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Antimicrobial potency of extracts from selected medicinal plants towards Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa

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ABSTRACT

Antibiotic resistance has become a very big threat to the existing first line antibiotics. Some of the infectious pathogens are becoming multidrug resistant including *Mycobacterium tuberculosis*. This has necessitated social, scientific and financial interventions from key players. The strain this puts on the fragile health care systems of developing nations is frustrating. Scientific interventions have involved campaigns for improved hygiene, use of combination therapies and revived search for new drugs with different modes of action. It is on this basis that this research was conducted as phase I into the search for antibiotic agents from nature. This was done by screening several plant extracts to identify bioactive extracts that can be developed into drugs or purified for better active single molecules in the second phase. Extracts were obtained by cold percolation of pulverized samples of different dried plant parts using different mono-solvents. Agar diffusion and froth floatation were used to measure the potency of the extracts. Many of the screened extracts had good to moderate activities. Five of the plant species; *Zanthoxylum chalybeum* and *gilletii*, *Diospyros abyssinica*, *Prunus africana*, *Peptadeniastrum africana* and *Blighia unijugata* showed very promising activities (1.9 to 9.4 mg/mL) against Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The other species had moderate activity (10.6 to 47.5 mg/mL). The species (*Albizzia coriaria*, *Maytenus senegalense* and *Kigellia africana*) that inspired this research from literature only demonstrated moderate activity against all the tested organisms, probably due to antagonistic effect of the active compounds within the extracts. In conclusion, *Z. chalybeum and gilletii*, *D. abyssinica*, *P. africana*, *Peptadeniastrum*. africana and B. unijugata have a very strong potential for drug development and are recommended for use in the management of infections caused by the tested microbes and purification to isolate the individual active compounds for better formulation, standardization and drug acceptability.

Keywords: *Antimicrobial, Antimicrobial Resistance, Plant Extracts*

Introduction

There is a high global burden of disease arising from infectious pathogens. New pathogens are emerging and there is re-emergency of the formerly controlled ones (Datta & Roy, 2021). The re-emerging pathogens are being resistant to the first line drugs available (Schneider, 2021). Combination therapies have been opted but still some of the microbes are becoming multidrug resistant. For example, multi-drug resistant

Mycobacterium tuberculosis strains which are on the increase and fast spreading around the world (Baluku *et al.*, 2022). There is reduced number of efficacious antibiotics which may be used in the treatment of many infections including pneumonia and tuberculosis (Baluku *et al.*, 2022). Multidrug-resistant microbes are a cause of the current emerging global diseases and a major threat to public health (Roca *et al.*, 2015). This calls for an immediate multipronged approach to bakeout a local solution to the African health challenges. Pharmaceutical scientists and phytochemists have refocused their attention to medicinal plants. The attention to the antimicrobial properties of plant extracts and their metabolites has grown owing to the rising rates of drug-resistant pathogens (Raza *et al.*, 2022). Journal articles have documented the therapeutic potential of natural compounds validating their claimed biological activities (Buyinza & Gumula, 2022). Medicinal plants have intrinsic capability to resist pathogenic microorganisms and this has motivated researchers to investigate their modes of action and subsequent isolation of the active compounds. This has informed the exploitation of medicinal plants in the development of new antimicrobial agents for the treatment of different microbial infections of both plants and humans (Hammer *et al.*, 1999). For centuries, medicinal plants have been used as therapies for human diseases and provide a new source of biologically active metabolites as antimicrobial agents (El-Saber Batiha *et al.*, 2021). Medicinal plants are the richest bio-resource for drugs in the traditional medicinal systems as well as in modern medicine, nutraceuticals, food supplements, pharmaceuticals and offer chemical template for synthetic drugs (Hammer *et al.*, 1999). It is estimated that over 20% of higher plant species are being used for curative purposes and that about 74% of the pharmacologically active plant derived entities were discovered based on the ethnomedicinal value of such plants (Das *et al.*, 2010). Most antibiotics in current clinical use (e.g. β-lactams, aminoglycosides, tetracyclines, macrolides) were discovered using the bioprospecting approach to drug discovery, and this is a strong argument to reprioritize bioprospecting over other strategies in the search for new antibacterial drugs (Cicka & Quave, 2019; Juan, 2017; Sharangi & Peter, 2023). Academic and research institutions are repositioning to take up the leading role in the early stages of these efforts (Cushnie *et al.*, 2020). The structural complexity of natural products having many stereo centres, polycycles and polyfunctional groups offers them superior specificity towards biological targets including microbial cell penetration. In addition to finding compounds capable of penetrating microbial cells, multitarget inhibitors that decrease the ease with which microbes develop antibiotic resistance are identified (Cushnie *et al.*, 2020). The rarity of new antibacterial is palpable. Natural products have showed powerful therapeutic potentials against pathogenic microbes and still forms the spine for the discovery of new antibiotic drugs (Dai *et al.*, 2020). Multidrug resistance is a global concern that has attracted attention of healthcare providers, putting pressure to the drug developers to find an immediate remedy. However, phytochemicals having provided standalone effective therapeutics like the quinine, penicillin, vinca cancer drugs among others with minimal registered resistance(Buyinza *et al.*, 2019, 2022), a search of effective antibiotic therapies with a new mode of action from natural sources is a promising avenue. On this background, extracts from different parts of thirteen medicinal plant species including K. africana, M. senegalensis, A. coriaria from Mabira forest central Uganda were investigated for their preliminary potency against five microbes.

