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Research Paper

Utilizing *Anacardium occidentale* Leaves Extract as an Alternative to Conventional Antibiotics Against Antimicrobial-Resistant Microorganisms

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Abstract

Antimicrobial resistance (AMR) is a critical global health crisis that compromises the efficacy of conventional antibiotics and increases the need for alternative therapeutic strategies. This study investigates the potential of *A. occidentale* leaf extracts as a natural replacement for conventional antibiotics, particularly against antibiotic-resistant bacteria. Extracts from young and mature leaves were prepared using aqueous and ethanolic methods and evaluated for their antimicrobial properties using the Kirby-Bauer disk diffusion method. The results showed that ethanolic extracts from young leaves exhibited a broad spectrum of antibacterial activity and effectively inhibited 17 bacterial strains, including *S. typhimurium* and *S. aureus*, with inhibition zones of up to 35.58 mm. This indicates that the young leaves are rich in bioactive compounds such as flavonoids, phenolic acids and terpenoids, which are known for their strong antimicrobial properties. In contrast, ethanolic extracts of mature leaves showed more specific antibacterial activity, particularly against *S. aureus* and methicillin-resistant *S. aureus* (MRSA), with zones of inhibition of 17.15 mm and 15.70 mm, respectively. These results suggest that mature leaves may contain unique bioactive compounds that are effective against specific pathogens, highlighting their potential for targeted antimicrobial therapies. This study highlights the importance of *A. occidentale* as a source of natural antimicrobial agents and emphasises the need to adhere to the principles of Islamic Sharia law during extraction. Given the ongoing threat of antibiotic resistance, the results support further research into the therapeutic applications of plant-derived compounds that combine traditional medical knowledge with modern scientific approaches to combat antibiotic-resistant infections.

Keywords *Antimicrobial resistance (AMR), A. occidentale, leaf extracts, inhibition zones, bioactive compounds, cultural and ethical considerations, halal*

INTRODUCTION

Antimicrobial resistance (AMR) is a major challenge worldwide. It undermines the effectiveness of conventional antibiotics and requires the development of innovative strategies to combat infectious diseases. The emergence of multidrug-resistant organisms (MDROs) has led to extensive research into alternative sources of antimicrobial agents, particularly plant-based compounds. *Anacardium occidentale*, commonly known as the cashew tree, has a rich history of traditional medicinal use. Studies have shown that extracts from the leaves of *A. occidentale* exhibit antibacterial, antifungal, anti-inflammatory, antioxidant and hypoglycemic properties, which has sparked interest in its potential as a reservoir of antimicrobial agents.

In this context, the present study aims to investigate the potential antimicrobial properties

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of extracts from young and mature leaves of the *A. occidentale* plant against a variety of pathogenic bacterial strains, including those resistant to conventional antibiotics. The methodology of the study includes a comprehensive evaluation of the antimicrobial activity of *A. occidentale* extracts by comparing their efficacy with seven established antibiotics. The measurement of inhibition zones, a standard measure in antimicrobial research, provides quantitative insights into the ability of the extracts to inhibit the growth of pathogenic microorganisms.

A notable aspect of this study is the adherence to the principles of Islamic Sharia during the extraction process, ensuring compliance with halal practices and ethical considerations. This underscores the importance of incorporating cultural and religious perspectives into scientific endeavours. With the emerging threat of AMR, research into alternative antimicrobial agents is essential. If successful, the results of this study could provide valuable insight into the use of *A. occidentale* extracts as a natural and potentially sustainable solution to combat antimicrobial resistance.

Furthermore, this study is not only promising for medical applications but is also in line with the broader goal of promoting environmentally conscious and culturally sensitive approaches in healthcare. By harnessing the therapeutic potential of herbal agents, this study contributes to ongoing efforts to combat antimicrobial resistance while respecting various cultural and ethical considerations.

LITERATURE REVIEW

Introduction to AMR

AMR is widely seen as the next global pandemic that humanity will face. According to Calvo-Villamanan et al. (2023), most believe that this pandemic is already underway and that the number of deaths caused by AMR will rise to almost 5 million worldwide in 2019. The World Health Organisation (WHO) has ranked AMR as one of the top 10 global threats to public health, putting it on par with the threat level of HIV, dengue, COVID-19 or future influenza pandemics. The progress made in public health through the introduction of antibiotics against infectious pathogens is now threatened by the global development of multidrug-resistant strains (Sharma et al., 2018). Indeed, AMR and MDROs are two interlinked issues in the field of infectious diseases, each of which poses a major challenge to public health.

