

The Potential of Medicinal Plants to Enhance Antimicrobial Resistance in Diarrheal Pathogens

Muhindo Anita

Department of Pharmacy Kampala International University Uganda
Email: anita.muhindo@studwc.kiu.ac.ug

ABSTRACT

The global rise of antimicrobial resistance (AMR), particularly among diarrheal pathogens, poses a severe public health threat, especially in developing regions where diarrheal diseases remain a leading cause of child mortality. Conventional antibiotics are increasingly losing efficacy against multi-drug resistant (MDR) strains of *Escherichia coli*, *Vibrio cholerae*, and *Salmonella* spp. This calls for alternative therapeutic interventions. Medicinal plants, long employed in traditional medicine, offer a promising, sustainable solution due to their diverse phytochemical profiles and antimicrobial potential. This paper explores the role of selected medicinal plants in enhancing the treatment of diarrheal diseases and inhibiting resistant microbial strains. Focusing on plants such as *Acacia nilotica*, *Julbernardia paniculata*, and *Maerua angolensis*, the study highlights their inhibitory effects on key diarrheagenic bacteria, supported by in vitro findings. Mechanistic insights into plant-based bioactives such as phenolics, terpenes, and flavonoids suggest these compounds interfere with microbial resistance pathways and membrane integrity. The paper concludes that integrating medicinal plant research into mainstream therapeutic frameworks could play a vital role in addressing AMR and improving diarrheal disease outcomes globally.

Keywords: Medicinal plants, Antimicrobial resistance (AMR), Diarrheal pathogens, Traditional medicine, Phytochemicals, Multi-drug resistant bacteria, *Escherichia coli*, *Vibrio cholera*.

INTRODUCTION

Man has struggled with diseases and premature death for centuries, burdened by various living conditions, despite being an evolutionary success. As humans transitioned from nomadic to stationary living, populations increased, necessitating faster food production and leading to new diseases from livestock. This created an urgent need for sanitation, with populations sleeping away from refuse, which became breeding grounds for pathogens. They recognized that diseases were contagious, but cleaning often came too late. Hence, herbal remedies were sought to counteract ailments. The advent of antibiotics offered hope to combat infections. However, dormant microbes evolved into harmful forms, and resistance to antibiotics emerged. Many diseases, including diarrhea, plague, syphilis, and ulcers, continue to disrupt lives. Diarrhea, the second leading cause of death in children under 5, can result in death within hours if untreated. It's characterized by the passage of three or more loose stools in less than 24 hours and poses a risk across all ages. Different types include secretory, osmotic, inflammatory, and motor diarrhea. Na⁺ and Cl⁻ transport across epithelial tissues requires ATP, involving three transporters: the Na⁺-glucose co-transporter, sodium-potassium pump, and Cl⁻ channel, with co-transport driving entry when Na⁺ secretory activity is absent [1, 2].

Understanding Antimicrobial Resistance

Microorganisms are essential in human life for bread making and curd formation, but some also cause infectious diseases. Major pathogens include bacteria, viruses, fungi, and protozoa, leading to significant morbidity and mortality, especially in developing countries with limited access to hygiene and antimicrobials. The misuse of antibiotics has resulted in multi-drug resistant (MDR) strains that are hard to treat. While new antibiotics are under development, they take years to be effective against resistant microbes, and their high costs limit usage. This situation emphasizes the need for alternative treatments for MDR bacterial infections, such as plant-derived antimicrobial agents. Many countries are exploring their flora for antimicrobial properties, with herbal medicines being used for centuries without promoting resistance. Most plant-derived antimicrobials identified are phenolic compounds, organic acids, terpenes, or fatty species. Their distinct chemical structures from traditional antibiotics suggest they could be effective against antibiotic-resistant pathogens. Utilizing antibacterial plants may also lessen the pressure on antibiotic-resistant strain development. For instance, diarrhea remains a major cause of death in children under five, particularly in low and middle-income countries, with *Escherichia coli* and *Vibrio cholerae* as leading pathogens [3, 4].