Literature Review

Antimicrobial Resistance

Manifestation of disease-causing microbes such as parasites, fungi, bacteria and viruses are on the increase (Aghababa & Nadi, 2021). The infections from these microbes have caused devastating ailments to both man and animal over the years across the globe (Munita & Arias, 2016) hence becoming a universal health

problem. To a large extent, the current treatments are failing pointing to the need for novel antipathogens (Datta & Roy, 2021). Basing on the disease condition, different antimicrobials and antiparasitic drugs have been developed and used. However, by the emergence of resistance (Anabela *et al*., 2015), host toxicity and various undesirable side effects (Buyinza *et al*., 2019) of the current antimicrobial drugs undermine their use and the search for new molecular compounds for new antimicrobial drugs is an undeniable global priority (Sanchez Armengol *et al*., 2021). Multidrug-resistant microbes are on the increase and are a cause of the current emerging global diseases and a major threat to public health (Roca *et al*., 2015), ending the golden era of antimicrobial superiority (Ayaz *et al*., 2019). The resistant microorganisms can emerge either by mutations or the acquisition of mobile genetic elements carrying resistant genes irrespective of the presence of antibacterial agents (Roca *et al*., 2015). The exposure to these drugs is likely to provide the necessary selective pressure for the rise and spread of resistant pathogens (Álvarez-Martínez *et al*., 2020). The increasing rates of resistance can be claimed on the abuse and misuse of antibacterial agents, whether used in patients and livestock or released into the environment (Roca *et al*., 2015). This is no longer a medical issue alone but a global health threat that will require the coordinated action of many different stakeholders to tackle antibiotic resistance at its very root.

As it is for other pathogens, antibiotic abuse has great contribution to the speedy development of antibiotic resistance and human medicine is a key player (Hassan & Olaoye, 2020). Roca *et al*., (2015) asserts that, obsolete guidelines and pharmaceutical pressures result into inappropriate prescription. Over the counter antibiotic accessibility and self-medication are a common contributor to antibiotic resistance (Reygaert, 2018) and are also a reflection of the little awareness people have to the danger this poses to our society. Major contributing factors to the emergence of antimicrobial resistance in low-income countries include; less potency of some antibacterial agents (some being counterfeit), over the counter accessibility, insufficient dosages, wrong prescription, poor diagnosis (Roca *et al*., 2015). These are coupled with family and community spread of resistant pathogens by means of low sanitation levels. Also there is an increasing number of immune-compromised individuals (comorbidities like cancer patients on immunosuppressive chemotherapy (Filipa *et al*., 2020), HIV patients on ARVs, aging), transplant complications and stress (Ayaz *et al*., 2019). It is believed that nearly 90% of S. aureus strains are resistant to penicillin, while 75% are said to be resistant to methicillin (Ayaz *et al*., 2019).