AMR refers to the ability of microorganisms (bacteria, viruses, fungi and parasites) to resist the action of drugs previously used to successfully treat infections caused by them. This resistance can develop naturally over time through genetic changes but is often accelerated by the overuse and misuse of antibiotics and other antimicrobials. MDROs are certain types of bacteria that have developed resistance to multiple antibiotics. These organisms are of particular concern because they limit treatment options for infections. Common examples include MRSA (methicillin-resistant *S. aureus*), VRE (vancomycin-resistant *enterococci*) and CRE (carbapenem-resistant *Enterobacteriaceae*). Both AMR and MDROs pose a significant threat to modern medicine. They complicate surgery, chemotherapy and the treatment of chronic diseases that require frequent or long-term treatment. They also increase the risk of disease spread, serious illness and death while increasing healthcare costs through longer hospital stays and more intensive care.

Several global strategies and initiatives have been developed to combat AMR and MDROs. For years, the approach to fight MDROs has been an almost exclusively scientific one. As AMR started spreading, a huge effort was placed in the search for new antibiotics and the development of new therapies. This includes research into novel compounds that act on bacteria in a different way to existing drugs.

Natural compounds, particularly those derived from plants, show promise as effective agents against AMR and MDROs. These compounds often target multiple microbial mechanisms and have been shown to be effective in overcoming resistance in various pathogens, making them an essential resource for the development of new antimicrobials (Jadimurthy, 2023; Ashraf, 2023). Many natural products, especially those derived from plant extracts, provide a diverse reservoir of secondary metabolites, such as flavonoids and alkaloids, that exhibit significant antibacterial and antifungal activities. These substances can interfere with bacterial quorum sensing and biofilm formation, which is crucial for combating resistant strains (Jadimurthy, 2023). The synergistic effects of phytochemicals with conventional antibiotics provide a compelling alternative to conventional therapies and reduce the evolutionary pressure on pathogens to develop resistance (Zhai et al., 2023).

The existence of substantial evidence for the development of drug resistance in microbes has attracted much attention in the scientific world. To address this issue, researchers have conducted experiments and tested strategies, including screening different molecules and using natural plant products, to see if these substances can serve as an untapped source of antibacterial, antiviral and antifungal agents. Since these substances are non-toxic, non-synthetic, have minimal side effects and are inexpensive, the development of new antimicrobials relies heavily on the use of many of these existing products and increases the need for new natural substances yet to be discovered. These natural plant-based products promise the best protection against infections and the pathogenesis of many diseases. In addition, the biodegradability of many of these products increases their chances of being used by farmers and plant biologists to combat microbial pathogenesis, especially AMR and MDROs.

The Potential of *A. occidentale*

A. occidentale, also known as the cashew tree, is a tropical evergreen tree that originally comes from north-eastern Brazil. In Asia, the tree is widespread in the tropical and subtropical regions of Myanmar, Thailand, Vietnam, Malaysia, Indonesia, India, Pakistan, Sri Lanka and the Philippines (Norzielawati, 2015). According to Heuze et al. (2016), *A. occidentale* is known as a multipurpose plant species, almost all parts of which are edible and have ethnomedicinal properties. Among the different parts of *A. occidentale*, the most special part is the fruit, commonly called the nut. Inside the dark nutshell is a large, edible, curved seed. Botanically speaking, the nut is the actual fruit, and the fleshy cashew apple is the swollen fruit stalk. The cultivation of the nuts and the products derived from them is widespread in Australia, Asia, and Africa (Ogunwolu et al., 2010; Liu et al., 2018).