Diarrheal Pathogens Overview

Diarrheal diseases are a serious burden to humankind. The burden of bacterial diarrhoea is greater than before because of increases in HIV, and antibiotic resistance as a result of the inappropriate use of antibiotics. Medicinal plants have until recently received little attention for their possible use in diarrheal disease. Drugs taken from plants have often proven to have lead better allopathic drugs and many with established better efficacy than their allopathic counterparts. One group of diseases responsible for significant morbidity and mortality worldwide are the diarrheagenic diseases. There are major syndromes of bacterial diarrheal disease and even a focused application of highly active antibiotics to treat them would leave populations vulnerable to disease caused by other bugs. Complications of diarrhoeal diseases also add to the multi-aspect burden these diseases impose on humankind. Special attention is drawn to those complications which appear to be most frequent and most serious in young children. Both the community and the individual bear sizeable costs for hospitalisation for diarrhoeal episodes, loss of days at school or work, and missed opportunities for social contact due to shyness or ostracism related to faecal incontinence. There has been a much failed search for an inexpensive safe pharmacological adjunct to the oral rehydration solution that is vital for the management of dehydration in diarrheal disease. Diarrhoea, after respiratory infections, is the second commonest cause of childhood mortality in developing countries. It is caused by a large number of microorganisms including bacteria, viruses, fungi, parasites and intestinal worms. Diarrhoea affected 7.5 million children less than 5 years old and resulted in 765054 deaths, globally. These cases and deaths represented 10% and 15% of all deaths in children less than 5 years old globally, respectively. *V. cholerae*, *E. coli*, *A. lumbricoides*, *G. lamblia* and *S. mansoni* were the leading causes of diarrhoea and mortality, respectively [5, 6].

Role of Medicinal Plants in Traditional Medicine

Medicinal plants hold great potential for discovering new antimicrobial agents, with over 90% of species historically used as medicine across cultures. Herbal medicine remains prevalent, particularly in developing countries, due to its accessibility, low-cost, and relative safety. These plants, classified as flowering plants, contain active compounds for treating various diseases, including infectious ones like diarrhea, which poses significant challenges and causes millions of deaths, especially in children in developing countries where contamination of food and water is common. Diarrhea leads to considerable morbidity and mortality and highlights the importance of medicinal plants, which have been utilized for thousands of years. Various plant parts, including leaves, roots, and seeds, can be employed in different forms such as powders or tinctures. Research on diarrheal pathogens like *Shigella dysenteriae* and *Salmonella typhimurium* reveals that all plant materials effectively inhibit growth, showcasing unique characteristics of each extract. The historical and potential uses of medicinal plants are significant, particularly in developing regions, though variations in species usage and sustainability are noted [7, 8].

Mechanisms of Action of Medicinal Plants

Previously unpublished, this dataset includes in vivo and in vitro molecular, biophysical, and structural analyses of a diverse selection of medicinal plants originally screened for inhibiting bacterial strains with resistance to extended-spectrum beta-lactams and methicillin, as well as for their combinations with known antibiotics. Emerging findings reveal sandalwood oil and farnesol significantly decrease counterselective polymyxin B resistance conferred by lipid a palmitoylation and phosphoethanolamine modification mechanisms, respectively. New combination treatments with farnesol, sandalwood oil, and

mupirocin increase susceptibility to both ampicillin and fusidic acid in resistant *Staphylococcus aureus* strains and decrease resistance development in *Escherichia coli* strains. Detailed analyses of sandalwood oil compounds and combinations with mupirocin identify α -santalol as an effective antimicrobial and show re-sensitisation of resistant strains permitted targeting of distinct resistance mechanisms. Structural and biophysical data reveal new targets for the reprogramming of membrane polarisation and intracellular pH in *Mycobacterium tuberculosis*. Exenatide is discovered as a compound with activity as a long-acting insulin secretagogue, while α -bisabolol is an exaggerated endogenous compound with higher intoxication in the context of polypharmaceutic regimens used in diabetes treatment. A unique antifungal/antioxidant activity is assigned to a novel structural class of azole-derived compounds, which displace lanosterol derivatives from both the fungal sterol C3-oxygenase and mammalian HSD11B1. These discoveries highlight these techniques and medicinal/functionalised compounds as novel strategies for targeting multidrug-resistant pathogens associated with serious human disease [9, 10].

