



# The Leaves and Seeds of *Vigna subterranean* (Bambara Nut) Inhibit the Growth of Selected Bacterial and Fungal Organisms

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

With increasing resistance of bacterial organisms to conventional antibiotics, it is crucial that alternative therapies are produced to combat this menace. One plant that has gathered the attention of researchers in recent time as a potential medicinal plant is Bambara nut (*Vigna subterranean*). Hence, this study was aimed at investigating the antimicrobial properties of *Vigna subterranea*, commonly known as the Bambara nut, as a potential alternative to synthetic antibiotics

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amid rising resistance levels. The study employed an *in vitro* experimental design, utilizing the agar well diffusion method for qualitative assessment and the broth microdilution method to determine the minimum inhibitory concentration (MIC) of extracts from the seeds and leaves of the plant. The findings reveal that both the leaf and seed extracts possess significant antimicrobial activities against various pathogens, including both Gram-positive and Gram-negative bacteria and fungi. The comparison of the results from both extracts showed that the leaf extract has a higher antibacterial effect than the seed extract. However, for the antifungal activity, whereas higher antifungal activity was seen in the leaf extract based on zones on inhibition, the MIC result of the seed extract was better. Chemical analysis identified phytochemicals such as flavonoids, tannins, and alkaloids, which are known to contribute to the observed antimicrobial activity. This shows that extracts from *Vigna subterranea* possess significant antimicrobial potential, with higher potency seen in the leaf extracts. This means that the components of this plant could be developed as natural alternatives to combat AMR. In conclusion, these findings underscore the importance of further research into plant-based antimicrobials, which may provide sustainable solutions to the growing challenge of drug resistance in clinical settings.

**Keywords:** Antibiotics; seeds; bacterial and fungal organisms; medicinal plant.

## 1. INTRODUCTION

The rise of antimicrobial resistance (AMR) poses a grave threat to global public health, affecting millions of lives annually. AMR occurs when microorganisms such as bacteria, fungi, viruses, and parasites develop resistance to drugs that were once effective against them. This resistance renders many traditional treatments ineffective, leading to prolonged infections, higher medical costs, and increased mortality (World Health Organization, 2023). According to the World Health Organization (WHO), AMR is one of the top 10 global public health threats facing humanity, and it is responsible for approximately 700,000 deaths per year worldwide (World Health Organisation, 2021; Oliveira et al., 2024). Without urgent action, this number could rise to an estimated 10 million deaths per year by 2050, surpassing deaths caused by cancer (O'Neill, 2016). Given AMR's significant health and economic burden, there is an urgent need to explore alternative antimicrobial therapies. One promising avenue is the use of natural antimicrobial agents derived from plants. Many plants produce secondary metabolites—bioactive compounds such as alkaloids, flavonoids, tannins, and saponins—that have evolved as defence mechanisms against pathogens. These compounds can inhibit the growth of bacteria, fungi, and viruses by targeting vital cellular processes, such as protein synthesis, cell wall formation, and DNA replication (Zou et al., 2023).

The potential of plant-based antimicrobials has garnered significant interest in recent years, particularly as researchers search for alternatives

to synthetic antibiotics. One such plant is *Vigna subterranea*, commonly known as the Bambara nut. This leguminous plant is widely grown in sub-Saharan Africa, where it serves as an essential crop for food security due to its high nutritional value. In addition to its rich content of proteins, carbohydrates, and essential amino acids, the Bambara nut contains bioactive compounds with potential antimicrobial properties (Tan et al., 2020; Majola et al., 2021). Preliminary studies have shown that extracts from the seeds and leaves of *Vigna subterranea* possess antimicrobial activity against a range of pathogens, including Gram-positive and Gram-negative bacteria (Ajiboye and Oyejobi, 2017; Taahir, 2017). The presence of phytochemicals such as flavonoids, tannins, and alkaloids in the plant is believed to contribute to its antimicrobial potential. Flavonoids, for instance, can disrupt bacterial cell walls, making them more permeable and leading to cell death. Alkaloids, on the other hand, can interfere with microbial DNA replication, preventing cell proliferation (Huang et al., 2022; Shamsudin et al., 2022).

The Bambara nut has long been used in traditional medicine to treat various ailments, including infections. Its seeds and leaves have been used as poultices to treat wounds and skin infections, and decoctions made from the plant have been employed to treat gastrointestinal infections. The plant's antimicrobial properties make it a promising candidate for further investigation as a natural alternative to conventional antibiotics, especially in light of the growing problem of drug resistance (Udeh et al., 2020; Majola et al., 2021; Oluwole et al., 2021).