Antimicrobial Resistance Mechanism

Microbes have and continue to develop complex drug resistance mechanisms involving several biochemical processes in a single cell (Munita & Arias, 2016). Attaining exogenous genetic material by HGT is among the most important drivers of bacterial evolution responsible for developing antimicrobial resistance. This includes bacterial transformation (uptake of free DNA by a "competent" bacterial cell), bacterial transduction (transfer of genetic material from donor bacteria to recipient one facilitated by bacteriophage) as well as bacterial conjugation (transfer of genetic material from one bacterial cell to another via direct physical contact;- most important mechanism for horizontal gene transfer) (Aghababa & Nadi, 2021). Antimicrobial drug resistance by pathogens can therefore be intrinsic or extrinsic as a transmission within bacterial species (Aghababa & Nadi, 2021; Hassan & Olaoye, 2020). The common mechanism of antimicrobial action is related to protein biosynthesis and alteration of cell walls and membranes (Álvarez-Martínez *et al*., 2020). Receptor modification mediated through mutation in the target site leading to a decline in the antimicrobial drug efficacy is a common resistance mechanism for the Gram positive bacteria (Munita & Arias, 2016). Meanwhile production of β-lactamases is the preferred mechanism of resistance to β-lactams in Gram

negative bacteria (Munita & Arias, 2016). Such kinds of resistance modes are seen in the structural alteration of the penicillin binding proteins and DNA gyrase mutations and RNA polymerase, which render many drugs sedentary. Another recognised resistance mechanism by pathogens is the active extracellular efflux mediated by efflux pumps. Intrinsic resistance main mechanisms are believed to be non-permeability of the cell membrane and activation of drug efflux pumps. Therefore, bacteria drug resistance can be developed via extracellular drug efflux facilitated by efflux pumps, target modification as well as enzymatic degradation of drugs (Ayaz *et al.*, 2019). Various natural compounds, especially phytochemicals, have shown synergistic capacity with antibiotics (Álvarez-Martínez *et al.*, 2020) and some compounds have successfully shown activity against resistant Gram-negative bacteria by deactivating the mechanism of resistance, as the case for the β-lactamase Inhibitor antibiotic adjuvants (Breijyeh *et al.*, 2020). Research efforts of this kind that meet the urgent need for new treatments must be intensified.

Plant antibiotics

The evolution of pathogens across the globe seem to be developing at a very fast rate, thus turning into a worldwide health problem. The current treatments have failed to a large extent, an indication that novel antibiotics are in high demand. Among the alternative molecules sought are antimicrobial peptides because of their wide-spectrum activity, rapid killing and cell selectivity (Datta & Roy, 2021). Some essential oils have been found to have superior antimicrobial properties than synthetic compounds as well as offering other beneficial properties with fewer side effects (Pateiro *et al.*, 2021). For centuries, medicinal plants have been used as therapies for human diseases and provide a new source of biologically active metabolites as antimicrobial agent. Medicinal plants are the richest bio-resource for drugs in the traditional medicinal systems as well as in modern medicine, nutraceuticals, food supplements, folk medicines, pharmaceuticals and offer chemical temperate for synthetic drugs (Hammer *et al.*, 1999). Pharmaceutical scientists and other research scientific are now refocusing their attention to medicinal plants. Attention to the antimicrobial properties of plant extracts and their metabolites has grown owing to the rising rates of drug-resistant pathogens. Journal articles have documented the therapeutic potential of natural compounds validating their claimed biological activities. Medicinal plants have intrinsic capability to resist pathogenic microorganisms and this has motivated researchers to investigate their modes of action and subsequent isolation of the active compounds. This has informed the exploitation of medicinal plants in the development of new antimicrobial agents for the treatment of different microbial infections of both plants and humans (Hammer *et al.*, 1999). It is estimated that over 20% of higher plant species are being used for curative purposes and that about 74% of the pharmacologically active plant derived entities were discovered based on the ethnomedicinal value of such plants (Das *et al.*, 2010). Most antibiotics in current clinical use (eg. β-lactams, aminoglycosides, tetracyclines, macrolides) were discovered using the bioprospecting approach to drug discovery and this offers a strong argument to reprioritize bioprospecting over other strategies in the search for new antibacterial drugs. Academic and research institutions should be well positioned to lead the early stages of such efforts (Cushnie *et al.*, 2020).

Phytochemicals as natural metabolites are more likely substrates for the transporter systems than synthetic compounds that will facilitate their entry into the microbial cell. The structural complexity of natural products having many stereo centres, polycycles and polyfunctional groups offers them superior specificity towards biological targets. In addition to finding compounds capable of penetrating microbial cells, multitarget inhibitors that decrease the ease with which microbes develop antibiotic resistance are identify (Cushnie *et al.*, 2020).

The rarity of new antibacterial is palpable. Natural products have showed powerful therapeutic potentials against pathogenic microbes and still forms the spine for the discovery of new antibiotic drugs (Dai *et al*., 2020).

