Evaluating the phytochemical composition and antimicrobial activity of *Dovyalis abyssinica* and *Oxygonum sinuatum* root extracts against *Proteus mirabilis* and *Trichophyton rubrum*

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ABSTRACT

Antimicrobial resistance (AMR) is an emerging global health crisis that indicates the inability of widely used antimicrobial treatments to work as intended. Microorganisms like Proteus mirabilis and Trichophyton rubrum, which is a causative agent of tinea pedis, are linked to antimicrobial resistance, so there is a need to look for an alternative antimicrobial agent from medicinal plants. The study was aimed at assessing the phytochemical composition and antimicrobial activity of the root extract of Dissotis abyssinica against P. mirabilis and T. rubrum, and also the root extract of *Oxyanthus sinuatum*. An experimental lab-based study was carried out to evaluate the antimicrobial property of methanolic root extracts of D. abyssinica and O. sinuatum. Two concentrations of the extract (0, 2, 4, 8 and 16 mg/mL) were tested. The antimicrobial activity was established by the agar well diffusion method and minimum inhibitory concentrations (MICs) by the broth microdilution method. Triplicate treatments were used for each treatment. There were positive controls of ciprofloxacin (5 µg/well) for P. mirabilis and fluconazole (25 µg/mL) for T. rubrum, and a negative control of dimethyl sulfoxide (DMSO). Phytochemical screening was also done in order to find out the presence of bioactive compounds in the extracts. Flavonoids, alkaloids, saponins and glycosides were present in the extracts. Plant extracts exhibited antimicrobial activity towards the test organisms with significantly higher antimicrobial activity as the extract concentration was raised ($p < 0.0001$). The antimicrobial activity of the composites was moderate to strong as per the composite mean analysis. These results showed that the methanolic extracts of roots do have some antimicrobial activity against both P. mirabilis and T. rubrum. It is the conclusion of this study that the root extract of D. abyssinica and O. sinuatum contains phytochemical constituents that exhibited antimicrobial activity against the tested microorganisms, which further supports their potential use as an alternative source of antimicrobial agents and offers a scientific basis for their ethnomedicinal uses. Further studies should be conducted by sequential extraction with at least three different solvents with different polarity, like hexane, ethyl acetate and methanol, or water, to have a more complete phytochemical profile and compare the antimicrobial activity of the extract type. A further investigation is also needed to find and characterize the specific bioactive compounds responsible for the observed antimicrobial effects.

Keywords: Antimicrobial resistance, phytochemicals, *Dissotis abyssinica*, *Oxyanthus sinuatum*, *Proteus mirabilis*, *Trichophyton rubrum*, tinea pedis.

I. INTRODUCTION

Antimicrobial resistance (AMR) is one of the most urgent global health threats of the 21st century, as it compromises the effectiveness of existing antibiotics and antifungal agents (World Health Organization [WHO], 2023). The WHO has warned that AMR is reaching pandemic proportions, threatening to reverse many advances in modern medicine. Recent estimates indicate that antibiotic-resistant infections directly cause over 1 million deaths annually and contribute to nearly 5 million deaths worldwide (WHO, 2025). Furthermore, if left unaddressed, AMR could reduce global economic output by up to $1.7 trillion by 2050 due to increased healthcare costs and reduced productivity (WHO, 2025). The burden of AMR is particularly severe in low- and middle-income countries, where limited access to advanced therapies and weak healthcare systems exacerbate the problem (Dhingra et al., 2020). In Africa, projections suggest that AMR could result in approximately 4.1 million deaths annually by 2050, alongside economic losses ranging between $1 trillion and $3.4 trillion (Africa Centres for Disease Control and Prevention [Africa CDC], 2024). High resistance rates to commonly used antibiotics, including cephalosporins, further complicate treatment strategies (WHO, 2025). These challenges highlight the urgent need to identify alternative and affordable antimicrobial agents.

Medicinal plants have long been recognized as valuable sources of bioactive compounds with therapeutic potential. Their use in combating resistant infections has gained renewed scientific attention due to their accessibility and chemical diversity (Atanasov et al., 2015; Cowan, 1999; Mensah et al., 2019). Among clinically significant

pathogens, *Proteus mirabilis* and *Trichophyton rubrum* represent major public health concerns. *Proteus mirabilis* is a Gram-negative opportunistic bacterium commonly associated with catheter-associated urinary tract infections (CAUTIs) and wound infections. Its pathogenicity is enhanced by virulence factors such as urease production, biofilm formation, and swarming motility (Cortes-Penfield et al., 2017). Alarmingly, resistance to fluoroquinolones, extended-spectrum cephalosporins, and other antibiotics is increasing, with some strains producing extended-spectrum β-lactamases (Hafiz et al., 2024; Tham et al., 2012).

Similarly, *Trichophyton rubrum*, the primary causative agent of tinea pedis (athlete's foot), is a widespread dermatophyte affecting millions globally (Leung et al., 2023). In Africa, the prevalence of dermatophytosis remains high, with tinea pedis accounting for a significant proportion of infections (Olusola-Makinde et al.,2021). Although antifungal agents such as azoles and allylamines are available, treatment failure and recurrence are common. Emerging resistance, particularly to terbinafine and azoles, has been reported, with increasing minimum inhibitory concentrations (MICs) observed (Coussement et al..,2021). In Kenya, AMR poses a substantial health burden, contributing to approximately 37,300 deaths annually, with about 8,500 deaths directly linked to resistant infections (Murray et al., 2022). High levels of antibiotic misuse, poor sanitation, and limited awareness further exacerbate the situation. For example, studies have shown that a significant proportion of individuals engage in self-medication, and many lack proper knowledge regarding antibiotic use (Murray et al., 2022). Additionally, urinary tract infections (UTIs) remain prevalent, with high resistance rates reported against commonly prescribed antibiotics such as ciprofloxacin (Kiiru et al., 2023).