Despite the known potential of natural antimicrobials, there exists a significant gap in knowledge regarding the specific antimicrobial mechanisms and efficacy of plant-derived compounds, particularly those from under-researched species like *Vigna subterranea*. Although preliminary studies indicate that extracts from this plant possess antimicrobial activity (Ajiboye and Oyejobi, 2017; Taahir, 2017), comprehensive investigations into its bioactive compounds, optimal extraction methods, and their modes of action against multidrug-resistant pathogens remain scarce. Furthermore, while *Vigna subterranea* has historical significance in traditional medicine, there is limited scientific validation of its use for treating specific infections or its comparative effectiveness against established antibiotics. Therefore, this study aims to address this knowledge gap by exploring the antimicrobial properties of *Vigna subterranea*. By contributing to the development of plant-based alternatives to conventional antibiotics, it aims to address the global AMR challenge. The findings may pave the way for more rigorous studies, potentially leading to the introduction of effective natural antimicrobial agents into clinical practice. This research aimed to explore the antimicrobial properties of *Vigna subterranea* extracts and determine their efficacy against selected microbial isolates. This aim was achieved by evaluating the antimicrobial effect of *Vigna subterranea* leaf and seed extracts on bacterial and fungal isolates known for causing infections in humans, identifying the phytochemical components within the extracts that contribute to their antimicrobial activity, and comparing the effectiveness of *Vigna subterranea* extracts with standard antibiotics to assess their potential as alternative treatments.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of *Vigna subterranea* Extracts

Healthy plant samples (leaves and seeds of Bambara nut) were selected to ensure the quality of extracts. These were collected from Imo State University farmland, typically during the peak growing season, to maximize the presence of the bioactive compounds. After collection, samples were rinsed with distilled water to remove contaminants like dust and soil particles, which could affect extract purity and reliability during antimicrobial testing. Collected samples were air-dried in a controlled environment to prevent

degradation of active phytochemicals, a method consistent with best practices for preserving plant materials used in medicinal studies. Once the leaves and seeds were prepared, an extraction process was initiated, utilizing ethanol, an organic solvent known to effectively solubilize a range of bioactive compounds, including flavonoids, alkaloids, and tannins. The dried plant materials were ground into a fine powder to increase the surface area, thus enhancing the extraction efficiency. A typical extraction involved soaking the powdered samples in ethanol at room temperature for 24 to 72 hours, with occasional stirring to facilitate compound release. Following this, the mixture was filtered to separate the liquid extract from plant residues, yielding a solution rich in active phytochemicals.

### 2.2 Concentration and Storage of the Extracts

After filtration, the liquid extracts were concentrated to remove excess solvent. This was done with a rotary evaporator under reduced pressure and low temperatures to protect heat-sensitive compounds. This concentration step produced a viscous residue, which was further dried to a semi-solid or powder form for easier handling. The dried extracts were then stored in airtight containers at a low temperature (usually 4°C) to prevent degradation. Proper storage was crucial, as exposure to light, heat, or air could diminish the potency of bioactive compounds. By following these steps, the prepared *Vigna subterranea* extracts maintained stability and efficacy for subsequent antimicrobial testing.

### 2.3 Microbial Isolates

The study included a range of microbial isolates commonly linked to infections in clinical and environmental settings. These isolates were selected due to their significant impact on human health, resistance profiles, and prevalence in various types of infections, such as wound and foodborne illnesses. The bacterial and fungal pathogens examined in this research included *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus* species. These isolates were sourced from both clinical and laboratory environments. Clinical strains provided a realistic model for infection due to their presence in patient populations and specific resistance mechanisms. Laboratory strains from microbial collections served as standardized controls to ensure experimental consistency and replicability.

## 2.4 Antimicrobial Susceptibility Testing

### 2.4.1 Agar Well Diffusion and Broth Microdilution Assay

The antimicrobial susceptibility of *Vigna subterranea* extracts was assessed using the agar well diffusion method and the broth microdilution method. The agar well diffusion method served as a preliminary qualitative assessment. In this technique, wells were created in an agar plate that had been pre-inoculated with the test microorganism. The plant extracts were then introduced into these wells, allowing them to diffuse through the agar medium. As the extract spreads, it establishes a concentration gradient, with higher concentrations near the well and lower concentrations further away. This arrangement facilitated clear visualization of antimicrobial activity by measuring the zones of inhibition, where bacterial or fungal growth is prevented around the wells.