Polyphenol plant extracts contain large amounts of bioactive compounds that inhibit the growth of microorganisms. Their mechanism of action is likely to be related to their chemical structure. They cause morphological changes in the microorganism, impair the bacterial cell wall in addition to influencing biofilm formation. Polyphenols also seen to influence protein biosynthesis, change metabolic processes in bacteria cells and inhibit ATP and DNA synthesis (suppressing DNA gyrase) (Efenberger-Szmechtyk *et al*., 2021). The upsurge of multidrug resistant (MDR) pathogens has become a global threat that has created hitches in providing satisfactory treatment for many of the infectious diseases. Though conventional antimicrobial agents are fairly effective against some pathogens, the need for additional effective antimicrobial agents to counter the MDR pathogens is real. Herbal medicines and phytochemicals have been used from ancient times as effective antimicrobial and the trend is increasing for developing plant based natural products meant for the prevention and treatment of pathogenic diseases (Anabela *et al*., 2015). To mitigate MDR, the use of antimicrobial drugs in combination with phytochemical agent that may neutralize the microbes' resistance mechanism have been suggested. In this respect, phytochemicals could work as inhibitors to target modifiers, drug degrading enzymes or efflux pumps. Many herbal extracts, essential oils and pure isolated compounds have been reported to act in synergy with existing antimicrobial agents and chemotherapeutics, augmenting well the activity of these drugs (Ayaz *et al*., 2019). In this respect, some useful drugs have been clinically approved on this basis, for example, beta-lactamase inhibitors (e.g. clavulanic acid, sulbactam, tazobactam) used in combination with amoxicillin (Ayaz *et al*., 2019, Álvarez-Martínez *et al*., 2020). Among the other alternative molecules sought are antimicrobial peptides because of their wide-spectrum activity, rapid killing and cell selectivity (Datta & Roy, 2021).

Materials and Methods

Plant Material

The leaves, fruits, stem and root barks of K. africana, M. senegalensis and A. coriaria along with nine (9) other species were collected from Mabira forest reserve central Uganda (Buganda) and voucher specimen samples were deposited to the Herbarium at Makerere University for authentication. The plant materials were packed in nylon bags for transportation to the Chemistry laboratory in Kyambogo University. They were then dried under shade and pulvalized before extraction. Analytical grade solvents were used in the extraction.

Extraction

Each dried plant material was ground using a blender and sequentially soaked in n-hexane, ethyl acetate, dichloromethane, acetone, methanol and water for 12 hours (each) to obtain crude extracts. The soaking and extraction were done three times to obtain sufficient extract (Sarker, *et al*., 2006). The extract was evaporated to remove the solvents using a rotary evaporator under reduced pressure. The extracts were then kept in a refrigerator at a temperature of -15˚ C for preservation before further analysis.

Bioassays

Media preparation

MHAgar and MHBroth was made following manufactures instructions in 1000mls of distilled water, stirred to dissolve with a magnetic stirrer, then autoclaved for 15minutes at 1210c. MHA was then dispensed in sterile plastic plates of 10 x 10cm wide left to stand and solidify for an hour. The plates were incubated at 370c for sterility test for 24hs before use, then stored at 40c.

Preparation of bacterial inoculum suspension.

The bacteria were grown on MHAgar medium to activate them. One bacteria colony was cultured in MHBroth medium, grown until the optical density of 0.5 corresponding to 108CFU is attained which was determined by the help of a UV spectrophotometer.

Antibacterial activity of extracts.

The effect and potency of the plant extracts on bacteria was determined using Agar well diffusion method described by Jones & Kinghorn, 2006 with modifications. 100 µL of standardized bacterial suspension OD = 0.5 was seeded over 4mm MHA with sterile swabs, left to dry for 5 minutes and three wells with a diameter of 0.5mm was drawn using sterile p200 pipette tips. Each was filled with about 100 µL of different plant extracts on separate plates. The empty two wells in each plate were filled with an equivalent volume of the positive and negative control, where the positive control had a concentration of 5mg/ml. All plates were incubated at 280c for 72 hrs. The inhibition zone in replicates were determined every 24 hrs measured in millimeters using a ruler.

Minimum Inhibitory Concentration of plant extracts.