Given the increasing resistance observed in *P. mirabilis* and *T. rubrum*, there is an urgent need to explore alternative antimicrobial agents derived from natural sources. Two medicinal plants commonly used in East African traditional medicine are *Dovyalis abyssinica* and *Oxygonum sinuatum*. *Dovyalis abyssinica* (family Salicaceae) is traditionally used to treat a wide range of conditions, including stomachaches, fevers, and skin infections (Baylie et al., 2024; Belitibo et al., 2025). Previous studies have demonstrated antibacterial activity of its leaf and fruit extracts against pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Legesse et al., 2019). Additionally, the plant has shown notable cytotoxic activity against cancer cell lines, suggesting the presence of potent bioactive compounds (Belitibo et al., 2025). However, the antimicrobial activity of its root extracts against *P. mirabilis* and *T. rubrum* remains inadequately investigated.

Oxygonum sinuatum (family Polygonaceae) is another medicinal plant widely used in Kenya for treating fungal infections, inflammatory conditions, and venereal diseases (Ochwang'i et al., 2014). Phytochemical investigations have identified compounds such as flavonoids, alkaloids, and saponins within the plant. Bioassay-guided studies have also identified emodin as a key bioactive compound with pharmacological potential (Elbermawi et al., 2025). Despite these findings, there is limited data on the antimicrobial efficacy of its root extracts, particularly against *P. mirabilis* and *T. rubrum*. This study was therefore designed to address these research gaps by evaluating the phytochemical composition and antimicrobial activity of methanolic root extracts of *D. abyssinica* and *O. sinuatum*, and by determining their minimum inhibitory concentrations (MICs) against *P. mirabilis* and *T. rubrum*.

1.1 Statement of the Problem

Despite the widespread ethnomedicinal use of *Dovyalis abyssinica* and *Oxygonum sinuatum*, several critical knowledge gaps remain. First, the phytochemical composition of their root extracts is not well characterized, as most existing studies have focused primarily on the leaves and fruits. Second, the antimicrobial activity of these root extracts against clinically relevant pathogens such as *Proteus mirabilis* and *Trichophyton rubrum* (the causative agent of tinea pedis) has not been adequately investigated, despite increasing resistance of these organisms to conventional therapies.

Although a recent study by Belitibo et al. (2025) evaluated the antimicrobial activity of *D. abyssinica* root extracts against *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, reporting minimum inhibitory concentrations (MICs) as low as 0.195 mg/mL, evidence against *P. mirabilis* and *T. rubrum* remains lacking. Additionally, the same study demonstrated notable antifungal activity using *Solanum dasyphyllum* root extract against *T. rubrum*, with an inhibition zone of 20.67 mm, suggesting that related plant species may possess promising activity against dermatophytes. Third, the minimum inhibitory concentrations (MICs)—which are essential for determining antimicrobial potency—have not been established for *D. abyssinica* and *O. sinuatum* root extracts against *P. mirabilis* and *T. rubrum*. This study therefore aims to address these gaps by systematically evaluating the phytochemical composition, antimicrobial activity, and minimum inhibitory concentrations of methanolic root extracts of *D. abyssinica* and *O. sinuatum* against *P. mirabilis* and *T. rubrum*.

1.2 Research Objectives

i. To evaluate the presence of phytochemical constituents of D. abyssinica and O. sinuatum root extracts.
ii. To evaluate the antimicrobial activity of root extracts of D. abyssinica and O. sinuatum against P. mirabilis and T. pedis.

iii. To determine the MICs of D. abyssinica and O. sinuatum root extracts.

II. LITERATURE REVIEW

2.1 Introduction

This chapter reviews the existing literature on the phytochemical composition and antimicrobial properties of *Dovyalis abyssinica* and *Oxygonum sinuatum*, with particular emphasis on root extracts. It further provides detailed information on the target pathogens, *Proteus mirabilis* and *Trichophyton rubrum* (the causative agent of tinea pedis), including their clinical relevance, pathogenic mechanisms, and antimicrobial resistance profiles. Additionally, the chapter situates the study within a broader microbiological context by examining resistance mechanisms and highlighting the growing need for plant-based therapeutic alternatives in the face of declining antibiotic efficacy.

2.1.1 Global Burden of Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges of the 21st century. It is estimated to have directly caused approximately 1.27 million deaths worldwide in 2019 (Murray et al., 2022). Beyond mortality, AMR imposes a substantial economic burden. A systematic review and meta-analysis by Poudel et al. (2023), covering 29 studies published between 2016 and 2021, reported that the cost attributable to resistant infections ranges from $2,371 to $29,289 per patient episode, with an average additional hospital stay of 7.4 days. Furthermore, resistant infections are associated with increased risks of mortality (1.84-fold) and hospital readmission (1.49-fold) compared to susceptible infections.

Microorganisms develop resistance through several mechanisms, including reduced drug uptake, target modification, enzymatic degradation of antimicrobials, and efflux pump activity (Dhingra et al., 2020). These mechanisms may be intrinsic or acquired through horizontal gene transfer. The World Health Organization (WHO, 2023) emphasizes that the burden of AMR is particularly severe in low- and middle-income countries, where access to second-line therapies is limited.

Resistance rates to commonly used antibiotics, such as fluoroquinolones and third-generation cephalosporins, range between 10% and 40% in certain Gram-negative infections (Hafiz et al., 2024). This trend threatens to reverse decades of medical progress by increasing morbidity, prolonging hospital stays, and elevating mortality rates. Additionally, the slow pace of new antibiotic development has widened the gap between emerging resistance and therapeutic innovation.