Following the agar well diffusion method, the broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) of the *Vigna subterranea* extracts. This procedure involved diluting the extracts in broth and inoculating them with the target microorganisms, enabling precise quantification of the lowest concentration that inhibits microbial growth. Together, these methods provided a comprehensive evaluation of the antimicrobial properties of the extracts.

### 2.4.2 Preparation of Culture Media and Inoculation with Microbial Isolates

Nutrient agar (for bacterial isolates) and Sabouraud dextrose agar (for fungal isolates) were prepared according to standard protocols. The agar was sterilized to eliminate any contamination, poured into sterile Petri dishes, and allowed to solidify. Once the media was set, it was inoculated with microbial isolates by evenly spreading a suspension of the test organism across the surface using a sterile swab. This process ensured a uniform microbial lawn, providing a consistent basis for measuring the effectiveness of the extracts. Each organism was cultured to its logarithmic growth phase, ensuring active growth for accurate susceptibility testing

### 2.4.3 Application of *Vigna subterranea* Extracts and Measurement of Zones of Inhibition

After inoculating the plates, wells were created using a sterile cork borer, typically 6 mm in diameter. Varying concentrations of *Vigna subterranea* extracts, prepared as described in the extraction process, were carefully pipetted into each well to assess their effectiveness across concentrations. Plates were then incubated at an optimal temperature for each microorganism (37°C for bacteria and 30°C for fungi) over 24 to 48 hours, depending on the growth rate of the pathogen. Following incubation, zones of inhibition were measured as the clear, circular areas around the wells where microbial growth was visibly suppressed. These zones indicated the antimicrobial potency of the extracts, with larger zones suggesting higher effectiveness. Results were recorded in millimetres (mm), and measurements were repeated to ensure accuracy and reproducibility

## 2.5 Phytochemical Analysis

### 2.5.1 Identification of Bioactive Compounds in the Extracts Using GC-MS

To characterize the bioactive compounds in *Vigna subterranea* extracts, advanced analytical technique like Gas Chromatography-Mass Spectrometry (GC-MS) was employed. GC-MS is particularly effective for identifying volatile compounds, separating and analyzing complex mixtures, and providing detailed molecular information on the chemical structure of each compound. The sample was vaporized and passed through a chromatographic column during GC-MS analysis. As each compound was separated based on its retention time, it was subsequently ionized and identified via mass spectrometry, where each compound's mass-to-charge ratio provided a unique signature. This process identified various classes of phytochemicals in *Vigna subterranea* extracts, including flavonoids, phenols, tannins, alkaloids, and saponins.

### 2.5.2 Analysis of the Antimicrobial Effects Linked to Specific Phytochemicals

Once identified, the antimicrobial properties of these phytochemicals were examined in the context of existing literature. Flavonoids and phenolic compounds, for example, are known to disrupt microbial cell walls and inhibit critical

enzyme functions, weakening microbial cells and preventing growth. Tannins, another key component, have demonstrated efficacy against a range of bacteria by binding to proteins on the cell wall, thereby inhibiting enzyme activity and reducing microbial adhesion to surfaces. Alkaloids, widely recognized for their antimicrobial effects, interfere with bacterial protein synthesis, making them effective against both Gram-positive and Gram-negative bacteria. Saponins exhibit antifungal and antibacterial activities, as they disrupt cell membrane integrity, causing leakage of cellular contents and eventual cell lysis. By correlating the specific phytochemicals identified in HPLC analysis with these mechanisms, the study highlighted the potential for *Vigna subterranean* extracts to inhibit microbial growth effectively

## 2.6 Data Analysis

The data from the study was analyzed statistically using Statistical Package for the Social Sciences (SPSS). One-way Analysis of Variance (ANOVA) was employed to compare the mean inhibition zones across different extract concentrations and between leaf and seed extracts. This method was particularly suitable for assessing whether there were statistically significant variations in antimicrobial activity across multiple experimental groups. Significance levels were set at  $p < 0.05$ , indicating that differences with a probability of occurring by chance less than 5% were considered statistically significant.

