prevalence of Multi-drug resistant P. mirabilis among the Diabetic Patients

Figure 2 illustrates the distribution of multidrug-resistant (MDR) and non-MDR *P. mirabilis* isolates from diabetic foot ulcers. Out of the total isolates, 61.5% (8 isolates) were classified as MDR, while 38.5% (5 isolates) were non-MDR. This data highlights that a significant majority of the isolates exhibited resistance to multiple antibiotics, indicating the prevalence of MDR strains among the tested *P. mirabilis* population.

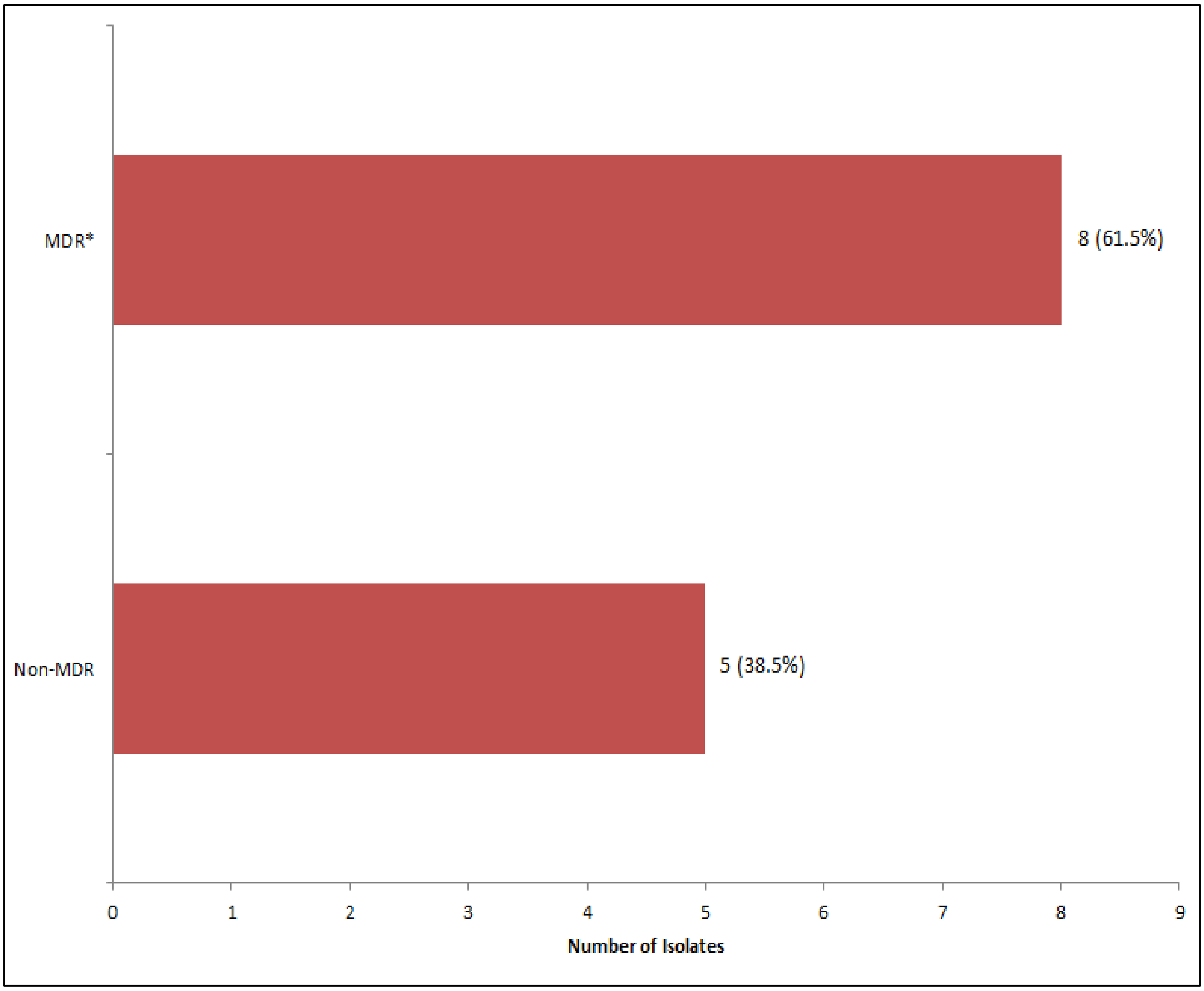

Figure 2: Distribution of multidrug-resistant (MDR) and non-MDR *Proteus mirabilis* isolates from diabetic foot ulcers
*Resistance to at least one antibiotic from three or more classes of antibiotics