The broth dilution method (Arendrup *et al.*, 2012) was used to determine the MIC for the extracts, where sets of 7 test tubes were arranged on the rack and labeled with the extract's initial letters; each tube was filled with 1ml MHB for each extract. Two-fold serial dilution (1:1) was made by introducing 1ml of test extracts at a predetermined concentration was added to the first tube and mixed by pipetting up and down several times to make sure there is uniform mixing of the sample. After, 1ml of the solution was taken from the first tube and transferred to the second tube to be diluted and the process was carried out onto the next tube until the eighth tube is reached. Micro pipette tips were changed before going to the next tube during mixing. The excess 1ml in the last tube was discarded to maintain equal volumes. 100µL of the standardized overnight bacterial suspension was introduced in each tube using a p200 micropipette except for the eighth tube which acted as a positive control (MHB + plant extracts). The ninth served as a negative control (MHB + bacterial suspension). All tubes were incubated at 280c for 24hrs. Any tube without turbidity after the incubation period with the lowest plant extract concentration was as charted as the MIC.

Minimum Bactericidal Concentration of plant extracts

Minimum bactericidal concentration was evaluated according to Chikezie, 2017 with modifications. The MBC of all the plant extracts was determined by sub-culturing all tubes that did not show any sign of growth from the MIC tubes observed due to lack of turbidity formation on to MHA media. This was then incubated overnight where the least concentration that does not show any sign of growth on the plate was taken to be the MBC.

Result and Discussion

Bioassay Results

The detailed bioassay test results for the different test organisms are found in supplementary information provided under the tables 1 to 3. In the tables, both the minimum inhibition concentrations (MIC) and minimum bactericidal concentrations (MBC) are shown.

Table 1: Bioassay Results for Escherichia coli.

Plant Species	Plant Part	Solvent	Mic (mg/ml)	Mbc (mg/ml)
Albizia coriaria	sb	Ethanol	25.0	0.0
"	"	EtoAc	17.2	34.4
Blighia unijugata	sb	Methanol	10.9	21.9
"	"	DCM	3.1	6.3
"	"	EtoAc	7.5	15.0
"	"	Ethanol	12.5	0.0
Diospyros abyssinica	sb	DCM	17.5	17.5
"	"	EtoAc	5.6	11.3
"	"	Ethanol	16.9	0.0
"	"	Methanol	15.3	30.6
"	rb	EtoAc	8.1	16.3
"	"	DCM	18.8	0.0
"	"	Methanol	8.8	8.8
"	"	Ethanol	9.1	18.1
Kigelia africana	lv	EtoAc	0.0	0.0
Mytenus senegalensis	rb	EtoAC	37.5	0.0
Peptadeniastrum africana	sb	Methanol	10.6	21.3
"	"	EtoAc	8.4	16.9
"	"	Ethanol	43.1	43.1
Prunus africana	sb	Methanol	18.8	37.5
"	"	Ethanol	47.5	47.5
"	"	EtoAc	5.3	10.6
"	"	DCM	7.5	15.0
Zanthoxylum chalybeum	"	DCM	4.1	8.1
"	rt	DCM	14.4	0.0
"	"	Methanol	7.2	14.4
Zanthoxylum gilletii	rb	Ethanol	8.8	17.5
"	"	Methanol	8.8	17.5
"	"	EtoAc	7.5	0.0
"	sb	DCM	1.9	3.8
"	"	EtoAc	4.4	0.0
"	"	Methanol	12.5	0.0
"	rw	Ethanol	4.4	8.8

NB: lv; leaves, sb; stem bark, rb; root bark, rw; root wood, rt; root, Mic; minimum inhibition concentration and Mbc; minimum bactericidal concentration

Table 2: Bioassay Results for Klebsiella pneumoniae.