## 3. RESULTS

### 3.1 Antibacterial and Antifungal Effects of *Vigna subterranea* Extracts on Selected Pathogens

The analysis of the antimicrobial activity of *Vigna subterranea* extracts reveals notable differences between the leaf and seed extracts against various microbial isolates, including both bacterial and fungal species (Table 1). For *Escherichia coli*, the leaf extract exhibited a zone of inhibition measuring  $15.0 \pm 1.2$  mm, reflecting moderate antimicrobial activity, while the seed extract had a lower inhibition zone of  $10.0 \pm 1.5$  mm. The leaf extract's minimum inhibitory concentration (MIC) was  $5.0 \pm 0.3$  mg/mL, indicating its effectiveness. The p-value of 0.002 suggests a statistically significant difference, reinforcing the leaf extract's superior efficacy.

Regarding *Staphylococcus aureus*, the leaf extract produced a significant inhibition zone of  $18.0 \pm 1.0$  mm compared to the seed extract's  $12.0 \pm 1.8$  mm. The MIC was lower for the leaf extract at  $4.0 \pm 0.2$  mg/mL, while the seed extract had an MIC of  $5.0 \pm 0.2$  mg/mL. A p-value of 0.001 confirms a significant difference, underscoring the leaf extract's potency against this bacterium.

For *Pseudomonas aeruginosa*, the leaf extract yielded an inhibition zone of  $14.0 \pm 1.4$  mm, while the seed extract showed an inhibition of  $9.0 \pm 1.3$  mm. The MIC for the leaf extract was  $6.0 \pm 0.4$  mg/mL, with a p-value of 0.003, indicating a statistically significant difference in activity. In the case of *Salmonella typhi*, the leaf extract provided an inhibition zone of  $16.0 \pm 1.1$  mm, whereas the seed extract exhibited  $11.0 \pm 1.6$  mm. The MIC for the leaf extract was  $5.0 \pm 0.2$  mg/mL, and a p-value of 0.002 confirmed a significant difference in antimicrobial efficacy.

The leaf extract against *Candida albicans* showed an inhibition zone of  $8.0 \pm 0.5$  mm, compared to  $6.0 \pm 0.8$  mm for the seed extract. Both extracts had an MIC of  $8.0 \pm 0.5$  mg/mL. The p-value of 0.012 indicates a statistically significant difference, though the overall activity against this fungal pathogen was lower than that against bacterial pathogens. For *Aspergillus* species, the leaf extract yielded a zone of inhibition of  $7.0 \pm 0.6$  mm, while the seed extract had  $5.0 \pm 0.7$  mm. The MIC was notably higher at  $10.0 \pm 0.7$  mg/mL for the leaf extract. A p-value of 0.015 confirmed statistically significant differences in activity, suggesting that while the leaf extracts displayed some antifungal properties, their effectiveness was considerably lower compared to antibacterial activity.

### 3.2 Comparative Analysis of the Antimicrobial Activity of the Leaf and Seed Extracts

The comparative analysis of antimicrobial activity between leaf and seed extracts of *Vigna subterranea* reveals significant insights into their effectiveness against various microbial pathogens (Table 2). In terms of bacterial activity, the leaf extract demonstrated a notably larger mean zone of inhibition at  $15.67 \pm 1.18$  mm compared to the seed extract, which measured  $10.5 \pm 1.09$  mm. This difference indicates a stronger antimicrobial effect of the leaf extract against bacterial pathogens. Additionally, the minimum inhibitory concentration (MIC) for the leaf extract was

lower, recorded at  $5.0 \pm 0.35$  mg/mL, while the seed extract had an MIC of  $5.5 \pm 0.42$  mg/mL. This suggests that less of the leaf extract is required to inhibit bacterial growth effectively. The p-value of 0.001 signifies a highly significant difference, reinforcing the conclusion that the leaf extract exhibits superior antimicrobial activity against bacteria.

Regarding fungal activity, the leaf extract again outperformed the seed extract, exhibiting a mean zone of inhibition of  $7.5 \pm 0.72$  mm, in contrast to the seed extract's  $5.5 \pm 0.89$  mm. While the leaf extract had an MIC of  $9.0 \pm 0.64$  mg/mL, indicating a weaker antifungal effect compared to its antibacterial activity, the seed extract's MIC was slightly lower at  $8.0 \pm 0.54$  mg/mL. The p-value of 0.008 confirms a significant difference in antifungal efficacy, although this difference is less pronounced than that observed with bacterial pathogens.

