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Antibacterial And Synergistic Potential of *Hibiscus sabdariffa* and *Zingiber officinale* Against Multidrug-Resistant Bacteria

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Abstract

Antimicrobial resistance (AMR) is a global health challenge, particularly in developing regions where access to effective drugs is constrained. Medicinal plants provide an alternative due to their affordability, availability, and wealth of bioactive compounds. This study investigated the antibacterial properties of *Zingiber officinale* (ginger) and *Hibiscus sabdariffa* (roselle), both widely used in African traditional medicine, against selected bacterial pathogens. Methanolic extracts were prepared from dried rhizomes of ginger and calyces of roselle, and concentrations of 100%, 95%, 90%, and 85% were tested. Antibacterial activity was determined against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* sp. using agar well diffusion. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were established by serial dilution and subculture techniques. Statistical significance was assessed using one-way ANOVA and Duncan's multiple range test at $p < 0.05$. Phytochemical analysis revealed steroids, terpenoids, and flavonoids in both plants. Ginger additionally contained saponins, alkaloids, tannins, and carbohydrates, whereas roselle uniquely presented cardiac glycosides. Antibacterial activity increased with concentration, with the 100% extracts showing the highest inhibition zones. The combined extract consistently produced larger inhibition zones compared to the individual extracts ($p < 0.05$). Ginger exhibited the strongest individual activity against *S. aureus*, while the combined extract was most effective against *E. coli*. *Pseudomonas* sp. was the least susceptible. MIC values confirmed these observations: *S. aureus* was the most sensitive (25 mg/mL), while *E. coli* and *Pseudomonas* sp. each recorded 50 mg/mL. MBC values were uniformly 50 mg/mL across all test organisms. The results demonstrate that ginger and roselle possess significant antibacterial properties, with ginger showing superior individual efficacy and roselle providing complementary metabolites that enhanced combination effects. Their synergistic action supports traditional polyherbal therapy and indicates potential as cost-effective, plant-based interventions against AMR. The findings underscore the need for further research to develop standardized formulations for therapeutic application.

Keywords: *Hibiscus sabdariffa*; *Zingiber officinale;* antibacterial activity; phytochemicals; synergistic effect; antimicrobial resistance.

INTRODUCTION

The effectiveness of antimicrobial agents, which target vital bacterial functions such as cell wall formation and DNA synthesis, has been significantly compromised by the rapid rise of antimicrobial resistance (AMR) (Ilechukwu *et al.,* 2025). The widespread misuse and overuse of these drugs drive this crisis. Resistance is a complex phenomenon; it differs from non-genetic tolerance observed in persistent cells and can develop through intrinsic mechanisms or be acquired via genetic mutations and horizontal gene transfer (Oliveira *et al.,* 2024). Bacteria have developed advanced defenses to evade antimicrobials, such as producing enzymes that inactivate the drug, altering target sites, decreasing membrane permeability to limit drug entry, and using efflux pumps to actively remove the compounds (Belay *et al.,* 2024). These defenses are often more robust in biofilms. To combat this challenge, the field is exploring innovative non-antibiotic strategies like bacteriophage therapy, probiotics, and phytomedicine, drawing on a long tradition of plant-based medicine, to find new ways to treat and prevent infections without worsening resistance (Lyimo and Sonola, 2025).

Ginger (*Zingiber officinale*) is a well-established botanical remedy in naturopathic medicine, recognized for its potent activity against a diverse spectrum of microbial pathogens (Edo *et al.,* 2025). This efficacy, demonstrated by the raw rhizome itself, underscores its potential as a powerful therapeutic agent for infectious diseases. As a member of the Zingiberaceae family, ginger shares the aromatic and medicinal qualities of its relatives, which are characterized by their unique rhizomes (Kadam and Shahi, 2025). Its worldwide availability, low cost, and high tolerability make it a common resource. The antimicrobial activity of ginger is largely attributed to its active constituents, primarily gingerols and shogaols, which are known to resolve gastric infections and inhibit the proliferation of harmful gut bacteria, including resistant strains of *Escherichia coli* and *Staphylococcus* species (Ayodeji *et al.,* 2024). Historically, ginger has been a cornerstone in treating intestinal and digestive ailments. Antimicrobial resistance represents a critical global health crisis, particularly in low- and middle-income nations, where it leads to increased treatment costs, extended hospitalizations, and higher mortality rates (Salam *et al.,* 2023).

