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In-vitro Evaluation of the Antimicrobial Properties of Selected Natural Spices Commonly Used for Culinary Purposes in Nigeria

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ABSTRACT
This study evaluated the antibacterial activity of five spices, Cloves (Syzygiumaromaticum), Rosemary (Rosmarinusofficinalis), Black Pepper (Piper nigrum), Cinnamon (Cinnamomumverum), and Bay Leaves (Laurusnobilis)against Escherichia coli, Klebsiella spp., Staphylococcus aureus, and Staphylococcus saprophyticus. Using disc diffusion and agar well diffusion methods, the spices' aqueous extracts were tested, and inhibition zones were measured in millimeters (mean ± SD).Cinnamon demonstrated the strongest antibacterial activity against E. coli (30.16 ± 1.30 mm), S. saprophyticus (24.43 ± 1.07 mm), and S. aureus (16.31 ± 0.46 mm), but was ineffective against Klebsiella spp. Cloves showed significant activity against S. saprophyticus (25 ± 1.2 mm), E. coli (24 ± 0.87 mm), and S. aureus (14 ± 0.63 mm), but was not effective against Klebsiella spp. Rosemary inhibited S. saprophyticus (26.51 ± 1.26 mm), S. aureus (22.16 ± 0.12 mm), and Klebsiella spp. (20.14 ± 0.11 mm). Black Pepper was effective against E. coli (21.65 ± 0.87 mm), Klebsiella spp. (19.16 ± 0.19 mm), and S. saprophyticus (18.32 ± 0.68 mm). Bay Leaves inhibited Klebsiella spp. (25.26 ± 1.28 mm), S. aureus (22.53 ± 1.04 mm), and S. saprophyticus (18.47 ± 0.88 mm).The study suggests that these spices possess varying antibacterial properties, potentially valuable for therapeutic applications and food preservation. These findings emphasize the importance of natural antimicrobial agents as alternatives to synthetic options.

Keywords: *Antimicrobial activity, Spices, Escherichia coli, Staphylococcus aureus, Agar well diffusion.*

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1.0 INTRODUCTION
Spices have been used in cooking for as long as there has been human civilization, and they are not only used to enhance flavour but also may have health benefits. The growing concern about foodborne illnesses and the emergence of antibiotic-resistant organisms has brought increased attention to these pantry staples' antibacterial properties in recent years. Novel antibacterial compounds are being explored as possible solutions to the worldwide issue of resistant microbes[1]. Both food producers and consumerscontinue to place a high priority on food safety. Foodborne illness outbreaks resulting from pathogenic and spoilage microorganisms persist in posing serious public health concerns, even with advancements in food preservation methods [2].

While synthetic antimicrobial agents have traditionally been used to inhibit microbial growth in food products, there is increasing consumer demand for natural alternatives due to concerns about the potential health risks associated with chemical additives [3].

The spices selected for this study were chosen based on their widespread use in culinary practices and their reported antimicrobial properties. Cloves (*Syzygium aromaticum*), known for their potent flavour and high eugenol content, have been traditionally used in both cooking and medicine[3] Rosemary (*Rosmarinus officinalis*), rich in rosmarinic acid and carnosic acid, is recognized for its antioxidant and antimicrobial properties. Black pepper (*Piper nigrum*), a staple in many cuisines, contains piperine, a compound with noted antimicrobial activity.

Cinnamon (*Cinnamomum verum*), with its characteristic cinnamaldehyde content, has been widely studied for its antimicrobial and antifungal properties and Bay leaves.

Bay leaves offer more than just flavour to dishes; they provide a range of health benefits: A study by [3] demonstrated that consumption of bay leaves can reduce glucose levels and improve lipid profiles in patients with type 2 diabetes, suggesting their role in managing blood sugar and reducing the risk of complications related to diabetes. The presence of polyphenols, such as caffeic acid and quercetin, in bay leaves has been linked to cardiovascular benefits. These compounds have antioxidant properties that help in reducing oxidative stress, a major factor in the development of cardiovascular diseases [3,4]. Regular consumption of bay leaves can help in lowering cholesterol levels and triglycerides, thereby protecting against heart diseases.These benefits are primarily attributed to the bioactive compounds present in bay leaves.