In Southeast Asian countries, especially Malaysia, Thailand, and Indonesia, the young and tender leaves of *A. occidentale* are regularly consumed as a spice. The young leaves are popular as *ulam*, which can be eaten raw. The Malays believe they can cure certain diseases and give a youthful appearance (Norzielawati, 2015). Numerous pharmacological properties have been reported for *A. occidentale*, including antioxidant, antibacterial, antiviral, antifungal, hypoglycemic, hypolipidemic, anticholesterolemic, anti-ulcerogenic, antihypertensive, analgesic, and anti-inflammatory activities (Chan et al., 2017). A detailed study of phytochemical and pharmacognostic parameters was conducted by Yogini et al. (2012), and the results of the study showed the presence of carbohydrates, proteins, saponin glycosides, flavonoids, alkaloids, tannins and phenolic compounds in the ethanol and aqueous extract of *A. occidentale* leaves. On the other hand, Chan et al. (2017) reported that flavonoids and phenolic acids are the major metabolites found in the leaves of *A. occidentale*. According to the study conducted by Sulong et al. (2022), the extracts from young and mature leaves of *A. occidentale* effectively reduce the activity of amylase, an enzyme important for carbohydrate digestion that affects blood glucose levels. The extracts prepared using aqueous extraction methods showed that mature leaves significantly reduced amylase activity within 15 to 30 minutes, while young leaves took up to 60 minutes to show comparable effects.

Based on the study by Dahake et al. (2009), the antibacterial and antifungal activities of *A. occidentale* were successfully tested against various bacteria such as *B. subtilis, S. aureus, P. aeruginosa, E. coli* and various fungi such as *C. albicans* and *A. niger*. These most common pathogenic bacteria cause infections, especially nosocomial infections, and are already resistant to most antibiotics. Methicillin-resistant *S. aureus* (MRSA), known for its resistance to a range of antibiotics, is frequently associated with infectious complications in hospital patients and medical staff.

E. coli is a bacterium commonly found in the intestines of humans and other warm-blooded animals. The National Centre for Emerging and Zoonotic Infectious Diseases reports that most *E. coli* strains are harmless. However, a few are known to contaminate food. Symptoms of illness include abdominal cramps, pain, bloody diarrhoea, and nausea. Fever and vomiting may also occur. Most people recover within two weeks, although the disease can become extremely dangerous in a few cases.

S. aureus causes various pyogenic (pus-forming) infections and toxinosis (microbial toxins) in humans. *S. aureus* causes superficial skin lesions such as pimples or boils and more serious infections such as osteomyelitis and endocarditis. It is an important community-acquired infection, nosocomial infections of surgical wounds and the most common cause of hospital-acquired infections such as surgical wounds and *S. aureus* in hospitals are becoming increasingly resistant to antibiotics. Recently, problems with microorganisms that are unaffected by drugs, side effects of conventional drugs and the development of diseases for which there are no drugs have reawakened awareness and curiosity about plants as an important source of new drugs.

Phytochemicals are plant metabolites that act as a natural defence system for host plants and provide characteristic colour, smell and taste in certain plant parts. They are a group of non-nutrient compounds biologically active when consumed by humans. Many phytochemicals are beneficial to health and can prevent many diseases. Phytochemical analysis revealed the presence of alkaloids, tannins, anthraquinones, glycosides, and phenols in both ethanol and aqueous extracts of *A. occidentale* dried fruit. The presence of alkaloids, tannins, and saponins in *A. occidentale* stem extract is also reported. Several studies have reported a rich variety of secondary metabolites in *A. occidentale* extracts. The pharmacological properties of medicinal plants have been attributed to their rich secondary metabolites. Plants generally produce many secondary metabolites, which constitute an important source of microbicides, pesticides, and pharmaceutical drugs. Flavonoids have been reported to significantly affect the cell wall of microorganisms, which may invariably lead to the collapse of the cell wall and, overall, affect the entire mechanism of the microbial cell. Alkaloids have also been reported to be involved in antimicrobial activities. Hassan et al. (2019) reported an appreciable antimicrobial effect of the ethanol leave extract of *A. occidentale* against *E. coli, S. mutans, B. cereus, S. typhi, and C. albicans* in addition to varying levels of phytochemicals in the leaves and stem bark of *A. occidentale*.