Healthcare-associated infections (HAIs) represent a significant economic burden in sub-Saharan Africa. A report by WaterAid and the World Bank (2024) estimated that HAIs cost the region up to USD 8.4 billion annually, accounting for approximately 1.1% of GDP and 4.5% of national health budgets. Catheter-associated urinary tract infections (CAUTIs), often caused by multidrug-resistant *P. mirabilis*, are among the most prevalent and costly HAIs. These findings underscore the urgent need for affordable and locally accessible antimicrobial alternatives.

2.2 Plant-Derived Antimicrobial Agents

Medicinal plants are recognized as valuable sources of bioactive compounds with diverse therapeutic properties. Unlike synthetic antibiotics, which typically act on single molecular targets, phytochemicals often exhibit multi-target mechanisms of action (Jadimurthy et al., 2023). These compounds—including alkaloids, flavonoids, terpenoids, tannins, and saponins—have demonstrated significant antimicrobial activity (Cowan, 1999). Natural products possess structural diversity and functional complexity, enabling them to interfere with multiple microbial pathways simultaneously. This reduces the likelihood of resistance development compared to conventional antibiotics (Boakye et al., 2019). Additionally, plant-derived compounds are generally biodegradable and biocompatible. One key advantage of crude plant extracts is the potential for synergistic interactions among phytochemicals. Synergy occurs when combined compounds produce an effect greater than the sum of their individual actions. This phenomenon enhances antimicrobial efficacy, reduces required dosages, and may overcome resistance mechanisms (Shahin et al., 2025).

Several studies highlight such synergistic effects. For instance, Shahin et al. (2025) demonstrated that combining essential oils with antibiotics significantly enhanced antibacterial activity against resistant strains, as confirmed by fractional inhibitory concentration index (FICI) analysis. Similarly, Matsuete et al. (2025) reported that *Kotschya strigosa* leaf extract exhibited synergistic and additive effects when combined with conventional antibiotics, alongside mechanisms such as membrane disruption and inhibition of ATPase activity. Elbermawi et al. (2025) further showed that although isolated compounds from *O. sinuatum* exhibited limited activity individually, the crude extract displayed notable antimicrobial effects, likely due to phytochemical synergy. These findings support the use of crude extracts in antimicrobial research.

2.3 Phytochemical Compounds and Antimicrobial Activity of *Dovyalis abyssinica*

Dovyalis abyssinica (family Salicaceae) is a thorny shrub native to East Africa and widely used in traditional medicine to treat infections and inflammatory conditions (Belitibo et al., 2025). Ethnomedicinal practices among Kenyan communities also utilize its roots for treating gonorrhea, bilharzia, and typhoid (Adongo, 2013). Phytochemical studies have identified flavonoids, alkaloids, saponins, tannins, and terpenoids in the leaves (Legesse et al., 2019). However, research on root extracts remains limited. Most antimicrobial studies have focused on leaves, fruits, and stems, with only recent investigations examining root activity.

Available evidence indicates that root extracts possess broad-spectrum antibacterial activity. Belitibo et al. (2025) reported MIC values as low as 0.195 mg/mL against *Escherichia coli* and *Pseudomonas aeruginosa*. The extract also demonstrated antifungal activity against *T. rubrum* and notable cytotoxicity against cancer cell lines. Further phytochemical investigations identified compounds such as tremulacin, cochinchiside A, and stigmasterol (Belitibo et al., 2024). Some isolated compounds exhibited moderate antibacterial activity, although the crude extract showed superior efficacy, again suggesting synergistic interactions. Despite these findings, no studies have evaluated *D. abyssinica* root extracts against *P. mirabilis*, highlighting a critical research gap.

2.4 Phytochemical Compounds and Antimicrobial Activity of *Oxygonum sinuatum*

Oxygonum sinuatum (family Polygonaceae) is an herbaceous plant widely distributed in East Africa and traditionally used to treat infections and inflammatory conditions (Ochwang'i et al., 2014). Phytochemical studies have identified alkaloids, flavonoids, tannins, and glycosides in the plant. Recent work by Elbermawi et al. (2025) identified sixteen compounds from the aerial parts, including a novel lignan glycoside (oxylignoside). However, individual compounds exhibited limited antimicrobial activity, suggesting that therapeutic effects may arise from synergistic interactions within the crude extract.Importantly, quantitative antimicrobial data—particularly MIC values—for root extracts of *O. sinuatum* against *P. mirabilis* and *T. rubrum* remain unavailable. This represents a significant knowledge gap addressed by the present study.

2.5 Biology of Target Pathogens and Resistance Mechanisms

2.5.1 *Proteus mirabilis*

Proteus mirabilis is a Gram-negative, rod-shaped bacterium belonging to the family Morganellaceae. It is a common opportunistic pathogen associated with catheter-associated urinary tract infections (CAUTIs), wound infections, and bacteremia (Armbruster et al., 2018). Pathogenicity: Key virulence factors include urease production, swarming motility, biofilm formation, and adhesins. Urease activity leads to increased urine pH and crystal formation, contributing to catheter blockage and stone formation (Schaffer & Pearson, 2017).

Antimicrobial Resistance: *P. mirabilis* exhibits intrinsic resistance to certain antibiotics and has increasingly acquired resistance to beta-lactams, fluoroquinolones, and aminoglycosides. The emergence of extended-spectrum beta-lactamase (ESBL)-producing strains has further complicated treatment (Hafiz et al., 2024). Biofilm Formation: The organism forms crystalline biofilms on catheter surfaces, which enhance resistance to antibiotics and immune responses. These biofilms limit drug penetration and promote persistence (Wasfi et al., 2020).

2.5.2 *Trichophyton rubrum*

Trichophyton rubrum is a dermatophytic fungus responsible for superficial infections such as tinea pedis. It infects keratinized tissues and is characterized by slow growth and chronic infection patterns. Resistance and Treatment Challenges:Resistance to conventional antifungals has been increasingly reported, necessitating the exploration of alternative therapies. Natural products, including plant extracts and essential oils, have shown promising antifungal activity through mechanisms such as membrane disruption and oxidative stress induction (Sun et al., 2026).