The high proportion of MDR isolates (61.5%) reflects the significant antimicrobial pressure likely exerted by the frequent use or misuse of antibiotics in clinical settings (Wang *et al*., 2019). This prevalence is consistent with global trends, where MDR Gram-negative bacteria are increasingly associated with chronic wound infections (Ilyas *et al*., 2024). The relatively lower percentage of non-MDR isolates (38.5%) suggests that a subset of the population may still be treatable with traditional antibiotics. However, the dominance of MDR strains emphasizes the need for alternative treatment approaches. These findings strongly support the exploration of novel therapeutic strategies, such as the antibacterial effects of *S. zeylanica* extracts, as highlighted in the study. Such plant-based interventions could provide effective and sustainable solutions to combat MDR pathogens, particularly in resource-limited settings where antibiotic options may be restricted.

Antibacterial Activity of Leaf and Root Extracts of *S. zeylanica* against MDR *P. mirabilis* Isolates

Table 3 presents the antibacterial activity of *S. zeylanica* leaf and root extracts (500 µg/mL) against eight MDR *P. mirabilis* isolates. For the Leaf Extract, the zones of inhibition ranged from 8 mm (MDR-6) to 13 mm (MDR-4), demonstrating moderate antibacterial activity. The root extract exhibited higher activity, with inhibition zones ranging from 10 mm (MDR-3 and MDR-6) to 17 mm (MDR-2).

Table 3: Antibacterial activity of *Sansevieria zeylanica* leaf and root extracts (500 µg/mL) against multidrug-resistant (MDR) *Proteus mirabilis* isolates

Zone of Inhibition (mm)		
Test bacteria	**Leaf Extract**	**Root Extract**
MDR-1	11	15
MDR-2	11	17
MDR-3	10	10
MDR-4	13	15
MDR-5	12	13
MDR-6	8	10
MDR-7	10	11
MDR-8	10	13

The antibacterial activity of *S. zeylanica* leaf and root extracts against MDR *P. mirabilis* was evaluated through agar well diffusion assays. The root extract of *S. zeylanica* consistently exhibited larger zones of inhibition than the leaf extract, suggesting that bioactive compounds with greater antibacterial potential are more concentrated in the root. This aligns with previous studies showing that roots of medicinal plants often harbor secondary metabolites like alkaloids and flavonoids in higher concentrations, which contribute to antimicrobial activity (Ugbomoiko *et al*., 2022). The observed inhibition zones (10–17 mm) indicate moderate effectiveness of the extracts at 500 µg/mL. Given that *P. mirabilis* is inherently resistant to multiple antibiotics (Liu *et al*., 2023), the ability of these extracts to inhibit growth supports their potential as adjunct therapies. These results underscore the possibility of developing plant-based antibacterial formulations to address multidrug resistance in chronic wound infections like diabetic foot ulcers. The use of plant extracts may reduce dependence on conventional antibiotics, thereby mitigating the emergence of further resistance.

Medicinal plants demonstrating similar antibacterial activity against *P. mirabilis* as observed in this study are reported elsewhere (Rawaa & Nidaa, 2023). *Moringa oleifera* leaf extract containing secondary metabolites similar to those observed in the *S. zeylanica* extracts has been reported to have good antibacterial activity against strains of *P. mirabilis* isolated from wound samples (Atef *et al*., 2019). Alam (2014) reported the use of honey in the effective management of diabetic wounds infected with *P. mirabilis*.