Plant Species	Plant Part	Solvent	mic (mg/ml)	mbc (mg/ml)
Albizia coriaria	sb	Ethanol	0.0	0.0
"	"	EtoAc	17.2	34.4
Blighia unijugata	sb	Methanol	10.9	21.9
"	"	DCM	3.1	6.3
"	"	EtoAc	3.8	7.5
"	"	Ethanol	12.5	0.0
"	rb	Methanol	17.5	0.0
Diospyros abyssinica	sb	DCM	8.8	17.5
"	"	EtoAc	11.3	0.0
"	"	Ethanol	16.9	0.0
"	"	Methanol	15.3	30.6
"	rb	EtoAc	8.1	16.3
"	"	DCM	18.8	0.0
"	"	Methanol	8.8	17.5
"	"	Ethanol	9.1	18.1
Kigelia africana	lv	EtoAc	0.0	0.0
Mytenus senegalensis	rb	EtoAc	37.5	0.0
Peptadeniastrum africana	sb	Methanol	10.6	21.3
"	"	EtoAc	8.4	16.9
"	"	Hexane	4.4	8.8
"	"	DCM	11.9	0.0
"	"	Ethanol	43.1	43.1
Prunus africana	sb	Methanol	18.8	37.5
"	"	Ethanol	23.8	47.5
"	"	EtoAc	5.3	10.6
"	"	DCM	7.5	15.0
Zanthoxylum chalybeum	rt	DCM	14.4	0.0
"	"	DCM	4.1	8.1
"	"	Ethanol	20.0	0.0
Zanthoxylum gilletii	rb	Ethanol	17.5	17.5
"	"	Methanol	8.8	17.5
"	"	EtoAc	7.5	0.0
"	"	EtoAc	4.4	0.0
"	sb	Methanol	12.5	0.0
"	"	DCM	1.9	3.8
"	rw	Ethanol	4.4	8.8

Table 3: Bioassay Results for Pseudomonas aeruginosa.

Plant Species	Plant Part	Solvent	mic (mg/ml)	mbc (mg/ml)
Albizia coriaria	sb	Ethanol	0.0	0.0
"	"	EtoAc	17.2	34.4
Blighia unijugata	sb	Methanol	10.9	21.9
"	"	DCM	6.3	6.3
"	"	Ethanol	12.5	0.0
"	"	EtoAC	7.5	15.0
Diospyros abyssinica	sb	DCM	17.5	17.5
"	"	EtoAC	11.3	0.0
"	"	Ethanol	16.9	0.0
"	"	Methanol	15.3	30.6
"	rb	EtoAc	8.1	16.3
"	"	DCM	18.8	0.0
"	"	Methanol	8.8	17.5
"	"	Ethanol	9.1	18.1
Kigelia africana	lv	EtoAc	12.5	0.0
Mytenus senegalensis	rb	EtoAC	0.0	0.0
Peptadeniastrum africana	sb	Methanol	10.6	21.3
"	"	EtoAc	8.4	16.9
"	"	Hexane	8.8	0.0
"	"	DCM	11.9	0.0
"	"	Ethanol	43.1	0.0
Prunus africana	sb	Methanol	18.8	37.5
"	"	Ethanol	23.8	47.5
"	"	EtoAc	5.3	10.6
Zanthoxylum chalybeum	rw	ethanol	7.8	15.6
"	"	Methanol	7.2	7.2
"	"	DCM	4.1	8.1
"	rt	DCM	14.4	0.0
"	"	Ethanol	3.6	7.2
Zanrhoxylum gilletii	"	EtoAc	7.5	0.0
"	sb	EtoAc	2.2	4.4
"	"	DCM	1.9	3.8
"	"	Methanol	12.5	0.0
"	rw	Ethanol	8.8	0.0

Discussion of Results

Different plant parts were studied based on their use in the traditional health practices where leaves, stem bark, root bark, whole roots, flowers and or fruits are used (Buyinza & Gumula, 2022; Iwu, 2014; Ozioma & Nwamaka, 2019; Pinn, 2001). This ruled out the uncertainty of the most valuable plant part. Multiple solvents were used for extraction to take care of the polar and non-polar compounds that could be present in the different parts. This enabled identification of single solvent extracts that demonstrated different antimicrobial activities.

For the studied extracts, antimicrobial activity is classified to be significant when MIC < 100 µg/mL, moderate if MIC is between 100 µg/mL and 625 µg/mL and low for MIC > 625 µg/mL (Cos *et al.*, 2006; Djeussi *et al.*, 2015; Kuete & Efferth, 2010).

Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa

The DCM stem bark extract of *Zanthoxylum gilletii* showed the highest activity with MIC of 1.9 mg/mL and a corresponding MBC of 3.8 mg/mL against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. While the root wood ethanol extract of Z. gilletii showed a MIC of 4.4 mg/m, a MBC of 8.8 mg/mL and its root bark methanol extract had a MIC of 8.8 mg/mL with a MBC of 17.5 mg/mL towards all the three microbes. Meanwhile the stem bark EtoAc extract was only active against *P. aeruginosa* with a MIC of 2.2 mg/mL and a MBC 4.4 mg/mL.