2.6 Summary of Literature Gaps

Despite the growing interest in medicinal plants as potential sources of alternative antimicrobial agents, several important gaps remain. First, phytochemical characterization of the root extracts remains limited, particularly regarding the identification and profiling of the bioactive constituents responsible for antimicrobial activity. Second, there is limited evidence on the antimicrobial activity of the extracts specifically against *Proteus mirabilis* and *Trichophyton rubrum*, despite their clinical importance. Third, MIC data for *Oxyanthus sinuatum* root extracts are lacking, limiting understanding of the minimum concentrations required to inhibit the growth of the target organisms. Finally, there is insufficient investigation of potential synergistic interactions among the phytochemical constituents, which may contribute to the overall antimicrobial effects of the extracts.These gaps justify the need for the present study, which evaluates the phytochemical constituents and antimicrobial activity of *Dissotis abyssinica* and *Oxyanthus sinuatum* root extracts against *P. mirabilis* and *T. rubrum*, including determination of their MICs.

2.7 Conclusion

The reviewed literature demonstrates the therapeutic potential of medicinal plants while highlighting critical gaps in current knowledge. Addressing these gaps is essential for developing alternative antimicrobial agents, particularly in resource-limited settings where drug resistance poses a significant threat.

III. METHODOLOGY

3.1 Research Design

This study employed an experimental laboratory-based design. Independent variable: Concentration of plant extracts (0, 2, 4, 8, and 16 mg/mL). Dependent variables: Diameter of inhibition zones (mm), Minimum inhibitory concentration (MIC) (mg/mL). All treatments were performed in triplicate to ensure reliability. Positive controls: Ciprofloxacin (5 µg/well) for bacterial assays, Fluconazole (25 µg/mL) for fungal assays. Negative control: Dimethyl sulfoxide (DMSO)

3.2 Study Area

The study was conducted at the Botany Laboratory, Maseno University, Kenya. Plant samples were collected from Mihuti Village, Mukurweini Sub-County, Nyeri County, Kenya. Nyeri County lies at approximately 0.42°S, 36.95°E, with an average elevation of 1,750 meters above sea level. The region experiences a cool highland climate, with temperatures ranging from 12°C to 26°C and annual rainfall between 1,200–1,500 mm.

3.3 Collection and Authentication of Plant Materials

Whole plants of *Dovyalis abyssinica* and *Oxygonum sinuatum* were collected from Mihuti Village. Authentication was conducted at the Maseno University Herbarium, and voucher specimens were assigned as follows:*D. abyssinica*: DA-2025-00 and *O. sinuatum*: OS-2025-002. The collected samples were transported to the laboratory for processing.

3.4 Preparation of Plant Material

Roots were washed thoroughly with tap water to remove soil and debris. They were then shade-dried at room temperature (25 ± 2°C) for 14 days. The dried roots were ground into coarse powder using a laboratory mill and stored in sterile, airtight containers at 4°C until extraction.

3.5 Extraction Procedure

Methanolic extraction was carried out using the maceration method as described by Geyid et al. (2005), with slight modifications. Briefly, 200 g of powdered root material was soaked in 500 mL of 95% methanol and left to stand for 72 hours, with intermittent shaking every 8 hours to enhance extraction efficiency. The mixture was first filtered through muslin cloth to remove coarse particles and subsequently through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was then concentrated using a rotary evaporator at 40°C under reduced pressure to remove the solvent. The resulting dried extract was stored in sterile vials at 4°C until further use. The percentage yield of the extract was subsequently calculated.

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of dry powder}} \times 100$$

Rationale for Solvent Selection: Methanol was selected due to its intermediate polarity, enabling extraction of a broad range of phytochemicals, including flavonoids, alkaloids, tannins, saponins, and glycosides (Cowan, 1999). owever, reliance on a single solvent may limit phytochemical diversity. Future studies should employ sequential solvent extraction (hexane → ethyl acetate → methanol/water) for comprehensive profiling (Belitibo et al., 2025).

3.6 Test Microorganisms

The study utilized standard microbial strains, namely *Proteus mirabilis* (ATCC 29906), a Gram-negative bacterium, and *Trichophyton rubrum* (ATCC 28188), a dermatophyte responsible for tinea pedis. Both strains were obtained from the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. The cultures were maintained under appropriate laboratory conditions, with bacterial strains preserved on nutrient agar slants at 4°C, while fungal strains were maintained on Sabouraud dextrose agar slants at 4°C.

3.7 Media Preparation

3.7.1 Mueller-Hinton Agar (MHA)

19 g of MHA powder was dissolved in 500 mL distilled water, heated, and autoclaved at 121°C for 15 minutes. The medium was poured into sterile Petri dishes to a uniform depth of 4 mm.

3.7.2 Sabouraud Dextrose Agar (SDA)

65 g of SDA powder was dissolved in 1000 mL distilled water, sterilized, and poured under aseptic conditions. Prepared media were stored at 4°C until use.

3.8 Inoculum Preparation

Inocula were standardized following CLSI guidelines. Bacteria: Adjusted to 0.5 McFarland (~1.5×10^8 CFU/mL) and diluted to ~5×10^5 CFU/Ml. Fungi: Spore suspension adjusted to ~1×10^6 spores/mL

3.9 Phytochemical Screening

Qualitative phytochemical screening was conducted using standard procedures as described by Harborne (1998), Sofowora (2008), and Evans (2009). The analysis involved testing for the presence of various phytochemical constituents, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, quinones, and anthraquinones. The results of the screening were recorded as either present (+) or absent (−) for each phytochemical component.