The result of the minimum bactericidal concentration (MBC) was similar to the report of Arekemase et al. (2020), who reported that the ethanolic extract was found to be bactericidal to all the test bacteria, while the aqueous extract was found to be bacteriostatic to the test bacteria. Methanol and ethyl acetate of leaf extract showed more sensitivity against *S. aureus* and *E. coli.* The leaf and bark of *A. occidentale* have been found to be effective against *S. aureus*, supporting the findings that the leaf and bark of *A. occidentale* can cure some of the diseases caused by *S. aureus*, such as urinary disorders and skin infections. The active compound from *A. occidentale* was also reported to have antimicrobial activity against pathogens, which can produce an active packaging film with a pH-triggered release (Lee et al., 2020). The antimicrobial activity of *A. occidentale* is related to the inhibition of beta-lactamase by anacardic acids. Beta-lactamases possess considerable hydrolytic activity and can inactivate a variety of beta-lactam antibiotics.

Anacardic acids have emerged as important alternatives for controlling microorganisms that employ this strategy as an escape and resistance mechanism against most commercially available antibiotics. Within this context, the present results suggest that the aerial parts of *A. occidentale*, particularly its flowers, show promise as materials for bioprospecting new broad-spectrum antimicrobial agents to treat diseases caused by microorganisms resistant to commercially available antibiotics. The most frequent antimicrobial activity was described in the leaves and barks against bacteria (*S. aureus, S. mutans, S. sobrinus, E. coli, E. faecalis*) and fungi (*C. albicans*). According to Sija et al. (2019), when using leaves, the highest activity in ethanolic extract was observed against *S. oralis, P. mirabilis,* and *E. coli*, while the lowest activity was against *S. epidermidis*. Therefore, there is an urgent need for studies on new antimicrobial agents capable of overcoming multidrug-resistant mechanisms. This study also focuses on the potential of young and mature leaves of *A. occidentale* for antimicrobial activity against antimicrobial-resistant pathogens.

Ethical and Cultural Considerations in Research

The importance of sustainable and ethical harvesting methods for medicinal plants such as *A. occidentale* cannot be overstated. Sustainable harvesting methods are crucial to ensure that the use of these plants does not lead to environmental degradation or depletion of natural resources. Ethical harvesting also includes fair trade practices that ensure local communities benefit economically from the resources they provide. In the case of medicinal plants, excessive harvesting can lead to species endangerment, biodiversity loss and ecosystem disruption. Therefore, applying sustainable practices helps maintain the ecological balance and ensure the availability of these resources for future generations (Papageorgiou et al., 2020). This aligns with the United Nations Sustainable Development Goal (SDG) 15 about Life on Land, which emphasizes the need to protect, restore and promote the sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and biodiversity loss.

The cultural and religious implications of using medicinal plants such as *A. occidentale* in medicine are profound. In many cultures, these plants have historical uses and significant cultural ties. It is essential to ensure that the extraction and use of these plants is in line with cultural and religious practices such as halal. This not only respects the beliefs and practices of the communities concerned, but also enhances the acceptability and marketability of pharmaceutical products derived from these plants. For example, adhering to Halal practices in the extraction processes means avoiding the use of prohibited substances and ensuring cleanliness and compliance with Islamic laws, which is crucial for products intended for consumption by the Muslim population. This also supports SDG 12 about Responsible Consumption and Production, which promotes sustainable consumption and production patterns. These ethical and cultural considerations are integral to responsible scientific research and commercialization that promotes respect, sustainability and inclusivity in the use of medicinal plants.

RESEARCH METHODS

This study was carried out in the Bioprocess Laboratory of the Department of Science and Biotechnology, Faculty of Engineering and Life Sciences at Universiti Selangor (UNISEL), located in Bestari Jaya, Selangor, Malaysia.

Sample of Plant Collection:

The plant material used in this study consisted of young and mature leaves of *A. occidentale.* These leaves were collected in Bestari Jaya Village, Kuala Selangor, Malaysia. A botanist in the Herbarium of Forest Research Institute Malaysia (FRIM), Kepong, Kuala Lumpur, Malaysia, sent the plant samples for identification and authentication with herbarium number PID081021-06.

Plant Extraction

Preparation for the leaves extraction: firstly, they were thoroughly washed, oven-dried for three days, and finely ground into a powder form. A total of 10 g of powder-dried leaves were dissolved in 100 ml of distilled water (aqueous) and ethanol solution (denatured 95%, HMBG brand), respectively, to facilitate the extraction of bioactive compounds. It is worth noting that the extraction process strictly adhered to the principles of Islamic Shariah in order to ensure adherence to halal practices. Once the dissolution was complete, the mixture underwent filtration using Whatman's filter paper No. 1 (Cytiva) to obtain the crude extract. Subsequently, the resulting extract was concentrated under reduced pressure using a rotary evaporator (Buchi) and stored at 4°C temperature until further analysis.