In our previous study, we identified a diverse array of bioactive compounds, including glycosides, phenols, betacyanins, flavonoids, and alkaloids in *S. zeylanica* extracts (Egunjobi *et al*., 2023). These phytoconstituents have been reported to possess antimicrobial properties and may contribute to the observed antibacterial activity of the extracts. Dsouza & Nanjaiah (2018) reported that 3,3'4'-Trihydroxyflavone, a flavonoid, isolated from *Justicia wynaadensis* demonstrated an effective antibacterial activity against MDR bacteria associated with diabetic patients (Dsouza & Nanjaiah, 2018). Similarly, flavonoids extracted from *Marrubium vulgare* L. leaves showed promising inhibition of pathogenic bacteria including *P. mirabilis*. This inhibition exceeded those produced by conventional antibiotics (Bouterfas *et al*., 2017).

Antagonistic Relationship of the Extracts and Ofloxacin

Figure 3 demonstrates the antagonistic interaction between the *S. zeylanica* leaf extract and Ofloxacin against *P. mirabilis*. The blue arrow indicates the zone of inhibition for Ofloxacin alone, measuring 22 mm, reflecting its potent antibacterial activity. The red arrow shows the lack of inhibition (0 mm) when Ofloxacin was combined with the Sansevieria zeylanica leaf extract, indicating an antagonistic effect of the combination. The result reveals that the co-administration of Ofloxacin with the leaf extract not only fails to enhance antibacterial activity but completely nullifies the effect of Ofloxacin.

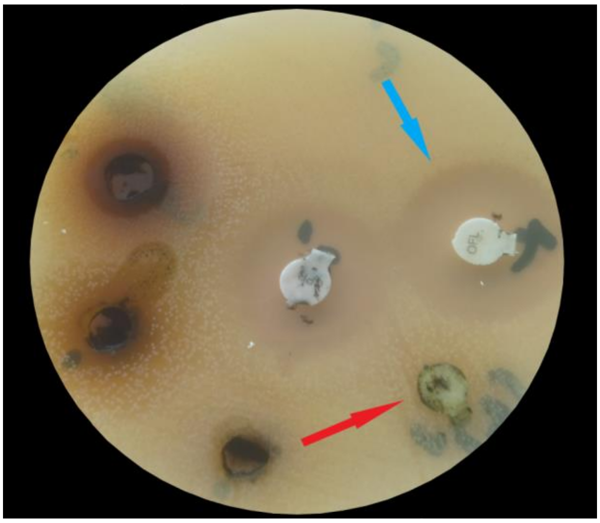

Figure 3: Antagonistic relationship of the leaf extract of *Sansevieria zeylanica* and Ofloxacin

The observed reduction in Ofloxacin's zone of inhibition from 22 mm to 0 mm suggests that certain compounds in the *S. zeylanica* leaf extract might interfere with the mechanism of action of Ofloxacin. This interference could occur at the molecular level (Tajer *et al*., 2024), potentially through competitive binding to bacterial targets, modulation of efflux pump activity, or alteration of cell wall permeability (Munita & Arias, 2016). The antagonism indicates that the simultaneous use of *S. zeylanica* leaf extract and Ofloxacin in therapeutic applications for *P. mirabilis* infections should be approached cautiously. Co-administration could compromise the efficacy of standard antibiotics, particularly fluoroquinolones. In our previous study, the root extract of *S. zeylanica* was observed to have a similar effect (antagonistic) with Gentamicin (Ugbomoiko *et al*., 2022). This phenomenon warrants further investigation to elucidate the underlying mechanisms and potential implications for combination therapy.

CONCLUSION

This study highlights the significant burden of MDR *P. mirabilis* infections in diabetic foot ulcers and the potential of *S. zeylanica* extracts as a novel antibacterial agent. Root extracts demonstrated superior antibacterial activity compared to leaf extracts, suggesting their greater therapeutic potential. However, the antagonistic interaction with Ofloxacin underscores the need for careful consideration in combination therapies. These findings emphasize the importance of alternative therapies in combating antimicrobial resistance, particularly in low-resource settings. While promising, further research is required to validate these results and advance *S. zeylanica* extracts toward clinical application.

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