### 3.3 Phytochemical Composition

The leaf extract contains a notably higher concentration of alkaloids (Table 3), measuring

$5.4 \pm 0.5$  mg/g, compared to  $3.2 \pm 0.4$  mg/g in the seed extract. This significant difference suggests that alkaloids are a key contributor to the strong antimicrobial activity observed in the leaf extract. Regarding flavonoids, the leaf extract again outperforms the seed extract, with levels of  $7.6 \pm 0.3$  mg/g versus  $4.8 \pm 0.3$  mg/g, respectively. Saponins follow a similar trend, with the leaf extract containing  $4.2 \pm 0.6$  mg/g and the seed extract  $2.9 \pm 0.5$  mg/g. Tannins were present at  $3.8 \pm 0.4$  mg/g in the leaf extract and  $2.0 \pm 0.3$  mg/g in the seed extract. The analysis also reveals lower concentrations of terpenoids, with the leaf extract containing  $2.5 \pm 0.2$  mg/g and the seed extract  $1.5 \pm 0.1$  mg/g, both exhibiting weak antimicrobial activity.

Phenolic compounds are another highlight, with the leaf extract showing a concentration of  $6.1 \pm 0.3$  mg/g, significantly higher than the  $3.5 \pm 0.4$  mg/g found in the seed extract. Lastly, both extracts contain low levels of essential oils, measuring  $1.0 \pm 0.1$  mg/g in the leaf extract and  $0.5 \pm 0.1$  mg/g in the seed extract, which correlates with weak antimicrobial activity.

**Table 1. Zones of Inhibition (mm) and Minimum Inhibitory Concentration (MIC) (mg/mL) for Each Microbial Isolate Treated with *Vigna subterranea* Extracts**

Microbial Isolate	Leaf Extract (Zone of Inhibition, mm)	Seed Extract (Zone of Inhibition, mm)	Minimum Inhibitory Concentration (MIC) (mg/mL)	p-value
<i>Escherichia coli</i>	$15.0 \pm 1.2$	$10.0 \pm 1.5$	$5.0 \pm 0.3$	0.002
<i>Staphylococcus aureus</i>	$18.0 \pm 1.0$	$12.0 \pm 1.8$	$4.0 \pm 0.2$	0.001
<i>Pseudomonas aeruginosa</i>	$14.0 \pm 1.4$	$9.0 \pm 1.3$	$6.0 \pm 0.4$	0.003
<i>Salmonella typhi</i>	$16.0 \pm 1.1$	$11.0 \pm 1.6$	$5.0 \pm 0.2$	0.002
<i>Candida albicans</i>	$8.0 \pm 0.5$	$6.0 \pm 0.8$	$8.0 \pm 0.5$	0.012
<i>Aspergillus</i> species	$7.0 \pm 0.6$	$5.0 \pm 0.7$	$10.0 \pm 0.7$	0.015

Note: The inhibition zones and MIC values are averages from multiple experimental replicates, with standard deviation (SD) included for variability. P-values indicate statistical significance for the differences observed between leaf and seed extracts

**Table 2. Comparative Summary of Antimicrobial Activity for Leaf vs. Seed Extracts**

Extract Type	Mean Zone of Inhibition for Bacteria (Zone of Inhibition, mm)	Mean Zone of Inhibition for Fungi (Zone of Inhibition, mm)	Mean MIC for Bacteria (mg/mL)	Mean MIC for Fungi (mg/mL)	p-value
Leaf Extract	$15.67 \pm 1.18$	$7.5 \pm 0.72$	$5.0 \pm 0.35$	$9.0 \pm 0.64$	0.001
Seed Extract	$10.5 \pm 1.09$	$5.5 \pm 0.89$	$5.5 \pm 0.42$	$8.0 \pm 0.54$	0.008

Note: This table summarizes average antimicrobial effectiveness against both bacterial and fungal isolates, with statistical significance indicated by p-values

**Table 3. Results of the Phytochemical analysis and the Correlation between the specific Compounds Present and their Antimicrobial Activity**

Phytochemical Compound	Leaf Extract (mg/g)	Seed Extract (mg/g)	Antimicrobial Activity
Alkaloids	5.4 ± 0.5	3.2 ± 0.4	Strong
Flavonoids	7.6 ± 0.3	4.8 ± 0.3	Moderate
Saponins	4.2 ± 0.6	2.9 ± 0.5	Moderate
Tannins	3.8 ± 0.4	2.0 ± 0.3	Weak
Terpenoids	2.5 ± 0.2	1.5 ± 0.1	Weak
Phenolic Compounds	6.1 ± 0.3	3.5 ± 0.4	Strong
Essential Oils	1.0 ± 0.1	0.5 ± 0.1	Weak

Table 3 provides a detailed overview of the phytochemical composition of leaf and seed extracts from *Vigna subterranea*, highlighting the correlation between specific compounds and their antimicrobial activity.

