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Antimicrobial activities of crude extracts from selected medicinal climbers against *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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Abstract

Antimicrobial resistance (AMR) is a major global health threat to humans, and combating it is increasingly challenging. Recent studies suggest that plant-derived antimicrobials could be effective in combating AMR. Commonly used as herbal medicine, *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia* are climbers with significant medicinal value for treating or managing various human diseases and conditions. This study aimed to evaluate the effect of methanol leaf, stem, and root extracts of these climbers on the growth of *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Plant samples were collected from Kisii, the shores of Lake Victoria-Kisumu, and Maseno University farm in Kenya, then shade-dried and extracted with methanol. Antimicrobial efficacy of the extracts was assessed using disc diffusion method at 25, 50, 75, and 100 mg/ml concentrations along with the determination of minimum inhibitory concentration (MIC) at 0.625, 1.25, 2.5, 5 and 10 mg/ml concentrations, minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC). Data was subjected to analysis of variance (ANOVA) followed by T-test analysis. Means were separated and compared using the least significant difference (LSD) at $P \leq 0.05$. Largest zones of inhibition were observed in 100 mg/ml *R. cordifolia* leaf extract against *P. aeruginosa* (21.00 mm), and *S. aureus* (15.57 mm), and 100 mg/ml *R. cordifolia* root extract against *C. albicans* (11.67 mm). MIC values for *R. cordifolia* extracts against *C. albicans* was 5 mg/ml for both leaf and root extracts and 10 mg/ml for the stem whereas for both *P. aeruginosa* and *S. aureus* they were all at 10 mg/ml. However, neither MBC nor MFC was observed. These research findings suggest that *A. lobata*, *I. aquatica*, and *R. cordifolia* have potential as antimicrobial agents, particularly *R. cordifolia*, with antimicrobial properties linked to phytochemicals like anthraquinones, terpenoids, alkaloids, and phenolic compounds, supporting their use in traditional herbal medicine.

1. Introduction

Antimicrobial resistance (AMR) poses a major public health challenge of the 21st century in the management of infectious diseases (Pennisi et al., 2025). Globally, antimicrobial resistance (AMR) infection is ranked third as the leading cause of death after cardiovascular diseases (Salam et al., 2023). It is estimated that by 2050 over 10 million people could be killed annually by antimicrobial resistance with a cost of \$300 billion to more than \$1 trillion (Dadgostar, 2019; Pennisi et al., 2025). The immense, unsuitable, non-uniform, and indiscriminate use of antibiotics has exacerbated the AMR, rendering many available medications ineffective (Reygaert, 2018).

Human beings can acquire antimicrobial-resistant bacteria and fungi from various sources and routes which include; human-to-human transmission, direct contact with food-producing animals and pets, food-borne transmission, and through the environment (Koutsoumanis et al., 2021; Mudenda, 2024). According to the World Health Organization (WHO), AMR is a critical priority that requires nations to implement innovative antimicrobial stewardship strategies to combat the emergence of multidrug-resistant strains "Superbugs" (Pennisi et al.,

2025). Worryingly, during this period of SARS-CoV-2 pandemic, the AMR situation has been aggravated mostly due to the mis- and over-use of antibiotics, treatments that induce immunosuppression, and prolonged hospitalization (Gajic et al., 2022).

According to Kadri (2020), *Candida* species (which include *C. albicans*), *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were listed by the United States Centers for Disease Control and Prevention (US CDC) among the serious antimicrobial drug resistance threats in the United States. In 2019, *S. aureus* and *P. aeruginosa* were listed among the top five leading pathogens responsible for 54.9% of deaths among the investigated bacteria (Ikuta et al., 2022). Kariuki et al. (2022) revealed that the prevalence of carbapenem-resistance *P. aeruginosa* from studies conducted in Uganda, Ghana, South Africa, Zambia, Nigeria, and Ethiopia ranged from 6% to 34.8%. In East Africa, methicillin resistance *S. aureus* (MRSA) prevalence rates among patients and healthcare workers were found to be high with a range of 31.5 to 42% (Wangai et al., 2019). In Kenya, MRSA, and vancomycin-resistant *S. aureus* were detected in environmental samples from one of Kenyan teaching hospitals with some studies reporting high proportions of MRSA (Lord et al., 2021). Additionally, from the same study, carbapenem-resistance *P.*

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aeruginosa isolates were also identified of which 13.7% were resistant to both imipenem and meropenem with meropenem resistance showing even higher levels. *C. albicans* resistance to amphotericin B has also been reported in the country at a rate of 25.6% (Africa & dos Santos Abrantes, 2016). This representative data is a clear indication that better strategies have to be established to control the AMR situation in Kenya and other developing countries.

Globally, an estimated 75-90% of the rural population (excluding Western countries) relies on traditional medicines as their only health system (Agidew, 2022). However, understanding medicinal herbs, including their phytochemical and pharmacological properties, and standardization is essential for determining appropriate dosages in an informed manner (Kiteme et al., 2023). Plants are considered to be a suitable approach in the control of AMR in that they are known to be inexhaustible and inexpensive (economical) source of antimicrobials with minimal toxicity, abundant chemical diversity and low chances of developing resistance in comparison to synthetic and semi-synthetic antimicrobials. The World Health Organization (WHO) has recorded over 20,000 species of medicinal plants from the total data of all known plant species, of which over 1340 plant species with known antimicrobial activity with over 30,000 antimicrobial compounds have been identified (Vaou et al., 2021). These plant-based antimicrobials (PBAs) contain a particular clinical value in that their bioactive components generally do not generate any resistance (Cheesman et al., 2017).

Climbing plants are among the obvious life forms in the different vegetation covers worldwide especially the tropical rain forests and even the modern temperate (Gianoli et al., 2012). Climbers are either woody (lianas) or non-woody (vines) plants that constitute one of the most conspicuous elements (Pandi et al., 2022). Climbers typically perform better under drought, and anthropogenic disturbances. Their ability for vegetative propagation, higher fecundity, and rigorous germination capabilities make climbers one of the highly competitive life forms in forest ecosystems (Pandi et al., 2022).

Several climbers have high medicinal value in addition to aesthetic use but they are widely used as herbal medicine in traditional systems (Deepa & Thomas, 2020). Interestingly, so far there has not been any report on estimated numbers of medicinal climbing plants used by human beings around the world in orthodox and traditional medicines (Ali et al., 2016). However, according to Eloff (2019), it is not a good practice to select plants to search for new antibiotics based solely on traditional usage in that there is no statistically significant difference in antimicrobial activity between plants used traditionally and plants randomly selected. All plants exhibit antimicrobial activity given a high enough dose. Thus, *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia*, the three climbers that were used in the present research study, were selected by considering various factors such as their ethnobotanical significance, environmental and social impacts, availability, and abundance.

Adenia lobata (Jacq.) Engl. is a large liana belonging to the Passifloraceae family (Fowa et al., 2019). *Ipomoea aquatica* is an often trailing vine belonging to the family Convolvulaceae with milky sap and it is known to be an aquatic or semi-aquatic consumable herb (Dewanjee et al., 2015). *Rubia cordifolia* is among the 70 species that belong to the Rubiaceae family, genus *Rubia* and its distribution is known to be extensively worldwide including Kenya (Gao et al., 2016; Jeremiah et al., 2023).