3.10 Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) analysis was performed using silica gel 60 F_{254} plates as the stationary phase. The mobile phase consisted of a solvent system of toluene and ethyl acetate in a ratio of 3:1 (v/v). After development, the chromatographic plates were visualized under ultraviolet (UV) light at wavelengths of 254 nm and 366 nm to detect separated compounds. The retention factor (Rf) values for the observed spots were subsequently determined.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

3.11 Antimicrobial Activity Assay

The antimicrobial activity of the extracts was evaluated using the agar well diffusion method as described by Anyanwu and Okoye (2017). Wells of 6 mm diameter were aseptically prepared in the agar plates, and different concentrations of the extract (2, 4, 8, and 16 mg/mL) were tested. A volume of 50 µL of each extract concentration was introduced into the respective wells. Ciprofloxacin was used as the positive control for bacterial assays, while fluconazole served as the positive control for fungal assays. Dimethyl sulfoxide (DMSO) was used as the negative control. The inoculated plates were incubated at 37°C for 24 hours for bacterial cultures and at 25°C for 48–72 hours for fungal cultures. Following incubation, the antimicrobial activity was determined by measuring the zones of inhibition around each well in millimeters.

3.12 Minimum Inhibitory Concentration (MIC)

3.12.1 Procedure

MIC was determined using the broth microdilution method (CLSI guidelines). Concentration range: 10.0 to 0.078125 mg/mL Resazurin dye used as indicator

3.12.2 MIC Determination

The MIC was defined as the lowest concentration with no color change (blue), indicating absence of microbial growth.

3.13 Data Collection and Statistical Analysis

3.13.1 Data Collection

The phytochemical screening results were recorded qualitatively as either present (+) or absent (−). Antimicrobial activity was assessed by measuring zones of inhibition in millimeters, expressed as mean ± standard deviation. The minimum inhibitory concentration (MIC) was determined and recorded as the lowest concentration of the extract that inhibited visible microbial growth. For thin-layer chromatography (TLC) analysis, retention factor (Rf) values were calculated for the separated compounds.

3.13.2 Statistical Analysis

Data analysis was performed using R software (version 4.2.1). One-way analysis of variance (ANOVA) was employed to compare the mean zones of inhibition across the different extract concentrations. Where significant differences were observed, Fisher's Least Significant Difference (LSD) post hoc test was applied for pairwise comparisons between

groups. Statistical significance was set at $p < 0.05$. All results were expressed as mean values accompanied by their standard deviations (mean ± SD).

IV. FINDINGS & DISCUSSION

4.1 Findings

4.1.1 Phytochemical Composition of *Dovyalis abyssinica* and *Oxygonum sinuatum* Root Extracts

Qualitative phytochemical screening of methanolic root extracts of *Dovyalis abyssinica* and *Oxygonum sinuatum* revealed the presence of several bioactive compounds. The results are summarized in Table 1. A representative image of the powdered root samples and a positive saponin test (persistent frothing) is presented in Figure 1

Figure 1. Powdered roots of *D. abyssinica* and *O. sinuatum* and a positive saponin test showing persistent frothing.

Table 1

Qualitative phytochemical constituents of methanolic root extracts

Phytochemical Compound	*D. abyssinica*	*O. sinuatum*
Glycosides	+	+
Steroids	+	+
Flavonoids	+	+
Terpenoids	−	−
Saponins	+	+
Alkaloids	+	+
Anthraquinones	−	−
Quinones	+	+
Cardiac glycosides	+	+
Tannins	+	−

Note. (+) = present; (−) = absent.

4.2 Antimicrobial Activity of Root Extracts

The antimicrobial activity of methanolic root extracts of D. abyssinica and O. sinuatum was evaluated against Proteus mirabilis and Trichophyton rubrum using the agar well diffusion method. The negative control (DMSO) produced a baseline inhibition zone of 6 mm (well diameter), which was subtracted from all measurements to obtain net inhibition zones**.**

4.2.1 Antimicrobial Activity of *D. abyssinica*

The inhibition zones produced by *D. abyssinica* root extract against *P. mirabilis* and *T. rubrum* are presented in Table 2 and Figure 1.

Table 2

Inhibition zones (mm) of D. abyssinica *root extract (mean ± SD, n = 3)*

Concentration (mg/mL)	*P. mirabilis*	*T. rubrum*
0 (DMSO control)	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
2	7.11 ± 0.84^{d}	4.94 ± 0.73^{d}
4	10.44 ± 1.01^{c}	7.28 ± 0.89^{c}
8	13.33 ± 0.88^{b}	15.56 ± 0.73^{b}
16	13.33 ± 0.88^{b}	15.11 ± 0.78^{b}
Positive control	24.56 ± 1.13^{a}	21.56 ± 0.88^{a}

LSD: 2.43 (*P. mirabilis*), 1.14 (*T. rubrum*) CV%: 14.68, 17.79, $p < 0.0001$

Note. Values represent net inhibition zones. Means with different superscripts within a column differ significantly at $p < 0.05$ (Fisher's LSD).