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was conducted using the disc diffusion method, based on the Kirby-Bauer method described by Bauer et al. (1966). The test was carried out by culturing each microorganism for 18 hours at 37°C in 2.5 mL of Mueller-Hinton broth (CM0405B) from Oxoid. The selected microorganism's inoculum was added into the test tube containing 1 mL of phosphate-buffered saline (PBS) solution (806552) from the Sigma-Aldrich brand. The cell turbidity in the test tube was compared to the McFarland standard No. 0.5 (Remel brand), which is equal to the cell density of 10^{-8} CFU mL^{-1}. Using a cotton swab on the surface of a plate containing Mueller-Hinton agar (CM0337B) from Oxoid, each inoculum was disseminated, spreading on the surface of the agar. Next, the wells were aseptically bored on each agar plate using a sterile cork borer (6mm) and were properly labelled. Each well was filled with approximately 20 µL of each leaf extract sample. The petri dishes were then incubated at 37°C for 24 hours. Experiments were performed in duplicate. The inhibition zones were measured, and their diameters, resulting from the antimicrobial effect of the extracts, were calculated in mm and recorded in a table. A negative result with no inhibition zone was recorded if microbial growth was present around the extract disc.

Conversely, a positive result, indicating a zone of inhibition, was recorded if there was no microbial growth around the extract disc. A blank disc filled with 20 µL of distilled water was used as a negative control. After the incubation period, the diameters of the inhibition zones (halos) were measured using an electronic LCD digital caliper (Mitutoyo Corporation). Inhibition zones were classified as moderate (+) if their diameter was within 6.0 mm to 10.0 mm, satisfy (++) if their diameter fell within the range of 11.0 mm to 15.0 mm, good (+++) if their diameter fell within the range of 16.0 mm to 20.0 mm, and excellence (++++) if their diameter exceeded 21.0 mm.

FINDINGS AND DISCUSSION

Antimicrobial Activity of *A. occidentale* Leaves Extract

According to Table 1, the aqueous extraction of young leaves has demonstrated inhibitory effects on four tested bacteria: *S. aureus* (25.93 mm), *L. monocytogenes ATCC 7644* (20.51 mm), *P. aeruginosa* ATCC 25619 (18.69 mm), and *E. faecalis* ATCC 51299 (15.85 mm). These findings indicate that the bacteria tested displayed a notable inhibition zone, with halo diameters exceeding 15 mm, suggesting significant antibacterial activity. This suggests that the aqueous extraction method effectively extracted bioactive compounds from young leaves, which exhibited antibacterial properties against various pathogenic bacteria. Unfortunately, there was no observed suppression of the tested bacterial strains. The results suggest that the aqueous extraction of mature leaves did not demonstrate any antibacterial properties, implying a potential disparity in the chemical makeup or concentration of bioactive substances compared to the young leaves.

Table 1. An Inhibition zone by *A. occidentale* Leaves Extract Against The Tested Microorganims

Tested Microorganisms	Young Leaves Extract Diameters (mm)	Matured Leaves Extract Diameters (mm)
	Aqueous (water) Method	
S. aureus	25.93 (++++)	6.0
L. monocytogene ATCC 7644	20.51 (++++)	6.0
P. aeruginosa ATCC 25619	18.69 (+++)	6.0
E. faecalis ATCC 51299	15.85 (++)	6.0
	Ethanol Method	
S. typhimurium ATCC 13311	35.58 (++++)	6.0
Salmonella sp	28.13 (++++)	6.0
S. aureus	25.76 (++++)	17.15 (+++)
E. faecalis ATCC 51299	25.10 (++++)	6.0
P. aeruginosa ATCC 25619	21.42 (++++)	6.0
E. coli O157	20.53 (+++)	6.0
K. pneumoniae Sub Pneumoniae ATCC 13883	20.52 (+++)	6.0
L. monocytogene ATCC 7644	20.13 (+++)	6.0
S. epidermis	18.01 (+++)	6.0
S. typhi	19.50 (+++)	6.0
E. coli V517	19.12 (+++)	6.0
P. multocida ATCC 12945	19.05 (+++)	6.0
E. coli ATCC 11775	17.11 (+++)	6.0
S. choleraesuis ATCC 10708	16.65 (+++)	6.0
E. faecalis ATCC 14506	16.17 (+++)	6.0
E. coli ATCC 25922	16.06 (+++)	6.0
S. paratyphi A ATCC 9150	15.47 (++)	6.0
Methicillin Resistance *S. aureus* (MRSA) ATCC 49476	6.0	15.70 (++)