Adenia lobata is extensively used in traditional medicine to treat abscesses and wounds, palpitations, coughs, bronchitis, fever, rheumatic pains, and abdominal pains (Agoreyo et al., 2012). The plant has been also used to treat syphilis, gonorrhea, and cancer of the nose (Agoreyo et al., 2012). In Kisii-Kenya, *A.*

lobata is locally used to treat rheumatism, gastrointestinal issues, abdominal pain, abscesses, boils, and fever-related conditions. It also serves as animal fodder, especially for goats and cattle, and providing shade in homesteads (Mayianda J. Meshack personal communication, 2024). *I. aquatica* in the traditional setup, has been used to treat/control liver diseases, constipation, diabetes, abscesses, high blood pressure, antimicrobial diseases, and inflammatory conditions (Malakar & Choudhury, 2015). In Kenya, *I. aquatica* is widely distributed and has mainly been used as a vegetable by humans, as well as for animal and fish feeds (Chepkirui et al., 2021; Owino, 1998). *R. cordifolia* is well-known as a traditional Indian and Chinese medicinal plant and it has been categorized in the Chinese pharmacopoeia (Wen et al., 2022). It's a beneficial medicinal plant with numerous important roles such as having antioxidant, potent blood purifier, antiplatelet, anti-diabetic, anti-stress, and vasodilating properties. Anti-cancer, anti-inflammatory, antioxidant, and antimicrobial properties have also been detected in this species (Ismail et al., 2016). In Kenya, *R. cordifolia*, has been used in traditional treatments for malaria, mouth ulcers, bleeding wounds, as an antiseptic, an antidote for general poisoning, diarrhea, and skin rashes among others (Barmao, 2024; Jeremiah et al., 2023; Mosoti & Gisesa, 2025). Additionally, it is worth noting that different geographic zones with variations like temperature, humidity, and soil type are known to affect the phytochemical composition in plants, which contribute to the therapeutic value of plants (Kader, 2024; Kandie, 2024; Mudau et al., 2022).

However, despite *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia* revealing to possess various therapeutic benefits including antimicrobial properties, particularly through the conventional way, there is insignificant information on antimicrobial activities of these climbers in Kenya against *C. albicans*, *P. aeruginosa* and *S. aureus*. Hence, the present study emphasized in investigating the antimicrobial activities of methanolic leaf, stem, and root extracts of these climbers against *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* as a suitable advent in controlling AMR.

2. Materials and Methods

2.1 Plant Identification, Collection and Preparation

Taxonomic identification and authentication of *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia* were performed with the assistance of a taxonomist, using taxonomic keys as per the procedure of Obila (2019). Voucher specimens were collected and stored in Maseno University herbarium. *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia* plant samples were randomly collected from Kisii (0°54'38.7"S 34°48'28.95"E), Lake Victoria shorelines-Kisumu city (0°7'33"S 34°44'33.6"E), and Maseno University farm (0°0'32.6"S 34°36'28.6"E) respectively. Although located in different geographical areas, the three sample collection sites are categorized under the same weather zone in Western Kenya, a Mesoclimate which is influenced by Lake Victoria (Ayugi et al., 2016; Kandie, 2024). This region receives rainfall throughout the year with a peak in April.

The samples were collected according to WHO 2003 ethical standard guidelines (Jeruto et al., 2017). Freshly collected samples of plant parts were packed in separate, well-labeled gunny bags and transported to the Maseno University botany laboratory on the same day of collection. Clusters of the mature climbers at the flowering and fruiting stage were selected for the experiment (Jeruto et al., 2017). The collected plant samples were air-dried separately under the shade for three weeks (Musa et al., 2017). Leaves, stems, and roots of the three climbers were separated from the whole plant bodies. The

samples were ground into fine powder form using a heavy-duty laboratory mill, and the powder was stored in airtight Zip lock bags at room temperature (25-30°C) in a dry and dark environment waiting for the extraction process (Buyela, 2017).

2.2 Extraction of Plant Crude Extracts

500 grams of each powder from *A. lobata*, *I. aquatica*, and *R. cordifolia* were weighed and soaked in 1L methanol (Analar 99.8%) separately (Nigussie et al., 2021). The samples were soaked for 7 days with intermediate agitation. Methanol was chosen as the solvent for crude extraction in this study in that it is known to be better at extracting more phytochemical when compared to other solvents like aqueous (Bhaigybati et al., 2020; Ismail et al., 2016; Mambe et al., 2016). The extracts were filtrated through a Whatman filter paper No. 1 on a Buchner funnel under a vacuum pump after which the filtrates were concentrated using a Rotary vapor pump (EYELA N-1000) (Buyela, 2017). After the extraction process, the crude extracts were stored in labeled vials and kept in a cool, dry, and dark environment until they were needed for the subsequent analysis (Musa et al., 2017).

2.3 Antimicrobial Assay

2.3.1 Test Organisms and preparation of inoculums

Test organisms for this study; *Candida albicans* (ATCC 10231); a fungus, *Pseudomonas aeruginosa* (ATCC 27853); a Gram-negative bacteria, and *Staphylococcus aureus* (ATCC 25923); a Gram-positive bacteria were sourced from Kenya Medical Research Institute (KEMRI). The bacterial test organisms and *C. albicans* were revived on plates containing Mueller Hinton agar (MHA) (Oxoid, UK) and Potato Dextrose Agar (PDA) (Oxoid, UK) respectively. The cultures were then incubated at 35°C±2°C for 24 hours for the bacterial strains and for 24-48 hours for the *C. albicans*. After the incubation period, well-isolated colonies of each test organism were collected and suspended in sterile sodium chloride (NaCl) solution (0.85%) in sterile culture tubes (with screw caps). The turbidity of the suspensions was adjusted to match that of the 0.5 McFarland standard. The test organisms on the culture tubes were maintained at 2 °C. (Putri et al., 2020).

2.3.2 Preparation of Extract Concentrations

Under aseptic conditions, a stock solution (100mg/ml) of each of the plant crude extracts was prepared according to the procedure of Osuntokun et al. (2020) by dissolving 1g of each methanol extract into 10ml of Dimethyl sulfoxide (DMSO). Using the stock solutions, 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml working concentrations were prepared.

2.3.3 Disc diffusion method

Antimicrobial efficacy test of the extracts was carried out using the disc diffusion method (Kirby Bauer method) according to Bhaigybati et al. (2020) and Nnaoma et al. (2019). 100µl of each prepared microbial suspension was spread uniformly with a sterile spreader on plates containing Mueller Hinton agar (MHA) for bacterial test organisms and Potato dextrose agar (PDA) for *C. albicans* and left to dry for 5 minutes. Sterile discs were impregnated with the 25, 50, 75, and 100 mg/ml extract concentrations from the various extracts. The impregnated discs were aseptically placed on the surfaces of the inoculated agar medium, with slight pressing to enhance the contact with the medium. Discs containing 25µg streptomycin (antibiotic) for *P. aeruginosa* and *S. aureus* and 10µg fluconazole (antifungal) for *C.*

albicans were used as positive controls. Discs immersed in DMSO were used as the negative controls. A completely randomized design (CRD) with three replicates was applied. The plates were incubated at 35°C±2°C 18-24 hours in an incubator (Mettler IF30M). After the incubation period, observation was made on the zones of inhibition (ZI) and their diameters were measured in millimeters using a standard ruler. The data was recorded appropriately for further analysis.