Review of Key Medicinal Plants

Antimicrobial activities of three medicinal plants against selected diarrheagenic pathogens

This paper discusses diarrhea as a burden to humankind. It focuses on diarrhea and its causative agents. This study will focus primarily on the biological activities of selected medicinal plants against microorganisms that cause diarrhea. Diarrhea is a serious health problem for children especially in developing countries where it is a leading cause of mortality and morbidity in preschool children. It is confirmed as the second leading cause of death in children under five years of age, resulting in the death of 1.5 million children per year; however, it is a burden in all age groups. The aim of this study is to evaluate the biological properties of selected medicinal plants. The main objectives of this study are to determine the antibiotic activity of the identified medicinal plants against diarrheagenic pathogens as well as to analyze the thin layer chromatography fingerprints for active compounds in different plant extracts. Diarrhea is an abnormal condition, characterized by frequent passage of loose or liquid stools. Diarrhea is defined as the passage of more than three loose or liquid stools per day. Diarrhea is a major burden on health in both industrialized and developing countries. It is still a leading cause of morbidity and mortality worldwide, especially among infants and young children in developing countries. Diarrhea is a state of fluctuation in the normal water transport in the gastrointestinal tract leading to the passing of liquid or fluid stools. Epidemiological studies confirmed that an infection by pathogens is a major cause of diarrhea. These pathogens include bacteria, protozoa and viruses. The major bacterial diarrhea-causing pathogens are *Escherichia coli*, *Shigella* spp. and *Salmonella* spp [11, 12].

Antimicrobial Properties of Medicinal Plants

The purpose of this study was to determine the activity of three selected medicinal plants on four selected diarrheagenic pathogens. The herbal plants investigated were the *Acacia nilotica*, *Julbernardia paniculata*, and *Maerua angolensis*. Of all the extracts tested on the four bacteria, all of the samples indicated inhibitory activity at different dosages. Methanol extracts of *Acacia nilotica* showed notable activity, especially against *E. coli* and *S. typhimurium* with 86% inhibition zones at 80 mg/ml. *Julbernardia paniculata* methanol extracts exhibited a notable inhibition of 90% at 80 mg/ml against *V. cholerae*. However, *Maerua angolensis* generally showed lower inhibition activity than the other two plants. In conclusion, methanol extracts from *Acacia nilotica* and *Julbernardia paniculata* had high activity against the selected diarrheagenic pathogens. This may result from the high polar nature of the solvent which extracts flavonoids and phenolic compounds with a known higher degree of activity. Further investigation on identifying and isolation of active compounds in the respective extracts will need to be performed. The presenter was born in 1984 in Gaborone, Botswana. He received his BSc. degree, following presentations at the University of Botswana and then the Advanced Degree and PhD in Biochemistry at the University of Cape Town in 2008 and 2015 respectively. After that, he served as a post-doctoral and research fellow at Botswana College of Agriculture on technofix of hazardous wastes, South Africa defence laboratories on persistent organic pollutants and biochemistry molecular research respectively. He is currently an Assistant Professor of the Department of Biological Sciences at the University of Botswana since 2016. His research interests include antimicrobial research, molecular biology and agricultural biochemistry [13, 14].

Challenges in Research and Application

The transmission route for pathogens is fecal-oral, and contaminations in drinking water with pathogens, such as *E.coli* diarrheagenic strains, are a global problem. Contaminated water can act as a mode of transmission of pathogens via the fecal-oral route. Diarrhea, defined as the passage of three or more loose stools, is a global health issue affecting people of all ages. Diarrhea is defined by the passage of three or

more loose stools, but it can also manifest as a decrease in stool consistency and frequency. While it is an important concern for global health, especially in developing countries, a lack of resources often leaves them vulnerable to outbreaks. In infants, diarrhea is often caused by rotavirus, but many other pathogens are responsible, including viruses, protozoa, and bacteria. An estimated 525,000 deaths occur globally in children aged 0–4 years due to diarrhea each year, with pathogens such as *Cryptosporidium*, *Shigella*, Enterotoxigenic *Escherichia coli*, and *Entamoeba histolytica* being responsible. With safer drinking water, sanitation, and hygiene, the burden has steadily decreased, but in 2017, 2 billion people still used drinking water from a source that was contaminated with feces, leading to 870,000 deaths worldwide. Loose stools can lead to dehydration and systemic infection, which can ultimately result in death. This chapter investigates the antimicrobial activities of three medicinal plants, namely *Leonotis ocyimifolia*, *Ptilimnium capillaceum*, and *Leonorus sibiricus*, against *E.coli* O157:H7 and ETEC strains, as well as their chemical ingredients. Diarrhea is recognized by WHO and UNICEF as the second leading cause of death in children. Antimicrobial resistance (AMR) can arise from intrinsic mechanisms that confer natural resistance to some antibiotics and/or by acquired mechanisms that result from the acquisition of genes encoding resistance determinants or by non-genetic changes that affect the permeability and/or activity of the antibiotic. Salinization of antibiotics can result in the formation of meso- gaps that enhance bioavailability, and a wide variety of bioactive compounds from medicinal plants with powerful anti-diarrheal efficacy and minimal side effects have been implicated. Herbal remedies are prescribed to enhance properties and speeds of chemical anti-diarrheal drugs in China, India, and African Traditional Medicine, but the mechanism remains unclear [15, 16].