Figure 1

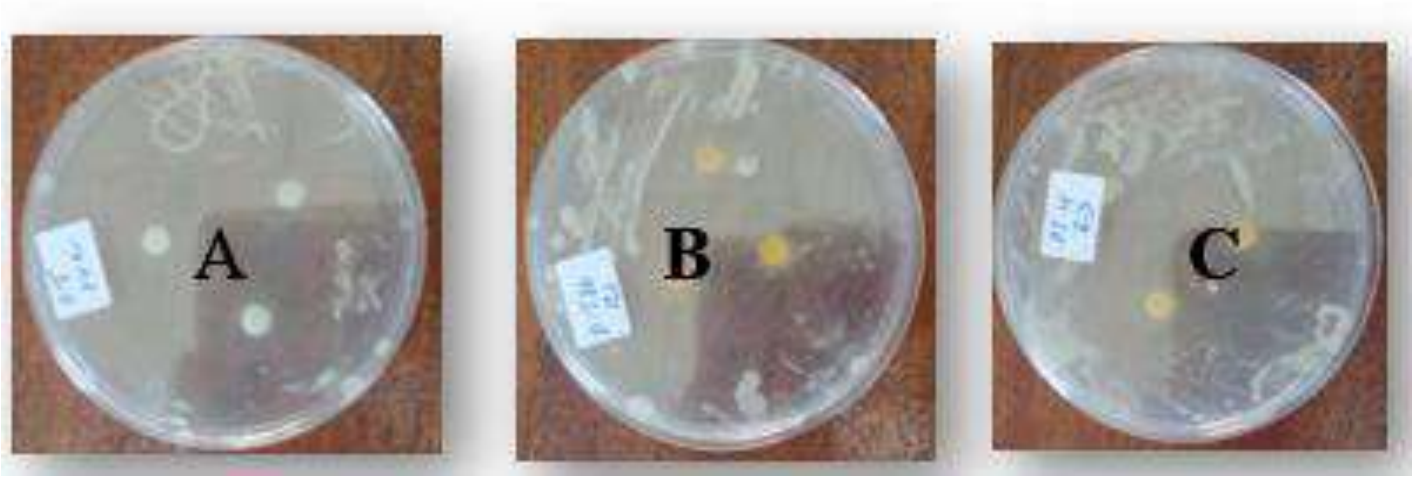

Plate 1

Inhibition zones of D. abyssinica root extract against (A) P. mirabilis, (B) T. rubrum, and (C) positive controls.

4.2.2 Antimicrobial Activity of *O. sinuatum*

The antimicrobial activity of *O. sinuatum* root extract is presented in Table 3 and Figure 3.

Table 3

Inhibition zones (mm) of* O. sinuatum *root extract (mean ± SD, n = 3)

Concentration (mg/mL)	*P. mirabilis*	*T. rubrum*
0 (DMSO control)	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
2	4.78 ± 0.83^{d}	4.94 ± 0.73^{d}
4	8.56 ± 0.73^{c}	7.28 ± 0.89^{c}
8	8.33 ± 0.88^{c}	15.56 ± 0.73^{b}
16	16.00 ± 1.15^{b}	15.11 ± 0.78^{b}
Positive control	24.56 ± 1.13^{a}	21.56 ± 0.88^{a}

LSD: 2.43 (*P. mirabilis*), 1.14 (*T. rubrum*) CV%: 14.68, 17.79, $p < 0.0001$

Note: *Values represent net inhibition zones. Means with different superscripts within a column differ significantly at $p < 0.05$.*

Figure 2

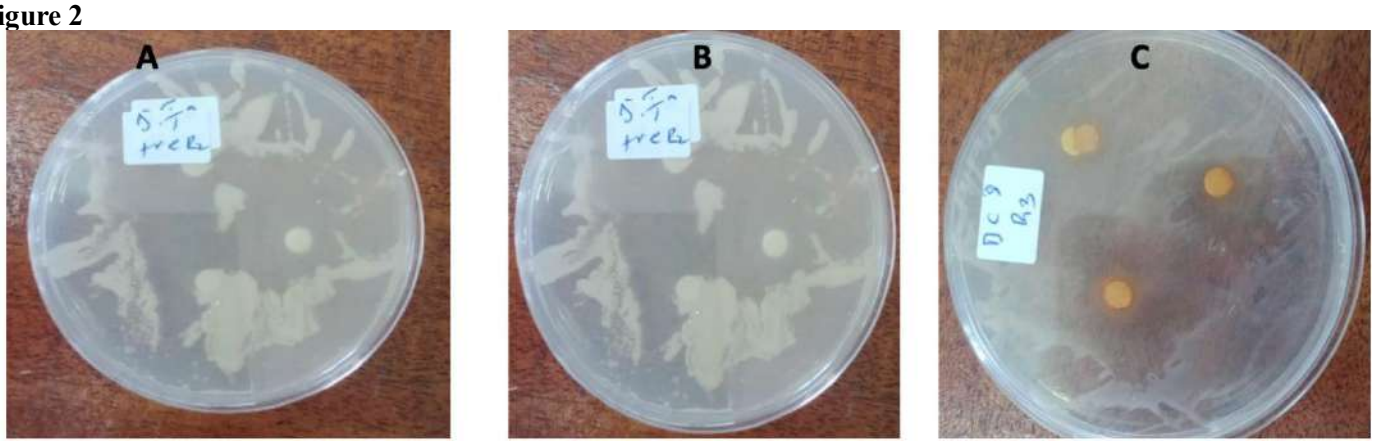

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Plate 2

Inhibition zones of O. sinuatum root extract at selected concentrations against test organisms.

4.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract that prevented a color change of resazurin (remained blue), indicating absence of visible microbial growth. MIC values were determined from a two-fold serial dilution series and are presented in Table 4.

Table 4

Minimum inhibitory concentrations (MICs) of root extracts (mg/mL)

Extract	*P. mirabilis*	*T. rubrum*
D. abyssinica	2.5	2.5
O. sinuatum	2.5	5.0

Note: *MIC values represent the lowest concentration inhibiting visible growth after incubation (24 h for bacteria; 48–72 h for fungi).*

4.4 Thin-Layer Chromatography (TLC) Profiles

Thin-layer chromatography (TLC) was performed to profile phytochemical constituents of the extracts. Chromatograms were visualized under UV light at 254 nm and 366 nm. Representative TLC plates are shown in Figure 4, and corresponding retention factor (Rf) values are presented in Table 5.