2.4 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of *R. cordifolia* leaf, stem, and root extracts that had exhibited strong antimicrobial activities compared to other extracts from *A. lobata* and *I. aquatica*, during the disc diffusion method were evaluated against *C. albicans*, *P. aeruginosa*, and *S. aureus* using broth dilution method (Ibrahim & Kebede, 2020). Stock solutions of 20mg/ml of *R. cordifolia* leaf, stem, and root extracts were made by dissolving 0.1 g of each dried crude powder in 5 ml of DMSO. A two-fold serial dilution method was used to prepare working solutions of 10, 5, 2.5, 1.25, and 0.625 mg/ml in sterile test tubes. Colonies from fresh bacterial and fungal cultures were picked with a sterile inoculating loop and transferred into a sterile saline solution, adjusting the turbidity to match the 0.5 McFarland standard by contrasting against white paper black line striped (Kebede & Shibeshi, 2022). 4ml of the Mueller Hinton broth (MHB) was pipetted into each of 5 sterile test tubes. 100µl ml of the prepared successive two-fold serial dilutions of the crude extract concentrations were mixed with the MHB. Thereafter, 100µl of the standardized inoculum of the test organisms was added into each of the test tubes containing the MHB, and the crude extract. The mixtures were mixed by pipetting. Growth controls, test tubes for positive control (MHB containing test microorganisms with no plant extract) and negative control (containing only MHB), were conducted parallel with each experiment. The study was done in triplicates. The vials were then properly corked and incubated at 35°C±2°C for 24 hours (Mettler incubator IF30M). Thereafter, growth observation was made on the test tubes. The minimal inhibitory concentration (MIC) was considered the lowest concentration of plant extracts that inhibited any visible growth (absence of turbidity) of the test organisms after 24h of incubation (Ibrahim & Kebede, 2020).

2.5 Minimum Bactericidal Concentration and Minimum Fungicidal Concentration

In determining MBC and MFC, 100µl of the post-incubation suspensions from the test dilutions which did not show any visible growth i.e., greater or equal to the lowest MIC, were sub-cultured onto freshly prepared MHA for bacterial test organisms and PDA for *C. albicans* using the spread plate technique aseptically. This was done in triplicates. The plates were incubated at 35±2°C for 24 hours. The lowest dilution that showed no single colony on the agar plates in triplicates was considered MBC for *P. aeruginosa* and *S. aureus* and MFC for *C. albicans* (Nivedhitha, 2021).

2.6 Data Analysis

The data that was obtained from the study was subjected to analysis of variance (ANOVA) followed by T-test analysis using SAS 9.4 statistical package. The means were separated and compared using the Least Significant Difference (LSD) at $P \leq 0.05$ to determine any significant difference between the treatments and the test organisms. The P-values < 0.05 were considered significant for the study (Mehta et al., 2020).

3. Results

The four concentrations (25, 50, 75, and 100 mg/ml) of *A. lobata*, *I. aquatica*, and *R. cordifolia* leaf extracts, in comparison to streptomycin the standard antibiotic, showed significant differences ($P \leq 0.05$) in the mean zone of inhibitions against *P. aeruginosa* and *S. aureus*. The standard antibiotic zone of inhibitions were significantly higher than those for plant leaf extracts across the four concentrations except for *R. cordifolia* against *P. aeruginosa* (21.00 mm) at 100 mg/ml. *R. cordifolia* at the four concentrations, consistently exhibited significantly highest antibacterial activity against *P. aeruginosa* and *S. aureus* in comparison to the other leaf extracts. *I. aquatica* constantly exhibited significantly the lowest antibacterial activity against both *P. aeruginosa* and *S. aureus*. The lowest antibacterial activity was observed in *I. aquatica* leaf extract against *S. aureus* (6.22 mm) at 25 mg/ml and with no antibacterial activity exhibited on *P. aeruginosa* (6.00mm) at 25 mg/ml (**Table 1**).

A. lobata, *I. aquatica*, and *R. cordifolia* leaf extracts at 25, 50, 75 and 100 mg/ml concentrations revealed significant differences ($P \leq 0.05$) in the mean zone of inhibitions against *C. albicans*. The standard antifungal zone of inhibitions were significantly higher than those exhibited by plant leaf extracts across the four concentrations except for *R. cordifolia* (9.22 mm) at 100 mg/ml. At 25 mg/ml concentration, *A. lobata*, *I. aquatica*, and *R. cordifolia* leaf extracts demonstrated no growth inhibition, each exhibiting a 6.00 mm zone of inhibition. At 50 mg/ml concentration *R. cordifolia* showed antifungal activity (6.77 mm), insignificantly higher than *I. aquatica* (6.44 mm) and *A. lobata* with no antifungal activity (6.00 mm). Notably, at 75 mg/ml concentration, *R. cordifolia* showed significantly higher

antifungal activity (8.78 mm), than *I. aquatica* (7.11 mm) and *A. lobata* (6.33 mm). At 100 mg/ml concentrations, *R. cordifolia* exhibited significantly higher antifungal activity (9.22 mm) than *A. lobata* (7.33 mm) and *I. aquatica* (7.33 mm) (**Table 2**).

All the four concentrations of *A. lobata*, *I. aquatica*, and *R. cordifolia* stem extracts, in comparison to standard antibiotic, showed significant differences ($P \leq 0.05$) in the mean zone of inhibitions against *P. aeruginosa* and *S. aureus*. The standard antibiotic was significantly higher than the extracts across the four concentrations. *R. cordifolia* at the four concentrations, consistently exhibited significantly highest antibacterial activity against *P. aeruginosa* and *S. aureus* in comparison to the other stem extracts. The highest plant stem extract antibacterial activity was observed in *R. cordifolia* at 75 mg/ml against *S. aureus* (15.22mm) and *R. cordifolia* at 100 mg/ml against *P. aeruginosa* (17.44 mm). *A. lobata*, *I. aquatica* stem extracts revealed insignificantly different antibacterial activities against both *P. aeruginosa* and *S. aureus* across the four concentration in most cases (**Table 3**).

The effectiveness of stem extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans* showed significant differences ($P \leq 0.05$) in the mean zone of inhibitions against *C. albicans*. The standard antifungal zone of inhibitions were significantly higher than those exhibited by plants leaf extracts across the four concentrations. At 25 mg/ml concentration, *A. lobata*, *I. aquatica*, and *R. cordifolia* stem extracts demonstrated no growth inhibition, each exhibiting 6.00 mm zone of inhibition. Notably, *A. lobata* consistently showed no growth inhibition across all concentrations tested (6.00 mm), whereas *R. cordifolia* and *I. aquatica* displayed increasing bio-activities, ranging from 6.00 mm to 8.78 mm zone of inhibitions (**Table 4**).