Hibiscus sabdariffa, commonly referred to as roselle, is a member of the Malvaceae family with considerable ethnomedicinal and nutritional value (Apaliya *et al.,* 2021). Traditionally, its various components have been utilized to address a wide array of health conditions, including but not limited to cardiovascular and nervous system ailments, digestive issues, and parasitic infections (Jabeur *et al.,* 2017). The plant is particularly prized for its edible calyces, which are a rich source of bioactive compounds. These include potent antioxidants like γ-tocopherol, as well as an abundance of polyphenols and flavonoids. Scientific investigations have substantiated that extracts derived from the plant exhibit significant pharmacological activities, such as antibacterial, antihypertensive, anticancer, and hepatoprotective effects, which are directly attributed to its complex phytochemical composition (McCalla and Smith, 2024).

Beyond its therapeutic applications, *H. sabdariffa* plays a crucial role in local diets and food security, particularly in West African regions such as Nigeria (Mukhtar *et al.,* 2025). The calyces are processed into popular food items such as the beverage "sobo," while the seeds are fermented into a condiment known as "daddawa" (Ajayi *et al.,* 2016). The leaves and green calyces are also commonly incorporated into soups, providing essential micronutrients including vitamins B and C. Phytochemical analysis has identified the presence of alkaloids, anthocyanins, tannins, and glycosides, which collectively contribute to its documented antifungal, antiviral, and antidiabetic properties (Salem *et al.,* 2022). With hundreds of species within the *Hibiscus* genus thriving in tropical climates, this group of plants represents a promising reservoir for developing novel functional foods and plant-based medicines (Sarwar, 2023).

METHODOLOGY

Samples collection, preparation, and extraction

The fresh calyces of *H. sabdariffa* and the rhizome *Z. officinale* were obtained from the Market of Yola South Local Government of Adamawa State. The plant leaf samples were shade-dried. The dried samples were blended into powder form using a blender. Then, 40 g of each powdered extract was soaked in 100 mL of methanol for 48 hours. Furthermore, 20 g of each of the powdered leaves was weighed and combined, resulting in a total of 40 g, which was then soaked in methanol for 48 hours. Then, it was filtered using a muslin cloth. The resultant filtrate was re-filtered using Whatman filter paper. The solvent was allowed to evaporate in a water bath.

Identification and Authentication

The plants were identified and authenticated by an experienced taxonomist with identification numbers ASP 1223 and ASP 1254 for *H. sabdariffa* and *Z. officinale,* respectively, at the Science Laboratory Technology Department, Adamawa State Polytechnic, Nigeria.

Dilution of the extracts

Dilutions of the extracts were made following the methods previously described by Izah et al. (2018). The original stock of the leaf extracts was considered as 100% concentration, and then it was further diluted into 95%, 90% and 85% of the original volume.

Standardization of the inocula

Mueller-Hinton Agar (MHA) served as a medium for sub-culturing the isolates on newly purchased sterile plates. The plates were incubated at 37 °C for 24 hours and thereafter preserved at 4 °C for subsequent overnight culturing, from which isolates were later suspended in broth culture for antimicrobial assay. The broth cultures of the isolates were adjusted to the exact turbidity equivalent to 0.5 McFarland Standard (Abaka *et al.,* 2025).

Antimicrobial Assay

Antimicrobial activity of the extracts was determined using the agar well diffusion assay method. Mueller Hinton Agar (MHA) was prepared as recommended by the manufacturer and allowed to cool to 50 °C before pouring on pre-labelled sterile petri plates on a level surface. One petri-plate was prepared per organism and done in triplicate, except that of the positive test control, using Maxi discs that were done in duplicate. A sterile 6 mm borer was used to punch seven equidistant wells: wells 1 – 6 for different concentrations of extracts. Well number seven (7) was bored at the center of the plates for the solvent used (negative control). A dip with sterile swabbing sticks of overnight broth cultures of each of the isolates was streaked on the surface of the prepared MHA plates. The volume of 0.2 mL of the extracts at preset concentration was introduced to each of the wells, and the plates were allowed to rest (and set) on the laboratory bench for 45 minutes, allowing for proper pre-diffusion of the extracts before 24 hours of incubation at 37 °C (Abaka *et al.,* 2025).

Determination of the Minimum Inhibitory Concentrations (MIC)

The MIC was determined using the tube dilution technique according to Kanu et al. (2018). The MIC was determined by taking 2ml of the concentrations of the extracts that inhibited the growth of the resistant strains and mixing it with 2ml of the nutrient broth containing 0.1ml of standardized inoculum of 0.5 McFarland standard. The tubes were incubated for 24 hours at 37 °C. It has a tube containing broth and inoculum without the extract as the control. The lowest concentration that showed no visible turbidity (inhibited microbial growth) is regarded as the MIC.