The stem bark DCM and Methanol extracts of *Zanthoxylum chalybeum* demonstrated a MIC of 4.1 mg/mL, MBC 8.1 mg/mL and MIC of 7.2 mg/mL, MBC 14.4 mg/mL respectively against both *E. coli* and *K. pneumoniae*. Its root bark ethanol extract had a MIC of 8.8 mg/mL with a MBC of 17.5 mg/mL against E. coli. However, the *Z. chalybeum* root bark ethanol extract showed a MIC of 3.6 mg/mL, MBC 7.2 mg/mL, its stem bark DCM and Methanol extracts demonstrated a MIC of 4.1 mg/mL, a MBC of 8.1 mg/mL and MIC 7.2 mg/mL, MBC 7.2 mg/mL respectively against *K. pneumoniae*. These results show that the stem bark and root bark extracts of this genus are the most active against both *E. coli, K. pneumoniae* and *K. pneumonia*e. The *benzophenanthridine alkaloids* predominant in the Zanthoxylum species (Sharma *et al.*, 2021; Tavares *et al.*, 2014) are likely to be responsible for the activity demonstrated by Z. gilletii and chalybeum.

The polyhydroxylated compounds in Blighia unijugata (Aquaisua *et al.*, 2020; Oloyede *et al.*, 2023; Sinmisola *et al.*, 2019) are likely to be responsible for the observed activity in the extracts of its species. This was evidenced by the activity of the stem bark DCM and EtoAc extracts of Blighia unijugata showing a MIC of 3.1 mg/mL, MBC of 6.3 mg/mL and MIC of 7.5 mg/mL respectively against both *E. coli, K. pneumoniae* and *K. pneumoniae.* But its DCM stem bark had a MIC of 6.3 mg/mL and MBC 15.6 mg/mL against only *P. aeruginosa*.

Prunus africana EtoAc stem bark extract had a MIC of 5.3 mg/mL and MBC of 10.6 mg/mL, yet the DCM extract showed MIC of 7.5 mg/mL with a MBC of15.0 mg/mL against *E. coli, K. pneumoniae* and P. aeruginosa. This activity is likely to be stemming from the prominent steroids characteristic species (Deresa *et al.*, 2022; Komakech *et al.*, 2017; Teshale, 2020).

Overall, the *Zanthoxylum* plant extracts were the most active against the tested pneumonia causing pathogens making it a good source of antibiotics including that for TB treatment.

Constraints

Delays in plant identification and classification due to very few experienced field botanists in the country. It was difficult to have a full broad microbial screening (including TB assays) due to shortage in testing centers and expert scientist.

Limited funding that hindered constructive scientific collaborations for advanced studies and bioactivity testing.

A lot of time was lost waiting for ethical approvals from Institutional Review Boards.
These factors combined, had a toll of the work schedule and funding.

Conclusions and Recommendations

Both the dichloromethane and ethyl acetate extract of the screened plants are active to all the tested organisms. Extracts from all parts of *diospyros abyssinica* and *zanthoxylum gilltie* were active against all the test organisms with very promising activities. Given such results, it is very possible that health remedies can be sought from plant sources. Such extracts can be purified to obtain bioactive compounds which can act as drug leads in the drug development.

The extracts demonstrated moderate activity (MIC > 100µg/mL) by the activity classification described above. However, the tested plant species have a very strong potential for drug development either in combination or after purification to isolate the individual active compounds which may even have a more superior activity.

There is an urgent need to respond to the disease challenges using a multidimensional approach including ethnobotanical and phytochemical approaches. If this is not done, then, human health will be threatened, economies challenged with low productivity from the sick and attendants and over expenditure on treatment and life support. Governments of African countries therefore, should take a deliberate move to entice their scientists into healthcare product development through funding and incentives. Nurture home grown solutions and interventions that suit African challenges (Buyinza & Gumula, 2022) through our indigenous knowledge coupled with the western knowledge from Universities.

Collecting all the species studied from a single forest reserve shows a promising medicinal plant diversity in Uganda, however, this is not well researched and yet some of the plants are becoming extinct due to population pressure. This demands preservation of the natural habitats of the flora, a call for selective harvesting of the plant materials and also commercial cultivation of the plants of value. There is need to generate more scientific evidence in the country to validate the use of herbal medicine in primary health care fostering their acceptance and integration in modern medical practices.

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