Table 1 Antibacterial activity of *A. lobata*, *I. aquatica*, and *R. cordifolia* leaf extracts against *P. aeruginosa* and *S. aureus*

Plant	25mg/ml		50mg/ml		75mg/ml		100mg/ml	
	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵
Positive control (Streptomycin)	20.50 ^a	18.00 ^a	19.44 ^a	17.55 ^a	21.22 ^a	18.67 ^a	20.67 ^a	23.01 ^a
<i>Adenia</i> ¹	8.89 ^c	9.22 ^c	9.44 ^c	9.78 ^c	9.89 ^c	10.67 ^c	9.56 ^b	10.11 ^c
<i>Ipomoea</i> ²	6.00 ^d	6.22 ^d	6.11 ^d	6.88 ^d	6.44 ^d	7.67 ^d	7.33 ^c	7.11 ^d
<i>Rubia</i> ³	15.78 ^b	11.67 ^b	18.00 ^b	14.00 ^b	18.56 ^b	15.00 ^b	21.00 ^a	15.57 ^b
Means	12.571	11.086	13.250	12.055	14.027	13.000	14.639	13.500
CV (%)	4.617	9.322	8.782	8.879	7.858	12.460	8.892	9.857
LSD ($\alpha=0.05$)	0.567	1.009	1.117	1.027	1.058	1.556	1.249	1.278

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*, ⁴ *P. aeruginosa*, ⁵ *S. aureus*

Table 2 Antifungal activity of leaf extracts of *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans*

Zones of Inhibition (mm)				
Plant	25mg/ml	50mg/ml	75mg/ml	100mg/ml
Positive control (Fluconazole)	9.56 ^a	9.56 ^a	11.11 ^a	9.67 ^a
<i>Adenia</i> ¹	6.00 ^b	6.00 ^{bc}	6.33 ^c	7.33 ^b
<i>Ipomoea</i> ²	6.00 ^b	6.44 ^b	7.11 ^c	7.33 ^b
<i>Rubia</i> ³	6.00 ^b	6.77 ^b	8.78 ^b	9.22 ^a
Means	6.889	7.194	8.333	8.389
CV (%)	3.825	6.949	11.874	12.207
LSD($\alpha=0.05$)	0.253	0.480	0.950	0.983

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*.

Table 3 Antibacterial activity of stem extracts of *A. lobata*, *I. aquatica*, and *R. cordifolia* against *P. aeruginosa* and *S. aureus*

Zones of Inhibition (mm)									
Plants		25mg/ml		50mg/ml		75mg/ml		100mg/ml	
		Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵
Positive control	(Streptomycin)	20.50 ^a	18.00 ^a	19.44 ^a	17.55 ^a	21.22 ^a	18.67 ^a	20.67 ^a	22.11 ^a
Adenia	¹	7.22 ^c	6.78 ^c	7.89 ^d	8.44 ^c	8.66 ^c	8.89 ^d	10.33 ^c	11.00 ^c
Ipomoea	²	6.56 ^c	6.89 ^c	9.11 ^c	8.33 ^c	12.00 ^c	10.67 ^c	11.78 ^c	9.67 ^d
Rubia	³	14.33 ^b	11.89 ^b	16.11 ^b	14.66 ^b	16.67 ^b	15.22 ^b	17.44 ^b	15.00 ^b
Means		11.914	10.686	13.139	12.250	14.638	13.361	15.055	14.444
CV (%)		5.988	9.408	8.811	7.696	8.150	9.762	9.228	7.228
LSD (α=0.05)		0.696	0.982	1.112	0.905	1.145	1.252	1.334	1.002

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*, ⁴ *P. aeruginosa*, ⁵ *S. aureus*

Table 4 Antifungal activity of stem extracts of *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans*

Zones of Inhibition (mm)				
Plant	25mg/ml	50mg/ml	75mg/ml	100mg/ml
Positive control (Fluconazole)	9.56 ^a	9.56 ^a	11.11 ^a	9.67 ^a
Adenia ¹	6.00 ^b	6.00 ^c	6.00 ^d	6.00 ^c
Ipomoea ²	6.00 ^b	6.56 ^b	7.11 ^c	8.78 ^b
Rubia ³	6.00 ^b	6.22 ^{bc}	8.33 ^b	8.67 ^b
Means	6.889	7.083	8.138	8.278
CV (%)	3.825	6.113	8.627	9.915
LSD ($\alpha=0.05$)	0.253	0.415	0.674	0.788

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*.

The antibacterial activity of root extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against *P. aeruginosa* and *S. aureus*, in comparison to streptomycin the standard antibiotic, was evaluated at 25, 50, 75 and 100 mg/ml concentrations. Significant differences ($P \leq 0.05$) in the mean zone of inhibitions against *P. aeruginosa* and *S. aureus* were observed among the extracts. The standard antibiotic was significantly higher than the screened plant extracts across the four concentrations. *R. cordifolia* at the four concentrations, consistently exhibited significantly highest antibacterial activity against *P. aeruginosa* and *S. aureus* in comparison to the other root extracts. The highest plant root extract antibacterial activity was observed in *R. cordifolia* at 75 mg/ml against *P. aeruginosa* (14 mm) and at 100 mg/ml against *S. aureus* (14 mm). The lowest bio-activity was observed in *I. aquatica* with inhibition zones of 6.33 mm for both *P. aeruginosa* and *S. aureus* at 50 mg/ml whereas there was no growth inhibition exhibited on both *P. aeruginosa* and *S. aureus* (6.00mm) by *I. aquatica* at 25 mg/ml (**Table 5**).

The antifungal activity of root extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans* revealed significant differences ($P \leq 0.05$) in their efficacy at the various concentrations. The standard antifungal zone of inhibitions was significantly higher than those exhibited by plants leaf extracts across the four concentrations except for *R. cordifolia* at 75 and 100 mg/ml concentrations. *R. cordifolia* at the four

concentrations, consistently exhibited significantly highest antifungal activity against *C. albicans* in comparison to the other root extracts. At 25mg/ml concentration, there was no growth inhibition observed for *A. lobata* and *I. aquatica* against *C. albicans* (6.00 mm each). Similarly, at 50mg/ml, there was no growth inhibition observed for *A. lobata* (6.00 mm) against *C. albicans*. (**Table 6**).

Inclusive bio-activity of leaf, stem, and root extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans*, *P. aeruginosa*, and *S. aureus* were evaluated at 100 mg/ml concentration. Significant differences ($P \leq 0.05$) in bio-activity were observed among the extracts against the test organisms. The highest growth inhibition against *C. albicans* was observed in *R. cordifolia* root extract (11.67 mm), which was significantly greater than the inhibition exhibited by Fluconazole (9.67 mm) and other plant extracts with *A. lobata* stem not revealing any growth inhibition (**Table 7**). In terms of antibacterial activity, *R. cordifolia* leaf extract exhibited the highest growth inhibition (21.00 mm) against *P. aeruginosa* which was significantly similar to that of Streptomycin (20.67 mm) but significantly higher than other extracts (**Table 7**). For *S. aureus*, Streptomycin demonstrated significantly higher growth inhibition (22.11 mm) than all the extracts. However, *R. cordifolia* leaves (15.57 mm), stems (15.00 mm), and roots (14.33 mm) exhibited significantly higher inhibitory activity than other extracts (**Table 7**).

Table 5 Antibacterial activity of root extracts of *A. lobata*, *I. aquatica*, and *R. cordifolia* against *P. aeruginosa* and *S. aureus*

Zones of Inhibition (mm)								
Plant	25mg/ml		50mg/ml		75mg/ml		100mg/ml	
	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵	Aerugi ⁴	Aureus ⁵
Positive control (Streptomycin)	20.50 ^a	18.00 ^a	19.44 ^a	17.55 ^a	21.22 ^a	18.67 ^a	20.67 ^a	22.11 ^a
<i>Adenia</i> ¹	7.33 ^c	7.22 ^c	8.11 ^c	9.33 ^c	8.89 ^c	10.56 ^c	9.56 ^c	12.67 ^c
<i>Ipomoea</i> ²	6.00 ^d	6.00 ^d	6.33 ^d	6.33 ^c	8.56 ^c	7.67 ^d	9.44 ^c	9.11 ^d
<i>Rubia</i> ³	8.57 ^b	9.56 ^b	11.22 ^b	11.89 ^b	14.00 ^b	12.56 ^b	12.89 ^b	14.33 ^b
Means	10.314	9.971	11.278	11.416	13.167	12.361	13.138	14.555
CV (%)	4.993	9.493	7.462	7.262	7.858	7.532	8.221	6.846
LSD ($\alpha=0.05$)	0.502	0.924	1.117	0.796	0.908	0.873	1.037	0.956

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*, ⁴ *P. aeruginosa*, ⁵ *S. aureus*.