Determination of the Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were incubated with samples from each of the test tubes that showed no visible growth from the MIC test. The plates were then incubated at 37 °C for 24 hours. The lowest concentration of the extract yielding no growth was recorded as the minimum bactericidal concentration (Islam *et al.,* 2008).

Statistical Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software version 20. The data were expressed as mean ± standard error, and one-way analysis of variance was carried out at $\alpha = 0.05$. Duncan's multiple range test was used to ascertain the source of the variation.

Table 1. Phytochemical screening of fractions of *Hibiscus sabdariffa* and *Z. officinale.*

Phytochemicals	*Hibiscus sabdariffa*	*Zingiber officinale*	Inferences
Steroids	+	+	Present in both
Terpenoids	+	+	Present in both
Saponins	-	+	Absent in *H. sabdariffa*, present in *Z. officinale*
Alkaloids	-	+	Absent in *H. sabdariffa*, present in *Z. officinale*
Tannins	-	+	Absent in *H. sabdariffa*, present in *Z. officinale*
Flavonoids	+	+	Present in both
Cardiac glycosides	+	-	Present in *H. sabdariffa*, absent *in Z. officinale*
Carbohydrates	-	+	Absent in *H. sabdariffa*, present in *Z. officinale*

Key: Positive = + Negative= -

Table 2. Mean Zone of Inhibition (mm ± SEM) of Plant Extracts against Bacterial Isolates.

Plant Extract	Isolate	100% Concentration	95% Concentration	90% Concentration	85% Concentration
Hibiscus sabdariffa	*E. coli*	12.82 ± 0.33^{b}	11.13 ± 0.34^{c}	8.80 ± 0.55^{c}	5.72 ± 0.26^{c}
	S. aureus	11.88 ± 0.27^{c}	9.23 ± 0.03^{d}	7.52 ± 0.35^{d}	5.74 ± 0.31^{c}
	Pseudomonas sp.	10.11 ± 0.07^{c}	8.03 ± 0.13^{e}	5.23 ± 0.07^{e}	3.63 ± 0.18^{d}
Z. officinale	*E. coli*	15.15 ± 0.09^{a}	9.85 ± 0.30^{d}	8.32 ± 0.51^{c}	5.48 ± 0.09^{c}
	S. aureus	14.30 ± 0.04^{b}	12.64 ± 0.24^{b}	9.52 ± 0.36^{c}	8.14 ± 0.08^{b}
	Pseudomonas sp.	14.81 ± 0.39^{a}	12.41 ± 0.19^{b}	9.89 ± 0.31^{c}	7.41 ± 0.22^{b}
H. sabdariffa + Z. officinale.	*E. coli*	17.57 ± 0.35^{a}	15.54 ± 0.15^{a}	12.10 ± 0.33^{a}	9.69 ± 0.07^{a}
	S. aureus	15.48 ± 0.19^{b}	14.31 ± 0.13^{a}	12.37 ± 0.13^{a}	9.14 ± 0.63^{a}
	Pseudomonas sp.	16.08 ± 0.52^{a}	11.86 ± 0.38^{b}	9.77 ± 0.33^{c}	7.41 ± 0.17^{b}

Different superscript letters (a, b, c) within the same column for a specific bacterium and concentration denote statistically significant differences ($p < 0.05$) according to Duncan's multiple range test.

Table 3. Minimum Inhibitory Concentration of Polyherbal Extracts of *Hibiscus sabdariffa* and *Zingiber officinale* against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa.*

Organisms	Extract	Concentration (mg/mL)						MIC	MBC
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL		
E. coli	ME	_	_*	+	+	+	+	50	50
S. aureus	ME	_	_	_*	+	+	+	25	50
Pseudomonas sp.	ME	_	_*	+	+	+	+	50	50

Discussion

The phytochemical analysis indicated that both *Hibiscus sabdariffa* and *Zingiber officinale* share common secondary metabolites such as steroids, terpenoids, and flavonoids, which are often linked to antimicrobial properties. Despite this similarity, *Z. officinale* exhibited a broader spectrum of bioactive compounds, including saponins, alkaloids, tannins, and carbohydrates, metabolites not observed in *H. sabdariffa*. In contrast, *H. sabdariffa* contained cardiac glycosides, which were absent in *Z. officinale*. These distinctions suggest that while both plants possess therapeutic constituents, the greater phytochemical diversity of *Z. officinale* could explain its relatively stronger antibacterial performance when evaluated independently.