Figure 3

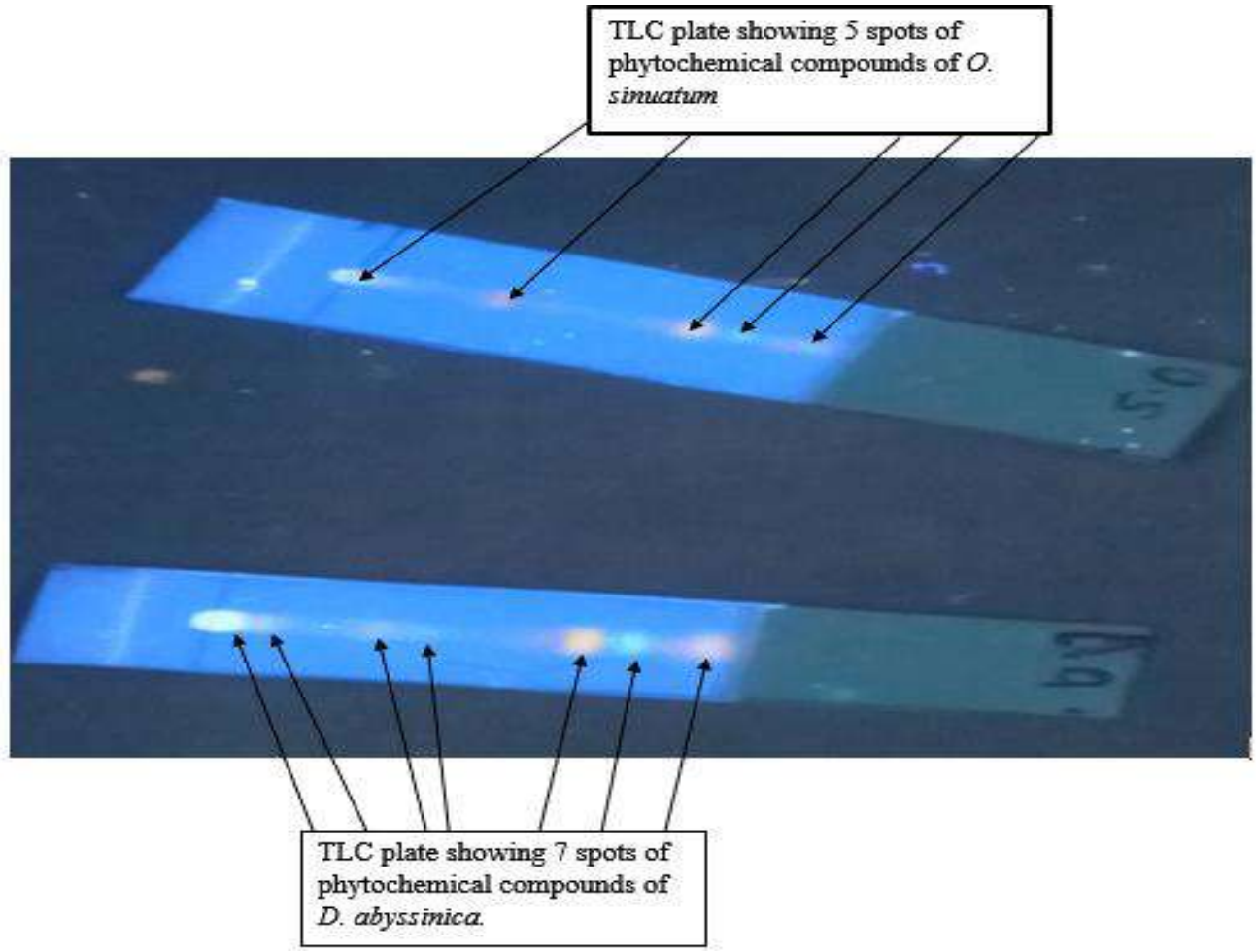

Figure 1

TLC chromatograms of (A) D. abyssinica (seven spots) and (B) O. sinuatum (five spots) under UV light (366 nm).

Table 5

Retention factor (Rf) values of detected compounds

Spot No.	***D. abyssinica***	***O. sinuatum***
1	0.0222	0.1111
2	0.0667	0.2667
3	0.2222	0.5556
4	0.3111	0.6222
5	0.5111	0.6889
6	0.5778	–
7	0.6667	–

4.5 Statistical Analysis

One-way analysis of variance (ANOVA) revealed statistically significant differences in inhibition zones across extract concentrations for both *D. abyssinica* and *O. sinuatum* against *P. mirabilis* and *T. rubrum* ($p < 0.0001$). Post hoc comparisons using Fisher's Least Significant Difference (LSD) test indicated that higher concentrations (8 and 16 mg/mL) produced significantly larger inhibition zones compared to lower concentrations (2 and 4 mg/mL). No statistically significant differences were observed between 8 mg/mL and 16 mg/mL in most cases. Positive controls (ciprofloxacin and fluconazole) exhibited significantly greater inhibition than all extract concentrations ($p < 0.05$).

4.6 Discussion

4.6.1 Phytochemical Composition of Root Extracts

Phytochemical Profile of *D. abyssinica* and *O. sinuatum:* Qualitative phytochemical screening revealed that the methanolic root extract of *Dovyalis abyssinica* contained glycosides, steroids, flavonoids, saponins, alkaloids, quinones, cardiac glycosides, and tannins, whereas *Oxygonum sinuatum* contained all the aforementioned compounds except tannins. Neither extract tested positive for terpenoids or anthraquinones. These findings are consistent with previous reports on *D. abyssinica*, particularly studies on leaf extracts that identified flavonoids, alkaloids, saponins, and tannins (Legesse et al., 2019). Similarly, earlier investigations of *O. sinuatum* have confirmed the presence of alkaloids, flavonoids, and saponins (Nantinda et al., 2025). However, this study provides novel insight into the phytochemical composition of the root extracts of both species, which has been largely underexplored.