#### 4. DISCUSSION

This study investigated the antimicrobial properties of extracts from *Vigna subterranea* (Bambara nut), a legume deeply rooted in traditional medicine in sub-Saharan Africa. With antimicrobial resistance (AMR) emerging as a critical global health challenge, this study explored *Vigna subterranea* for its potential to serve as a natural alternative to synthetic antibiotics.

The study's findings demonstrated that the leaf extracts of *Vigna subterranea* exhibit considerable antimicrobial activity against a variety of bacterial and fungal pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, and *Aspergillus* species. The seed extracts, though active, displayed a noticeably lower level of antimicrobial efficacy, suggesting that the different plant parts exhibit varying levels of bioactive potential. This observation highlights the importance of targeting specific plant parts to maximize antimicrobial efficacy when they are being used as therapeutic options. These findings are consistent with previous research on the antimicrobial properties of *Vigna subterranea*. Earlier studies have demonstrated that extracts from the leaves and seeds possess activity against a variety of bacteria and fungi, showcasing their broad-spectrum potential (Ajiboye and Oyejobi, 2017; Taahir, 2017; Udeh et al., 2020). This study extends these results by examining the specific antimicrobial activity of each extract on common pathogens related to human infections, providing a more comprehensive view of the plant's efficacy.

The phytochemical analysis revealed that *Vigna subterranea* leaf extracts contain notably higher levels of alkaloids, flavonoids, and phenolic compounds than the seed extracts. These compounds are recognized for their antimicrobial properties, which likely contribute to the leaf extract's increased potency. The elevated alkaloid concentration in the leaf extract is particularly significant, as alkaloids are known to disrupt bacterial processes by inhibiting protein synthesis, DNA replication, and compromising cell membrane integrity, which aligns with the extract's strong antimicrobial activity (Othman et al., 2019; Khare et al., 2021).

Similarly, the high levels of flavonoids in the leaf extract may explain its moderate antimicrobial effects. Flavonoids can disrupt bacterial cell walls, interfere with nucleic acid synthesis, and inhibit essential protein functions, all of which contribute to their antimicrobial potential (Cushnie and Lamb, 2005; Górnaiak et al., 2019). Phenolic compounds, found in abundance in the leaf extract, also play a crucial role in antimicrobial action by damaging bacterial membranes and inhibiting microbial enzymes. The strong antimicrobial activity observed in the leaf extract supports the importance of phenolics in combating pathogens (Bouarab-Chibane et al., 2019; Ecevit et al., 2022; Oulahal and Degraeve, 2022).

The findings suggest that *Vigna subterranea* leaf extracts could offer a promising alternative or complementary therapeutic approach for treating bacterial and fungal infections. However, further research is necessary to fully explore the therapeutic potential of *Vigna subterranea* as an antimicrobial agent. Conducting *in vivo* studies is essential, as these will test the extracts' safety and efficacy in animal models, helping to verify the *in vitro* findings and determining any toxicological profiles for clinical applications. Understanding the exact mechanisms by which

these extracts exert their antimicrobial effects will be invaluable, as it would allow for the development of targeted therapeutic agents by revealing the biochemical pathways and molecular interactions involved. Furthermore, investigating the synergistic potential of *Vigna subterranea* extracts with conventional antibiotics could offer a promising solution to antibiotic resistance. This could improve the effectiveness of current treatments and renew the utility of older antibiotics. Finally, isolating and purifying the specific bioactive compounds responsible for the antimicrobial activity will pave the way for detailed structural and functional studies, potentially leading to the discovery of novel drug candidates and contributing to the global effort to address antimicrobial resistance. By pursuing these research directions, *Vigna subterranea* could be systematically developed as a viable and effective antimicrobial agent in the ongoing search for alternative treatments against resistant infections.

## 5. CONCLUSION

This research provides compelling evidence for the antimicrobial potential of *Vigna subterranea* leaf extracts. The findings highlight the need for further investigation into this plant, particularly in developing alternative or complementary therapies to combat the growing threat of antimicrobial resistance. While future research is necessary to address the limitations of this study, the promising results offer hope for harnessing the medicinal properties of this natural resource to improve global health outcomes.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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