The differences in phytochemical profiles between the two plants are particularly important in understanding their varying antimicrobial activities. Compounds found in *Z. officinale*, such as saponins, alkaloids, and tannins, are known to disrupt microbial membranes, interfere with nucleic acid synthesis, and inactivate microbial proteins, thereby providing potent antibacterial effects (Akinmoladun *et al.,* 2018; Teke *et al.,* 2020). On the other hand, the presence of cardiac glycosides in *H. sabdariffa*, though primarily associated with cardiovascular health, has been reported to exhibit antimicrobial effects through disruption of microbial respiration and enzymatic pathways (Nwachukwu *et al.,* 2014; Iroha *et al.,* 2019). These complementary differences suggest that combining extracts from both plants could yield a synergistic effect. This is consistent with evidence from African ethnomedicine, where multi-plant formulations are often employed to enhance therapeutic activity through the interaction of diverse bioactive molecules (Okwu and Ndu, 2006; Ibrahim *et al.,* 2021). Consequently, the observed higher antibacterial activity of the combined extracts of *H. sabdariffa* and *Z. officinale* may be attributed to this phytochemical synergy.

The combined extract of *Hibiscus sabdariffa* and *Zingiber officinale* demonstrated the most pronounced antibacterial effect, consistently producing the largest mean zones of inhibition across *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* species. This synergistic effect was statistically significant ($p < 0.05$), particularly when compared with the activity of the individual plant extracts, which showed reduced inhibitory capacity. These results corroborate the findings of Akinmoladun et al. (2020), who highlighted that polyherbal formulations exhibit stronger antimicrobial activity than single extracts because of complementary interactions between diverse phytochemicals. Similarly, Oladunmoye and Komolafe (2020) emphasized that combining plant extracts can potentiate antibacterial efficacy by targeting multiple microbial pathways simultaneously, thereby enhancing therapeutic action and limiting the risk of microbial resistance. The enhanced performance of the combination in the present study suggests that the interaction of bioactive constituents from both plants likely underpins the broad-spectrum activity observed.

Furthermore, the study revealed a concentration-dependent decline in antibacterial potency across all extracts, with inhibition zones decreasing progressively from 100% to 85% concentrations. This dose-response relationship was statistically significant ($p < 0.05$), confirming that higher concentrations of extracts are more effective in suppressing bacterial growth. Such findings are consistent with the general pharmacological principle that antimicrobial efficacy is positively correlated with bioactive compound concentration until an optimal threshold is reached (Eze *et al.,* 2017). The superior efficacy of the combined extract across different concentrations indicates its potential as a more reliable and broad-spectrum antibacterial agent compared to individual plant preparations. These results strengthen the argument for polyherbal therapies in antimicrobial drug development, particularly in regions where resistance to conventional antibiotics is prevalent.

In contrast, *Hibiscus sabdariffa* alone was the least effective extract in this study, as it produced the smallest inhibition zones against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* spp. This outcome corroborates the findings of Ologundudu et al. (2010), who reported modest antibacterial activity of *H. sabdariffa* against clinical isolates, attributing the reduced activity to comparatively low concentrations of phenolic acids relative to other medicinal plants. However, this differs from Aliyu et al. (2016), who observed strong antibacterial activity of *H. sabdariffa* calyx extracts, particularly against *E. coli*. Such variations in outcomes may arise from differences in extraction solvents, phytochemical composition due to geographical factors, or variations in bacterial strains, as highlighted by Abba et al. (2021). These discrepancies suggest that while *H. Sabdariffa* holds antimicrobial potential; its effectiveness may be context-dependent and influenced by methodological and ecological factors.

The study further established that the antibacterial efficacy of all extracts was concentration-dependent, with higher concentrations (100%) producing larger inhibition zones compared to lower concentrations (85%). This concentration-effect trend is consistent with the findings of Nwachukwu and Uzoeto (2010), who reported that the antimicrobial activity of plant extracts diminishes with dilution due to reduced levels of bioactive compounds such as flavonoids, tannins, and saponins. Similarly, Eze and Okoye (2019) demonstrated a direct relationship between extract concentration and inhibition diameters when evaluating ginger and garlic extracts against enteric pathogens. Taken together, these consistencies reinforce the importance of maintaining high concentrations of extracts to achieve optimal therapeutic efficacy, highlighting a critical consideration for the development of standardized herbal formulations.