Table 6 Bio-activity of root extracts of *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans*

Zones of Inhibition (mm)				
Plant	25mg/ml	50mg/ml	75mg/ml	100mg/ml
Positive control (Fluconazole)	9.56 ^a	9.56 ^a	11.11 ^a	9.67 ^b
<i>Adenia</i> ¹	6.00 ^c	6.00 ^c	6.44 ^c	7.89 ^c
<i>Ipomoea</i> ²	6.00 ^c	6.44 ^c	7.44 ^b	8.11 ^c
<i>Rubia</i> ³	8.00 ^b	8.56 ^b	11.44 ^a	11.67 ^a
Means	7.388	7.527	9.111	9.33
CV (%)	5.968	5.961	8.580	9.982
LSD ($\alpha=0.05$)	0.423	0.431	0.751	0.894

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$). ¹ *Adenia lobata*, ² *Ipomoea aquatica*, ³ *Rubia cordifolia*.

Table 7 Bio-activity of Leaf, Stem, and Root Extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against *C. albicans*, *P. aeruginosa*, and *S. aureus* at the concentration of 100 mg/ml

Plant	N	<i>C. albicans</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
<i>Rubia cordifolia</i> Roots	9	11.67 ^a	12.89 ^c	14.33 ^b
Positive control (Fluconazole)	9	9.67 ^b	-	-
<i>Rubia cordifolia</i> Leaves	9	9.22 ^{bc}	21.00 ^a	15.57 ^b
<i>Ipomoea aquatica</i> Stems	9	8.78 ^{cd}	11.78 ^c	9.67 ^e
<i>Rubia cordifolia</i> Stems	9	8.67 ^{cde}	17.44 ^b	15.00 ^b
<i>Ipomoea aquatica</i> Roots	9	8.11 ^{def}	9.44 ^d	9.11 ^e
<i>Adenia lobata</i> Roots	9	7.89 ^{ef}	9.56 ^d	12.67 ^c
<i>Ipomoea aquatica</i> Leaves	9	7.33 ^f	7.33 ^e	7.11 ^f
<i>Adenia lobata</i> Leaves	9	7.33 ^f	9.56 ^d	10.11 ^{de}
<i>Adenia lobata</i> Stems	9	6.00 ^g	10.33 ^d	11.00 ^d
Positive control (Streptomycin)	9	-	20.67 ^a	22.11 ^a
Means		8.466	13.000	12.578
CV (%)		11.118	10.509	9.303
LSD ($\alpha=0.05$)		0.883	1.282	1.098

^a Means followed with the same letters in columns are not significantly different ($\alpha=0.05$).

A series of t-tests were performed to compare the zone of inhibition against *P. aeruginosa* and *S. aureus* for the antimicrobial activity of *A. lobata* extracts at a 100 mg/ml concentration. For the leaves, using the pooled variance method,

the analysis revealed a non-significant difference in antimicrobial activity between the two bacterial strains (t-value = -0.94; p-value = 0.36; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 9.56 mm, while for *S. aureus*, it

was 10.11 mm (**Figure 1**). For the stems, given the unequal variances between the two groups, Satterthwaite's method was used for the analysis. The results showed a non-significant difference in antimicrobial activity between the bacterial strains (t-value = -1.51; p-value = 0.16; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 10.33 mm, while for *S. aureus*, it was 11.00 mm (**Figure 1**). Lastly, for the roots, using the pooled variance method, the analysis indicated a significant difference in antimicrobial activity between the two bacterial strains (t-value = -7.55; p-value < 0.0001; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 9.56 mm, while for *S. aureus*, it was 12.67 mm (**Figure 1**).

A series of t-tests were also performed to compare the zone of inhibition against *P. aeruginosa* and *S. aureus* for the antimicrobial activity of *I. aquatica* extracts at a 100mg/ml concentration. For the leaves, using the pooled variance method, the analysis revealed a non-significant difference in antimicrobial activity between the two bacterial strains (t-value = -0.63; p-value = 0.54; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 7.33 mm, while for *S. aureus*, it was 7.11 mm (**Figure 2**). For the stems, using Satterthwaite's method, the results showed a significant difference in antimicrobial activity between the bacterial strains (t-value = 2.71; p-value = 0.02; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 11.78 mm, while for *S. aureus*, it was 9.67 mm (**Figure 2**). For the roots, using the pooled variance method,

the analysis indicated a non-significant difference in antimicrobial activity between the two bacterial strains (t-value = 0.73; p-value = 0.48; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 9.44 mm, while for *S. aureus*, it was 9.11 mm (**Figure 2**).

A series of t-tests were performed to compare the zone of inhibition against *P. aeruginosa* and *S. aureus* for the antimicrobial activity of *R. cordifolia* extracts at a 100% concentration. For the leaves, using the pooled variance method, the analysis revealed a significant difference in antimicrobial activity between the two bacterial strains (t-value = 6.52; p-value < 0.0001; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 21.00 mm, while for *S. aureus*, it was 15.57 mm (**Figure 3**). For the stems, using the pooled variance method, revealed a significant difference in antimicrobial activity between the two bacterial strains (t-value = 3.77; p-value < 0.0017; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 17.44 mm, while for *S. aureus*, it was 15.00 mm (**Figure 3**). Lastly, for the roots, using the pooled variance method, the analysis indicated a significant difference in antimicrobial activity between the two bacterial strains (t-value = -2.14; p-value < 0.0484; with $\alpha = 0.05$). The mean zone of inhibition for *P. aeruginosa* was 12.89 mm, while for *S. aureus*, it was 14.33 mm (**Figure 3**).

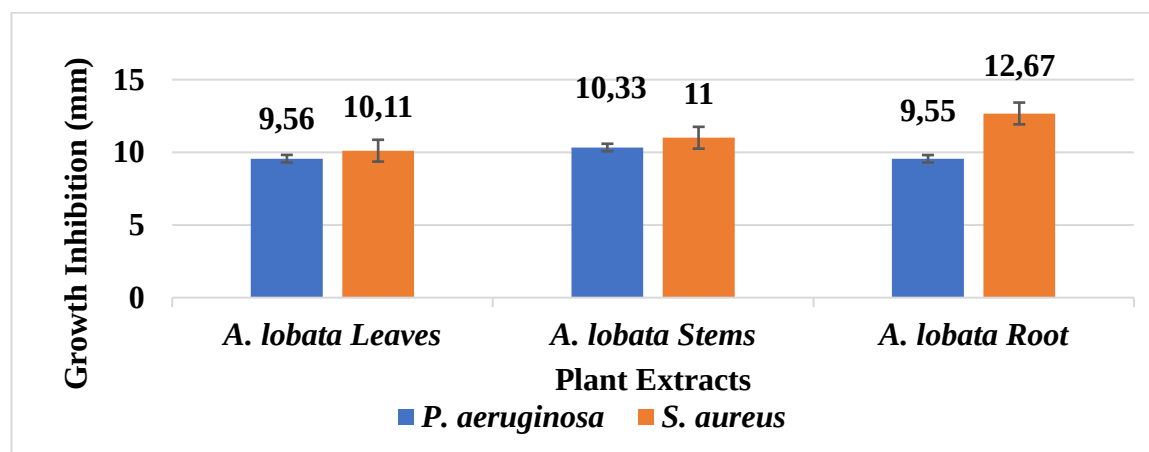


Figure 1 T-test Comparison of Bio-activity of *A. lobata* Extracts against *P. aeruginosa* and *S. aureus* at 100 mg/ml Concentration.