Case Studies and Clinical Trials

Diarrhea is a common ailment worldwide, especially in developing countries. Diarrheal diseases are a major public health challenge; they are the second leading cause of childhood mortality worldwide and a leading cause of malnutrition among children younger than 5 years old. Although dietary changes can improve outcomes in cases of mild to moderate dehydration, treatment often includes the use of antibiotics for serious diarrhea. Currently, treatment of diarrhea caused by bacteria and parasites in humans primarily involves the use of antibiotics. Unfortunately, widespread use of antimicrobial agents has resulted in increased antimicrobial resistance. Besides resistance development amongst pathogenic bacteria, the potential for drug resistance development in gut flora resulting in high levels of resistance in human pathogens is also a great concern. In turn, multiresistant bacteria can prevail due to their survival advantage in the presence of antibiotics. An increased prevalence of resistant bacteria, diminished effectiveness of antibiotics, and a significant rise in the cost of treatments is observed. Currently, treatment of diarrhea caused by bacteria and parasites in humans primarily involves the use of antibiotics. Found throughout the world, many pathogenic bacteria and parasites cause diarrhea in humans and animals and lead to the death of millions annually. Amongst other mechanisms, bacterial pathogens synthesize virulence factors that aid attachment or invasion and direct tissue damage, resulting in diarrhea. Peaks are observed when high levels of virulence factors are produced. Available treatments comprise rest, fluid and electrolyte intake, and antibiotics. In developing countries, challenges in obtaining an efficient solution include the inability to prevent dehydration and the nonavailability of electrolytes. Although dietary changes may improve outcomes in cases of mild to moderate dehydration, serious diarrhea traditionally requires the use of antibiotics [17, 18].

Future Directions in Research

Antimicrobial resistance (AMR) is a major public health threat. WHO estimates that 3,500,000 deaths are caused by resistant bacterial pathogens each year. The threat from AMR is exacerbated by the limited number of new antibiotics in the drug development pipeline. The medicinal plants reviewed in this chapter could be potential sources of new antibiotic agents. Further investigation, especially of the active phytochemicals at clinically relevant concentrations, is warranted. Natural products derived from plants represent a promising source of new antibacterial drugs targeting resistant bacteria. Due to the several pros of medicinal plants, they are a point of focus by researchers. In this study, fruits (*Psidium guajava* and *Annona muricata*), leaf (*Azadirachta indica*) and root (*Zingiber officinale*) extracts of the mentioned medicinal plants were evaluated against two Gram-negative bacteria strains. Agar disc diffusion technique was used to determine the antibacterial activity of the extracts and the Serial Tube Dilution Technique (STDT) for minimum inhibitory concentration (MIC) was also determined. Most of the extracts exhibited an antibacterial activity with the alcohol extracts showing better activity against both strains. The ethanol extracts were further fractionated using solvent partition and the active fractions were subjected to GC-MS analysis. While comparing the active fractions (against each pathogen), 25

phytochemicals were identified which could contribute to the antibacterial activity. Plant extracts at a concentration of 100% listed above were prepared and tested for their antibacterial activity against *E. coli* and *S. typhi*. The medicinal plants were obtained locally. Ethanol was purchased from a local pharmacy. Extracts were obtained following maceration in ethanol and filtering with Whatman No. 1 filter paper. The extracts were then sterilized using 0.22 μm filter and then dried on sterile Whatman No.1 filter paper [19, 20].