The detected phytochemicals are widely recognized for their antimicrobial properties. Tannins, identified only in *D. abyssinica*, are known to precipitate microbial proteins and disrupt cell wall synthesis, which may partly explain its comparatively stronger activity at lower concentrations (Cowan, 1999; Baylie et al., 2024). Flavonoids and alkaloids, present in both extracts, are associated with inhibition of nucleic acid synthesis and interference with microbial enzymatic systems (Wink, 2020). Saponins may enhance antimicrobial activity by increasing membrane permeability through interactions with membrane sterols, a mechanism particularly relevant in fungal pathogens (Singh et al., 2017). Quinones, also detected in both extracts, are capable of generating reactive oxygen species, contributing to oxidative damage in microbial cells.

Comparison with Existing Literature: The phytochemical profile observed in *D. abyssinica* roots aligns with earlier reports on aerial plant parts, although the absence of terpenoids contrasts with some previous findings (Legesse et al., 2019). This discrepancy may be attributed to differences in plant part, geographical origin, or extraction solvent. Although anthraquinones were not detected in the present study, previous research has identified anthraquinone derivatives such as emodin in *O. sinuatum* (Crawford et al., 2011). Their absence in the current extracts may reflect variation in plant chemistry due to environmental factors, seasonal changes, or extraction conditions. This highlights the well-documented variability of secondary metabolites in medicinal plants. Thin-layer chromatography (TLC) further supported the diversity of phytochemicals present. *D. abyssinica* exhibited a greater number of bands, including highly polar compounds, compared to *O. sinuatum*. This broader chemical diversity may contribute to its observed antimicrobial potency, particularly against fungal pathogens.

4.6.2 Antimicrobial Activity of Root Extracts

Both plant extracts demonstrated antimicrobial activity against *Proteus mirabilis* and *Trichophyton pedis*, with effects increasing significantly with concentration ($p < 0.0001$). This dose-dependent response is characteristic of plant-derived extracts, where higher concentrations provide greater availability of bioactive compounds, potentially enhancing synergistic interactions (Boakye et al., 2019). Activity of *D. abyssinica:* The root extract of *D. abyssinica* exhibited strong antimicrobial activity against both test organisms. The observed increase in inhibition zones with concentration indicates a clear dose-response relationship. Notably, maximal activity was reached at 8 mg/mL, beyond which no significant increase was observed. This plateau effect may be attributed to diffusion limitations inherent in agar-based assays, as previously described by Eloff (1998), rather than a true lack of increased antimicrobial potency.

The relatively strong antifungal activity against *T. pedis* may be linked to the presence of tannins and flavonoids, which are known to disrupt fungal cell wall integrity and inhibit spore germination (Aboody & Mickymaray, 2022). Additionally, saponins may enhance membrane permeability, further potentiating antifungal effects. The antibacterial activity against *P. mirabilis* is likely associated with alkaloids and flavonoids, which interfere with DNA replication and protein synthesis. Quinones may also contribute through oxidative stress mechanisms, which are particularly effective against Gram-negative bacteria due to their membrane-disruptive properties.

Activity of *O. sinuatum: O. sinuatum* also demonstrated significant antimicrobial activity, with larger inhibition zones observed against *P. mirabilis* at higher concentrations compared to *D. abyssinica*. Despite the absence of tannins, its comparable minimum inhibitory concentration (MIC) against *P. mirabilis* suggests that other phytochemicals, such as flavonoids, alkaloids, and quinones, may compensate through synergistic effects.

The antifungal activity of *O. sinuatum* was similar to that of *D. abyssinica* at higher concentrations, supporting its traditional use in the treatment of skin infections. However, its higher MIC against *T. pedis* suggests relatively lower intrinsic potency, despite comparable inhibition zone sizes.

4.6.3 Comparative Evaluation with Other Plant Extracts

The MIC values obtained in this study (2.5–5.0 mg/mL) fall within the range reported for many crude plant extracts. For instance, fermented plant extracts have demonstrated MIC values between 0.98 and 3.92 mg/mL against *P. mirabilis* (Hai & Khuong, 2025), while other methanolic extracts have shown higher MICs. These comparisons suggest that both *D. abyssinica* and *O. sinuatum* possess moderate but comparable antimicrobial efficacy. Interestingly, although *O. sinuatum* produced larger inhibition zones against *P. mirabilis*, both extracts exhibited identical MIC values. This discrepancy highlights the limitations of diffusion-based assays, where zone size may reflect compound diffusibility rather than intrinsic antimicrobial potency. Conversely, *D. abyssinica* demonstrated a lower MIC against *T. pedis*, indicating greater antifungal potency despite similar inhibition zones. Comparisons with purified compounds, such as kobusin (MIC = 0.039 mg/mL), reveal that isolated phytochemicals may exhibit substantially higher potency than crude extracts. However, crude extracts may offer advantages through synergistic interactions among multiple compounds, which can enhance efficacy and reduce resistance development.

V. CONCLUSION & RECOMMENDATONS

5.1 Conclusion

In summary, both *D. abyssinica* and *O. sinuatum* root extracts demonstrated significant antimicrobial activity, attributable to their diverse phytochemical composition. While *O. sinuatum* exhibited greater diffusion-based activity against *P. mirabilis*, *D. abyssinica* showed higher intrinsic potency against *T. pedis*, as reflected by lower MIC values. These findings support the continued investigation of these plants as potential sources of novel antimicrobial agents.

5.2 Recommendations

Based on the findings and limitations of this study, the following recommendations are made:
Use of multiple solvents for extraction – Future studies should employ sequential extraction with at least three solvents of varying polarity (e.g., hexane, ethyl acetate, and methanol or water) to obtain a more comprehensive phytochemical profile and to compare the antimicrobial activities of each extract type. The present study used only methanol due to resource constraints, bioassay-guided fractionation – The active compounds responsible for the observed antimicrobial activity should be isolated and characterized using chromatographic and spectroscopic techniques to identify lead molecules for drug development and in vivo studies – Animal model studies are necessary to confirm the efficacy, safety, and pharmacokinetics of the active extracts before clinical trials can be considered.

Declaration of Interest

The authors declare that they do not have any known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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