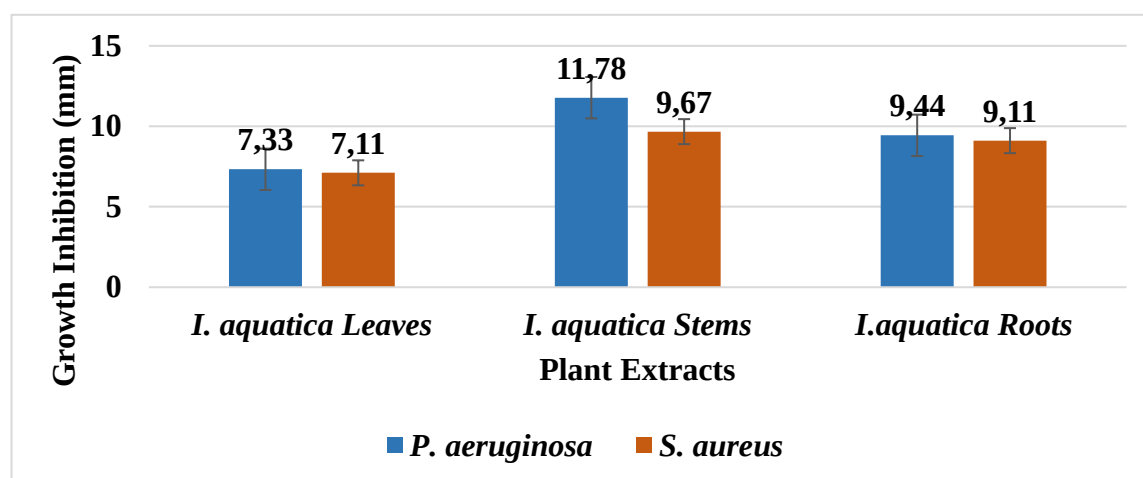


Figure 1 T-test Comparison of Bio-activity of *I. aquatica* Extracts against *P. aeruginosa* and *S. aureus* at 100 mg/ml Concentration.

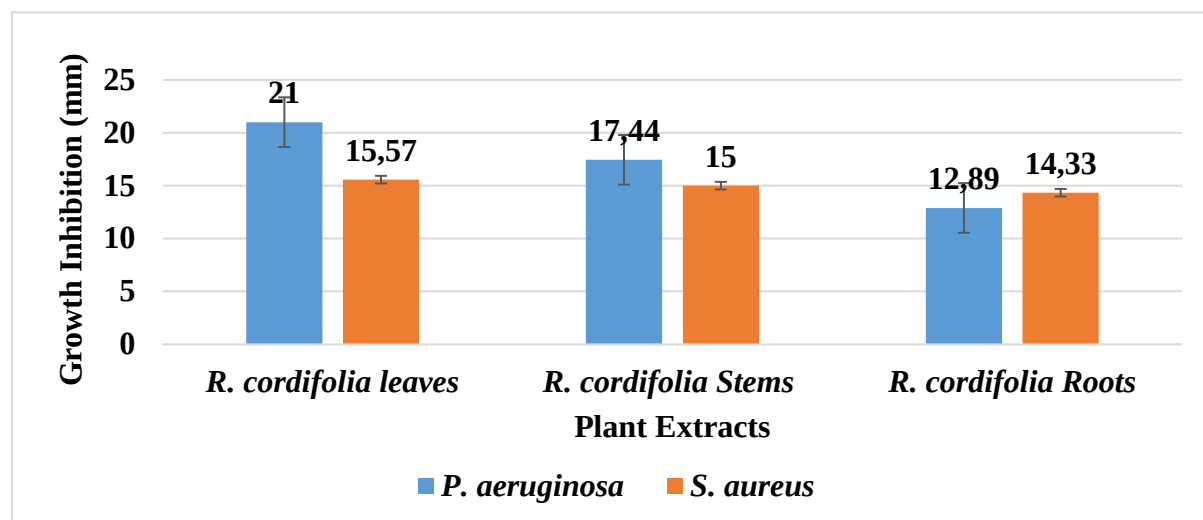


Figure 2 T-test Comparison of Bio-activity of *R. cordifolia* Extracts against *P. aeruginosa* and *S. aureus* at 100 mg/ml Concentration.

The results from the MIC analysis revealed variations among the *R. cordifolia* leaf, stem, and root extracts against the test organisms. The MIC of leaf and root extracts against *C. albicans* was 5 mg/ml, whereas for the stem extract it was 10 mg/ml. MIC values of 10 mg/ml for both *P. aeruginosa* and *S. aureus* were revealed from leaf, stem and root *R. cordifolia* extracts. According to the MBC, and MFC analysis, there was neither MBC nor MFC that was observed. However, the leaf, stem and root *R. cordifolia* extracts at 10mg/ml showed some positive results against the test organisms in some of the replicates with no or few colony growth.

4. Discussion

Medicinal plants have played an essential role in providing both curative and preventive medical therapy preparations for humans through the extraction of important bioactive compounds, which are crucial for maintaining good health. In Kenya, more than 70% of the people use local homemade remedies as their first source of medicine, while more than 90% use plant-related remedies at one time or another (Mbuni et al., 2020). Phytochemicals like alkaloids, phenols, flavonoids, tannins, saponins, and terpenoids are revealed to be of value in the human body by causing vital physiological actions hence effective in the treatment of diseases (Khan et al., 2021). According to the current study, the various leaf, stem, and root extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* exhibited antimicrobial activity against *C. albicans*, *P. aeruginosa* and *S. aureus* at different concentrations though with variations. According to Odongo et al. (2022), zones of inhibition (ZOIs) can be interpreted as low activity (1 mm–6 mm), moderate activity (7 mm–10 mm), high activity (11 mm–15 mm), and very high activity (>16 mm). Generally, *R. cordifolia* leaf, stem, and root extracts revealed the strongest antimicrobial activity against the test organisms. These results correlate with limited available earlier research studies that have also revealed the antimicrobial properties of these climbers.

According to Fowa et al. (2019), hydroethanolic leaf extract of *A. lobata* exhibited antibacterial potential by completely inhibiting *Salmonella typhi* a causative agent of typhoid fever from a 5mg/kg dose. Additionally, the same study demonstrated that various extracts of *A. lobata* leaf possess different degrees of antibacterial activity against various bacteria from the *Salmonella* genus i.e., *Salmonella typhi* collection "American Type Culture Collection" (ATCC 6539) and isolates of *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, and *Salmonella typhimurium*. The scant scientific data

concerning the antimicrobial activities of *A. lobata* leaf indicates that fewer research studies have been done on this climber despite its potential to suppress pathogenic microorganisms.