Public Health Implications

Public health has become a cause for concern over the years owing to the gradual increase in pathogenic microorganisms. Especially the advent of antibiotics and their subsequent revelation of resistance mechanisms has left mankind in a difficult position to combat infectious diseases. The latest one being the ample availability of synthetic antimicrobial agents that target various sites. The medical fraternity presumed that infections will be a thing of the past but permanent control of any microbial infections was not achieved. This has led to the discovery of medicinal plants that have been used in traditional medicine worldwide. Several plants were recognized for their therapeutic and healing properties since antiquity. Phytomedicine has gained specific interest due to its role as an alternative source of infectious disease control in conventional medicine. Medicinal plants have a long history of their use in treating human ailments. The constant exposure of bacterium to antibiotics facilitates the selection pressure for antimicrobial resistance (AMR). The increase in complacency in the effective use of antibiotics also has great implications towards AMR. However, the misuse and overuse of antibiotics in livestock and agriculture have conspicuously augmented rates of AMR in human bacterial pathogens. AMR bacteria are implicated in causing infections that are difficult to treat, leading to increased morbidity and mortality amongst infected individuals. Diarrhea, dehydration, and its complications lead to the death of millions of children worldwide, especially in developing countries. Modified Orals Rehydration Therapy (m-ORT) with Glucose-Electrolyte Solutions (GES) is beneficial. Chronic diarrhea is common in children, is unrecognized, and has economic consequences. Treating acute diarrhea prevents the progression of Chronic Gastroenteritis (CG). However, infectious diarrhea requires more comprehensive management, pushing the limits of standard m-ORT. Public health practitioners have noted that the discovery of antibiotics has not made a substantive dent in this social ill. An essential feature of the infectious diseases, recurrent episodes led to the formation of an AntiDiarrheal Drug Declarations Workshop in 1982 [21, 22].

Ethical Considerations

The medicinal plants identified by traditional healers for treating diarrhea were evaluated for their biological activities. Three plants, *Eucalyptus citriodora*, *Cinnamomum camphora*, and *Berzelia fruticulosa*, were selected based on local availability. Crude extracts from these plants were prepared using methanol, acetone, and distilled water. Organisms associated with diarrhea were obtained from a Reference culture collection. The antimicrobial properties of the extracts were screened using the agar well diffusion method, and those showing activity were tested for minimum inhibition concentration (MIC) using broth dilution. Thin layer chromatography (TLC) was employed to analyze the extracts and identify bioactive compounds. Biological assays revealed that all extracts exhibited antimicrobial activity against Coliforms and *Salmonella typhi*, but not against Gram-positive bacteria like *Staphylococcus aureus* and *Enterococcus faecalis*. Methanol extracts displayed the highest inhibition against all pathogens except *B. cereus*. TLC analysis indicated the presence of various compounds. Alcoholic extracts outperformed aqueous extracts in both antimicrobial and medicinal activities; the aqueous extracts showed no activity against *B. subtilis*, enterotoxigenic *E. coli*, and limited activity against *E. faecalis*. Although all extracts demonstrated some antimicrobial properties, aqueous extracts were less effective than acetone and methanol extracts. Methanol extracts yielded outstanding results overall. The rise of antibiotic-resistant bacterial pathogens related to diarrhea poses a significant public health problem globally, including South Africa. Resistance, characterized by reduced susceptibility to antimicrobial agents, can be either primary or secondary, with primary resistance inherent to the organism [23-28].

CONCLUSION

The escalating threat of antimicrobial resistance among diarrheal pathogens demands urgent and innovative approaches. Medicinal plants, historically used across cultures for their healing properties, provide a viable complementary or alternative pathway to antibiotic therapy. Studies reveal significant antibacterial activity in plant extracts like *Acacia nilotica* and *Julbernardia paniculata*, which exhibit strong inhibitory effects on resistant diarrheagenic microbes. The unique bioactive compounds found in these plants can target resistance mechanisms differently than conventional antibiotics, potentially reducing

resistance development. To fully harness their potential, systematic scientific validation, compound isolation, and clinical trials are essential. Future integration of plant-derived antimicrobials into global health strategies may help mitigate the burden of diarrheal diseases and curb the spread of resistance, especially in resource-limited settings where such solutions are most urgently needed.

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