Indicators:

	Range diameter of inhibition zone	Indicates
+	6-10 mm	Moderate
++	11-15 mm	Satisfied
+++	16-20 mm	Good
++++	> 21 mm	Excellence

Based on the results presented in Table 1, the extraction using the ethanol method revealed inhibitory effects on 17 of the tested microorganisms. These microorganisms include *S. typhimurium* ATCC 13311, *Salmonella sp*, *S. aureus*, *E. faecalis* ATCC 51299, *P. aeruginosa* ATCC 25619, *E. coli* O157, *K. Pneumoniae* Sub *Pneumoniae* ATCC 13883, *L. monocytogenes* ATCC 7644, *S. epidermis*, *S. typhi*, *E. coli* V517, *P. multocida* ATCC 12945, *E. coli* ATCC 11775, *S. choleraesuis* ATCC 10708, *E. faecalis* ATCC 14506, *E. coli* ATCC 25922, and *S. paratyphi* A ATCC 9150. These findings suggest that the ethanolic extraction method yielded extracts with broad-spectrum antibacterial activity, effectively targeting a diverse range of bacterial species. Additionally, the data demonstrated significant inhibition of these tested microorganisms by the ethanolic leaf extract (Shobha et al., 2018).

Furthermore, a study conducted by Ifenkwe et al. (2023) suggested that the presence of anthraquinone, resins, terpenoids, glycosides, and phenolic compounds in the crude extract of *A. occidentale* leaves may have contributed to its antimicrobial activity against Gram-negative bacterial strains such as *Salmonella sp*. and *E.coli*.

In contrast, the extraction of mature leaves using the ethanol method only inhibited the growth of *S. aureus* and Methicillin-Resistant *S. aureus* (MRSA) ATCC 49476. This indicates that mature leaves did not exhibit broad-spectrum antibacterial activities. However, they displayed effectiveness against the significant pathogen MRSA, indicating therapeutic potential. The comparison between young and mature leaves revealed that young leaves exhibited superior antibacterial activity, especially when extracted using the ethanol method. This difference in effectiveness suggests that it may be attributed to variations in the phytochemical composition, with young leaves potentially containing higher levels of bioactive compounds compared to mature leaves. Some common phytochemical compounds found in both young and mature leaves of *A. occidentale* include flavonoids, phenolic acids, tannins, triterpenoids, saponins, and essential oils.

Phenolic compounds, such as flavonoids and phenolic acids, are known for their antioxidant and anti-inflammatory properties and are abundant in both young and mature leaves of *A. occidentale*. Additionally, tannins, found in cashew leaves, contribute to the plant's defence against herbivores and pathogens, while triterpenoids exhibit diverse biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. On the other hand, Saponins are glycosides with detergent-like properties and have been studied for their potential health benefits (Nisa et al., 2024).

Young leaves of *A. occidentale* may contain essential oils rich in volatile compounds such as terpenes, which also possess antimicrobial and insecticidal properties (Dahake et al., 2009). These oils are responsible for the characteristic aroma of the leaves. Overall, both young and mature leaves of *A. occidentale* contain a rich array of phytochemical compounds with various biological activities, contributing to the plant's defence mechanisms and potential therapeutic properties.

The ethanol extraction method appeared to be more effective in extracting antibacterial compounds compared to the aqueous method for both young and mature leaves. This method may solubilize certain antibacterial constituents present in young leaves more efficiently.