Among the bacterial species tested, *Pseudomonas* sp. exhibited the greatest resistance, consistently producing the smallest inhibition zones across treatments. This aligns with the observations of Nsofor and Iroegbu (2013), who attributed the resilience of *Pseudomonas* to its efficient efflux systems and low outer membrane permeability. In contrast, *Staphylococcus aureus* showed higher susceptibility, particularly to *Zingiber officinale* extracts at lower concentrations, which is consistent with findings by Ibrahim et al. (2021) that linked ginger's strong activity against Gram-positive bacteria to phenolic compounds such as gingerol and shogaol. Meanwhile, *Escherichia coli* responded most favorably to the combined extracts, supporting Adebolu and Oladimeji's (2005) report that plant extract combinations often produce synergistic inhibition of Gram-negative organisms.

The polyherbal extract demonstrated inhibitory activity against *E. coli*, *S. aureus*, and *Pseudomonas* sp., with varying MIC and MBC values. The MIC was lowest for *S. aureus* (25 mg/mL), indicating the highest sensitivity, while *E. coli* and *Pseudomonas* sp. showed MIC values of 50 mg/mL. The MBC was 50 mg/mL for all tested organisms. Overall, the extract was most effective against *S. aureus*, but required higher concentrations to inhibit and kill *E. coli* and *Pseudomonas* sp.

The combined extract of *Hibiscus sabdariffa* and *Zingiber officinale* exhibited considerable antibacterial activity against *E. coli*, *S. aureus*, and *Pseudomonas* sp., though the degree of susceptibility differed among the organisms. The lowest MIC was recorded against *S. aureus* (25 mg/mL), indicating that the Gram-positive strain was more responsive compared to the Gram-negative species. This observation aligns with established knowledge that the lipopolysaccharide barrier in Gram-negative bacteria enhances their resistance to antimicrobial compounds (Oladunmoye and Komolafe, 2020). The higher MIC values observed for *E. coli* and *Pseudomonas* sp. (50 mg/mL each) further emphasize the inherent resilience of Gram-negative organisms, consistent with earlier findings on the therapeutic difficulties posed by these pathogens (Akinmoladun *et al.,* 2021).

The uniform MBC value of 50 mg/mL across all test organisms suggests that while inhibitory effects occurred at varying concentrations, complete bactericidal activity required higher doses of the extract. The greater sensitivity of *S. aureus* highlights the potential of this polyherbal formulation in treating Gram-positive infections, supporting previous reports of synergistic plant extract activity against *Staphylococcus* species (Okeke *et al.,* 2020). The enhanced efficacy may be attributed to the combined phytochemicals in *Z. officinale* and the unique bioactive compounds of *H. sabdariffa*. Furthermore, the ability of the extract to suppress *Pseudomonas* sp., a pathogen well known for its multidrug resistance, illustrates its therapeutic promise, albeit at higher concentrations. Overall, these findings underscore the potential role of polyherbal therapy as a complementary or alternative strategy to conventional antibiotics, particularly in addressing the growing challenge of antimicrobial resistance in Nigeria (Ezeigbo *et al.,* 2019).

The combined extract of *Hibiscus sabdariffa* and *Z. officinale* consistently yielded the largest inhibition zones across different concentrations, significantly outperforming either extract alone ($p < 0.05$), as confirmed by Duncan's multiple range test. While *H. sabdariffa* alone demonstrated the weakest antibacterial activity, its effectiveness was markedly enhanced when paired with ginger. These results underscore the importance of synergistic herbal formulations and highlight the role of extract concentration in therapeutic efficacy. The one-way ANOVA further confirmed significant main effects of extract type on antibacterial activity, reinforcing that the combined formulation was the most potent, followed by ginger alone, and lastly roselle. Collectively, these findings validate the traditional use of these plants while providing scientific evidence for their potential development into standardized phytomedicines within Nigeria and across Africa.

CONCLUSION

This study confirmed that *Hibiscus sabdariffa* and *Zingiber officinale* possess notable antimicrobial properties, with ginger showing stronger individual activity while roselle contributed unique phytochemicals. The combined extracts produced the most significant antibacterial effects against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* sp., highlighting a clear synergistic interaction. Antibacterial activity was concentration dependent, with higher doses producing greater inhibition zones. Among the test organisms, *Pseudomonas* sp. remained the most resistant, while *E. coli* and *S. aureus* were more susceptible to the synergistic and ginger extracts, respectively. Overall, the findings validate traditional use of these plants, emphasize the benefits of polyherbal formulations, and support their potential as affordable alternatives for managing antimicrobial resistance in Africa.

Competing Interests: The authors declare that there are no competing interests.

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