Ipomoea aquatica leaf extract on the other side has proved to possess antimicrobial activities against microorganisms like *Escherichia coli*, *P. aeruginosa*, and *Bacillus subtilis* (Malakar & Choudhury, 2015). According to Sivaraman et al. (2010), methanolic and aqueous leaf extract from *I. aquatica* possess antimicrobial activities against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa*, *Proteus vulgaris*, and *Escherichia coli*. It is worth noting that Bhaigybati et al. (2020) found that both the methanolic and aqueous extracts from a mixture of leaves and stems of *I. aquatica* Forsk have antibacterial activity in one or the other extracts against *E. coli*, *P. aeruginosa*, and *S. aureus*. Aqueous, ethanol, benzene, and chloroform *I. aquatica* leaf extracts were found to contain antimicrobial activity but with variations against *Escherichia coli*, *Klebsiella pneumonia*, *P. aeruginosa*, *S. aureus*, *Streptococcus pyogenes*, and *Enterococcus faecalis* (Velmurugan & Suresh, 2020). Focusing on *R. cordifolia*, methanolic leaf extract of *R. cordifolia* was found to be effective against *Salmonella typhi*, *S. aureus*, *Proteus sp.*, *E. coli*, and *Klebsiella oxytoca* (Niyonzima et al., 2024). Rani et al. (2010) revealed that chloroform, and ethanolic leaf extracts of *R. cordifolia* possess antibacterial activity against *B. subtilis*, *P. aeruginosa*, *S. aureus*, *P. vulgaris*, *K. pneumonia*, and *E. coli*. According to this study, it was found that the extracts from *R. cordifolia* and two other plant species; *Syzygium alternifolium* and *Phyllanthus niruri*, were found to be more active against Gram-positive bacteria than the Gram-negative. However, this study does not concur with the current study which has revealed that the leaf extract from *R. cordifolia* is more active against *P. aeruginosa* (Gram-negative) than *S. aureus* (Gram-positive) at the various concentrations.

Currently, information concerning the antifungal activity of *A. lobata* leaf extracts against *C. albicans* and even other fungal pathogens is unknown. In contrast, a couple of earlier research studies on *I. aquatica* and *R. cordifolia*, complement the current findings although interestingly only a fraction of them concentrated on antifungal activity against *C. albicans*. In determining the *In vitro* antifungal activity of extracts of fresh and dry apical buds and flowers of red-stemmed *I. aquatica* Forsk; the extracts of dry apical buds and flowers inhibited *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum gypseum*, and *Malassezia globosa* (Raksamat et al., 2018). Oon et al. (2021), revealed that hexane, chloroform, ethyl acetate, ethanol, methanol, and aqueous leaf extracts from *I. aquatica* exhibit antifungal activity against *Trichophyton interdigitale*, *C.*

albicans, *Candida parapsilosis*, *Candida krusei* and, *Cryptococcus neoformans* human fungal pathogens. Based on nanotechnology, an emerging paradigm of the twenty-first century, biogenic silver nanoparticles from aqueous leaf extract of *R. cordifolia* were effective against *Aspergillus flavus*; a food-spoiling, saprophytic, pathogenic, aflatoxigenic and ubiquitous fungi (Chandraker et al., 2022). Additionally, it was found that the effectiveness was concentration-dependent. In another study, methanolic extract of *R. cordifolia* among other six plant extracts was found to be more effective against thirteen phytopathogens of *Gossypium hirsutum* (cotton) which included ten fungi and three bacteria (Naidu et al., 2009).

Overall, there has been limited research on the antibacterial properties of stem extracts from *A. lobata*, *I. aquatica*, and *R. cordifolia* against the specified microorganisms and other human pathogens. For instance, Fowa et al. (2019), only focused on determining the antibacterial activity of hydroethanolic leaf extract of *A. lobata* against bacteria of the genus *Salmonella*. *I. aquatica*, leaf, and stem methanol extracts of this plant species were found to exhibit antibacterial properties against *S. aureus*, *Streptococcus pyogenes*, and *E. coli* (Wan et al., 2020). Additionally, at 100mg/ml concentration, the leaf extract exhibited higher antibacterial activity than the stem extract against the three pathogens. However, this study does not coincide with the current one in that the stem extract of *I. aquatica* at 100 mg/ml exhibited a significantly higher antibacterial activity than the leaf extract at the same concentration for both *P. aeruginosa* and *S. aureus*. On the other side of *R. cordifolia*, biosynthesized silver nanoparticles from aqueous stem and root extracts were found to be effective against Gram-positive bacteria *S. aureus*, *B. subtilis*, and Gram-negative bacteria *P. aeruginosa*, *E. coli* (Mariswamy et al., 2021).

Presently, little is known concerning the antifungal activity of *A. lobata*, *I. aquatica*, and *R. cordifolia* stem extracts against *C. albicans* and even other fungal pathogens. Interestingly, according to the current study, some stem extracts demonstrated greater antifungal activity compared to leaf extracts, which are typically the focus of several ethnomedicinal studies (Kipkore et al., 2014; Maiyo et al., 2024). Notably, the stem extract of *I. aquatica* stem extract exhibited significantly higher antifungal activity than the leaf extract against *C. albicans*. Interestingly, *A. lobata* stem extract has been used in determining different bioactivities like anti-oxytotic/ferroptonic (Pamela et al., 2024). It is worth noting that a formulated shampoo using a mixture of leaf and stem ethanolic extract from *I. aquatica* proved to have antifungal activity against *Pityrosporum ovale*, one of the fungal pathogens responsible for dandruff infection (Aisali et al., 2024). However, this study did not consider *C. albicans*, one of *Candida* spp. fungal pathogen that is responsible for most superficial infections (Talapko et al., 2021).

Similarly, there is scanty information regarding the antibacterial activities of *A. lobata* and *I. aquatica* root extracts against *P. aeruginosa*, *S. aureus* and other pathogenic bacterial microbes. However, for *R. cordifolia*, various preceding studies concerning antibacterial potential from the root extracts support the findings from the current study. Ali et al. (2020) revealed that the root extract of *R. cordifolia* exhibited broad antibacterial activities against several pathogenic bacteria such as *Klebsiella pneumoniae*. It is worth noting that *R. cordifolia* root methanol extract has antibacterial activity against Gram-negative bacteria; *B. subtilis*, *E. faecalis*, and *S. aureus*, and Gram-negative bacteria; *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Proteus mirabilis* and *P. aeruginosa* while it did not show antibacterial activity against *E. coli*, *K. pneumoniae*, and *Salmonella enteritidis* (Ismail et al., 2016). Chloroform and methanolic extracts were found to be significantly active against *B. subtilis*, and *S. aureus*

when compared to streptomycin and penicillin G (Ali et al., 2020). According to Chandrasekhar et al. (2023), five (5) out of ten (10) compounds extracted from methanolic *R. cordifolia* root extract exhibited antibacterial activity against *S. aureus* from a panel of bacteria including members of ESKAPE pathogens (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* species). However, no compound was active against Gram-negative bacterial pathogens that were tested.

Research studies concerning the antifungal potential of *A. lobata* and *I. aquatica* root extracts against *C. albicans* are currently unknown. However, considering *R. cordifolia*, Ismail et al. (2016), revealed that *R. cordifolia* root methanolic extract exhibited antifungal activity against *C. albicans* other than exhibiting antibacterial activity against three Gram-positive bacteria (*B. subtilis*, *E. faecalis* and *S. aureus*) and Gram-negative bacteria (*A. baumannii*, *E. aerogenes*, *P. mirabilis* and *P. aeruginosa*). This study concurs with the current study which has proven the antifungal activity of *R. cordifolia* against *C. albicans* in addition to antibacterial activities against *P. aeruginosa* and *S. aureus*.

Findings from bio-activity of leaf, stem, and root extracts from *A. lobata*, *I. aquatica* and *R. cordifolia* against *C. albicans*, *P. aeruginosa*, and *S. aureus* that were evaluated at 100 mg/ml revealed that there is significant differences ($P \leq 0.05$) in their efficacy. The differences in antimicrobial properties between various plant part extracts within a species and between plant species is mainly contributed by variations in phytochemical composition between various plant parts within a species and between plant species (Dubale et al., 2023).