In conclusion, our findings suggest that young leaves tend to have higher concentrations of certain antioxidants, such as flavonoids, phenolic acids, and carotenoids. These compounds protect the plant from oxidative stress during early growth stages. Additionally, young leaves may contain higher levels of terpenoids, including essential oils, contributing to their characteristic aroma and potential antimicrobial properties. On the other hand, mature leaves accumulate higher levels of tannins, which contribute to their astringent taste and serve as a defence mechanism against herbivores and pathogens. While alkaloids may be present in both young and mature leaves, their concentration tends to remain more stable in mature leaves due to less active growth and metabolic activity. The types and concentrations of flavonoids in mature leaves may differ from those in young leaves, potentially shifting towards compounds with specialized functions related to defence and stress response.

Various factors, such as metabolic activity, environmental conditions, genetic differences, and cultivation practices, may influence the composition of phytochemicals in leaves. Understanding these differences is crucial for maximizing their potential applications in medicine, nutrition, and agriculture.

Although commercial products derived from *A. occidentale* leaves may not be readily available, continuous research in this area may lead to the development of innovative products and applications. Additionally, traditional knowledge suggests that local communities in regions with abundant *A. occidentale* trees may already utilize the leaves for various purposes.

Future research could explore the potential applications of *A. occidentale* leaf extracts in functional foods, dietary supplements, cosmetics, agricultural products, and industrial processes. The expanding market demand for natural and plant-based products may generate more opportunities for the commercialization of products incorporating *A. occidentale* leaf extracts. Overall, our study contributes to understanding the antibacterial properties of *A. occidentale* leaves and their potential applications in diverse fields, including medicine, agriculture, and environmental sustainability.

CONCLUSIONS

The findings of this study demonstrate that ethanolic extracts from young leaves exhibit potent antibacterial effects against various microorganisms, including antibiotic-resistant strains. However, the extraction of mature leaves using the ethanol method only inhibited the growth of *S. aureus* and Methicillin-Resistant *S. aureus* (MRSA) ATCC 49476. This indicates that mature leaves did not exhibit broad-spectrum antibacterial activities. However, they displayed effectiveness against the significant pathogen MRSA, indicating therapeutic potential.

Future research endeavours may focus on isolating and characterizing the antibacterial bioactive components present in both young and mature *A. occidentale* leaves. Additionally, investigating the potential synergistic actions between these substances could provide further insights into their therapeutic efficacy. Exploring *A. occidentale* leaves for their bioactive components and pharmacological characteristics holds promise for advancing our understanding of natural remedies and medication discoveries. Furthermore, delving into the multifaceted uses of *A. occidentale* leaves presents an exciting and diverse opportunity with potential benefits spanning various domains, including science, medicine, economic growth, sustainability, culture, environment, and wellness.

LIMITATION & FURTHER RESEARCH

The study pointed out several limitations, including the variability in antimicrobial activity between extracts from young and mature leaves, suggesting differences in bioactive compounds depending on the maturity of the leaves. In addition, the exclusive use of ethanolic and aqueous extraction methods may not have extracted all potentially bioactive compounds. The research was also limited to laboratory scale, which raises questions about the scalability and practical application of the results to larger production and clinical settings. In addition, the study did not comprehensively investigate the mechanisms underlying the extracts' antimicrobial effects, which is crucial for the development of effective therapeutics. Finally, the lack of clinical studies to validate the efficacy and safety of these extracts in humans represents a significant gap before pharmaceutical applications can be considered.

The study on the leaf extracts of *A. occidentale* suggests several avenues for further research to improve their potential as alternative antibiotics. Future studies should focus on the isolation and characterization of the specific bioactive compounds in both young and mature leaves to understand their individual and synergistic antimicrobial effects. Exploring alternative extraction methods could potentially increase the spectrum and potency of the extracted bioactive compounds and lead to a better understanding of the plant's antimicrobial capabilities. Detailed mechanistic studies are required to elucidate the biochemical pathways and mechanisms behind the antimicrobial effects of the extracts, which would contribute to more targeted therapeutic applications. In addition, conducting pharmacokinetic and pharmacodynamic studies as well as clinical trials is crucial to evaluate the safety and efficacy of these extracts in the human population and facilitate their development into viable pharmaceutical agents. As interest in plant-based antimicrobials grows, it will also be important to ensure sustainable harvesting and ethical considerations in the sourcing and production of these materials.

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