Emphasizing the findings from the series of t-tests analysis, it is evident that different plant parts within a species possess antimicrobial properties but with variations. It was also evident that some plant extracts can be more active against Gram-positive than Gram-negative and vice versa. From the current study; *A. lobata* root and *R. cordifolia* root extracts were found to be more active against *S. aureus* than *P. aeruginosa* while *I. aquatica* stem, *R. cordifolia* leaf and stem extracts exhibited more antimicrobial activity against *P. aeruginosa* than *S. aureus* at 100 mg/ml. These findings are supported by other earlier studies with similar findings. For instance, Rani et al. (2010), revealed that the extracts of *Rubia cordifolia*, *Syzygium alternifolium*, and *Phyllanthus niruri* were found to be more active against Gram-positive bacteria than the Gram-negative bacteria in a study that involved *B. subtilis*, *P. aeruginosa*, *S. aureus*, *P. vulgaris*, *K. pneumoniae*, and *E. coli*. In another related study, different selected medicinal plants used as herbals in Pakistan, were found to be more sensitive to *S. aureus* (Gram positive) than *E. coli*, and *P. aeruginosa* (Gram negative) bacteria which may be attributed by the difference in their cell wall composition (Walter et al., 2011). However, some of the plant extracts were found to be more effective to the Gram negative bacteria than the Gram positive bacteria. Similarly, Abd Razik et al. (2012), revealed that Gram positive bacteria (*Staphylococcus aureus*, *Lactobacillus* sp.) were more susceptible to *Plantago major* and *Ceratonia siliqua* plant methanol extracts than the Gram negative bacteria (*Proteus* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus* sp.). It was also concluded that these differences could be attributed by great complexity of the double membrane-containing cell envelope in Gram negative bacteria in comparison to the single membrane structure of Gram positive bacteria or the presence of lipopolysaccharides in their outer membrane. Generally, various studies have argued that the cell walls of Gram-positive bacteria are more sensitive to many of anti-boutiques, antimicrobial chemical compounds and even many herbal drugs compared to that of Gram-negative bacteria (Koohsari et al., 2015).

The correlation of MIC resulting from the leaf, stem, and root extracts of *R. cordifolia* against *C. albicans*, *P. aeruginosa*, and *S. aureus* may be primarily due to the similarities in the phytochemical composition of these plant parts. The composition of particular compounds like tannins, alkaloids, terpenoids, and saponins probably affects the antimicrobial activities across different parts of a plant (Abebe et al., 2024). Given that plant extracts with a MIC value less than 8 mg/ml are regarded as active, *R. cordifolia* MIC values significantly indicate that this species is a promising one for the production of plant-based antimicrobials (Zouine et al., 2024). The MIC results revealed that increasing concentration increases the growth inhibitory effect on the microorganisms. It is worth noting that, MIC values indicate the defined nature of the antimicrobial activities of the selected plants while the inhibition zone values only indicate the extent of effectiveness of the extracts with increasing concentration (Zouine et al., 2024). This supports the definition of MIC which is recognized as the least concentration of antimicrobial agent that prevents microbial growth (Mossie, 2024). Thus, MIC is normally incorporated with the agar diffusion method to provide a more comprehensive evaluation of antimicrobial efficacy in plants in that some of the non-polar compounds can fail to diffuse hence leading to false results when using agar diffusion method alone. However, the agar diffusion method can be useful with a single compound with a known polarity (Zouine et al., 2024).

According to Eloff, (2019), it was evident that there is a bigger variation in results obtained in determining the zone of inhibition by agar diffusion as a parameter of antimicrobial activity and the MIC determined by serial dilution analysis. However, observing turbidity as an indicator of microbial growth using the tube dilution method may not be effective, particularly when precipitation occurs after adding plant extracts to complex microbial culture media (Eloff, 2019). Similar issues arise when using the microplate method, where cells can clump at the bottom of the microplate, affecting the accuracy of turbidity measurements. Additionally, the color of the plant extracts, depending on the extractant used, may further complicate turbidity quantification. This was evident in the present study. Interestingly, a study conducted by Nivedhitha (2021) was extended to evaluate the MIC and Minimum Bactericidal Concentration (MBC) values of *R. cordifolia* distillate (colorless) against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus*, revealed a significant increase in both MIC and MBC values, indicating that the red dye of the root extract possesses antimicrobial activity. This is a clear indication that the distillation of plant extracts with the aim to have clear distillate affects their bioactivity.

Based on the present study, it is evident that the concentrations of the serial dilutions from the *R. cordifolia* leaf, stem, and root extracts, which showed positive MIC results, were not strong enough to completely kill *C. albicans*, *P. aeruginosa*, and *S. aureus* thus leading to negative MBC and MFC results. According to Mossie (2024) and Scorzoni et al. (2016), MBC is considered the least concentration of antimicrobial agent needed to kill 99.9% of microbial inoculum while MFC is the lowest concentration of a substance in which no visible fungal growth of subculture occurs respectively. This indicates that the MBC and MFC primarily depend on the concentration of essential phytochemicals present in the plant that have antimicrobial activities.

Studies have suggested that components of plants, such as terpenoids, alkaloids, and phenolic compounds, interact with bacterial cell membranes and the proteins present within them which can lead to bacterial lysis by inducing the efflux of protons outside the cell or by inhibiting key enzymes responsible for amino acid synthesis (Imran et al., 2021). Phytochemicals like anthraquinones and flavonoids are known to work by modifying

the fluidity of the cell membrane and disrupting the cell wall. They inhibit DNA and RNA synthesis by binding to the phosphate groups of DNA and also hinder metabolic processes by affecting various bacterial cell processes and enzymes (Nivedhitha, 2021). Additionally, another study attributed the effects of plant extracts to their hydrophobic properties, which cause reactions between proteins in the bacterial cell membrane and mitochondria which can result in lysis, alteration of bacterial structure, and changes in membrane permeability (Imran et al., 2021). However, the lack of standardization in the MFC has significantly hindered the further development of the test in clinical practice, primarily due to the difficulty in determining the starting inoculum, especially for *Candida* sp. (Franconi & Lupetti, 2023).

5. Conclusion

According to the results from this study, it is evident that methanolic leaf, stem, and root extracts of the three climbers; *Adenia lobata*, *Ipomoea aquatica*, and *Rubia cordifolia* possess antimicrobial properties against *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* though with variations. Overall, the leaf, stem, and root extracts from *R. cordifolia* demonstrated the strongest antimicrobial properties against the test organisms, revealing their potential for developing plant-based antimicrobials through the pharmaceutical industries. The variations in antimicrobial properties among different plant parts and species are primarily due to differences in phytochemical composition. Thus, advancement in phytochemical analysis is necessary to purify and characterize the bioactive compounds responsible for the antimicrobial properties in plants. Interestingly, some plant extracts can be more active against Gram-positive than Gram-negative and vice versa. Moreover, microbial growth inhibition by plant extracts is dose-dependent, with higher concentrations leading to greater effects in both the Kirby-Bauer method and minimum inhibitory concentration (MIC) tests. Thus, by combining the two methods, a more comprehensive evaluation of antimicrobial efficacy in plants is achieved. Additionally, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) negative results were probably contributed by the lack of enough concentrations of vital phytochemicals in the extracts responsible for the complete killing of microorganisms.

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Declaration of interest

The authors alone are responsible for the content and writing of the paper.

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