Drug discovery and therapeutic outcome prediction can benefit from the assessment of antimicrobial activity of plants, plant derivatives, and other substances. Novel antimicrobial chemicals are being considered as potential answers to the global problem of drug-The in-vitro investigation of the extracts will go a long way in determining their antibacterial potency and as such be recommended for treatment of infections [3,4].Antimicrobial resistance is a major global concern that is made worse by infectious diseases. As a result, scientists are currently investigating novel antibacterial chemicals as possible remedies. This study aims to provide a comprehensive evaluation of the antimicrobial properties cum bactericidal effects of these spices, focusing on their effectiveness against four common pathogens: *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Staphylococcus saprophyticus*.By systematically assessing the antimicrobial activity of these spices, this research seeks to contribute to the growing body of knowledge on natural food preservatives and explore their potential applications in enhancing food safety and extending shelf life.

2. Materials and Methods

2.1.Collection and Preparation of Spices

Each local spice: Cloves (*Syzygium aromaticum*), Rosemary (*Rosmarinus officinalis*), Black pepper (*Piper nigrum*),Cinnamon (*Cinnamomum verum*), and Bay leaves (*Laurus nobilis*) was purchased from a local market and authenticated by a botanist. The spices were then ground into a fine powder using a laboratory-grade grinder to ensure uniformity. For the preparation of aqueous extracts, 10 grams of each spice were mixed with 40 mL of distilled water in sterilized conical flasks. The mixtures were shaken vigorously for 2–3 minutes to ensure thorough mixing and then allowed to stand for 24 hours in the dark to prevent light-induced degradation of active compounds. After 24 hours, the extracts were filtered using Whatman No. 1 filter paper, and the filtrates were stored at 4°C until further use.

2.2. Microorganism Preparation

The microorganisms used in this study were obtained from the Microbiology Laboratory at the Federal University of Technology Owerri. The selected strains included *Escherichia coli*, *Klebsiella pneumonia,Staphylococcus aureus*, and *Staphylococcus saprophyticus*. Pure cultures of the organisms were obtained using standard microbiology methods and later subjected to series of biochemical tests for proper identification of the isolates. Some selective media were also used to identify the isolates: Eosin Methylene Blue (EMB) Agar for *E. coli.*

Each microorganism was subcultured on MacConkey agar and incubated at 37°C for 24 hours to ensure optimal growth. The bacterial cultures were then prepared as inoculums by suspending a loopful of each culture in 2ml of sterile saline solution. The optical density of the suspensions was adjusted to match a 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/ml, ensuring uniformity in the bacterial load used for the assays.

2.3. Disc Diffusion Method

The disc diffusion method reported by Udensi et al. (2024)with slight modifications was employed in this study. Sterile filter paper discs (6 mm in diameter) were impregnated with 50 µl of each spice extract and placed on the surface of agar plates that had been previously inoculated with the respective bacterial strains using a sterile swab. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition (the clear areas around the discs where bacterial growth was inhibited) were measured using a digital caliper. The diameter of the inhibition zones was recorded in millimeters, and each test was performed in triplicate to ensure accuracy and reproducibility.

2.4. Agar Well Diffusion Method

In this method, 50 µL of each spice extract was directly incorporated into molten agar (MacConkey agar) before it was poured into sterile Petri dishes. The solidified agar plates were inoculated with bacterial strains using a sterile swab, and the plates were incubated at 37°C for 24 hours. Zones of inhibition were measured as in the disc diffusion method, providing additional data on the efficacy of the spice extracts.

2.5. Statistical Analysis

The data obtained from the disc diffusion and agar diffusion assays were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine the significance of differences between the inhibition zones of different spice extracts. A p-value of less than 0.05 was considered statistically significant. The results were presented as mean ± standard deviation (SD) for each group of experiments.

3. Results

3.1. Disc Diffusion Assay

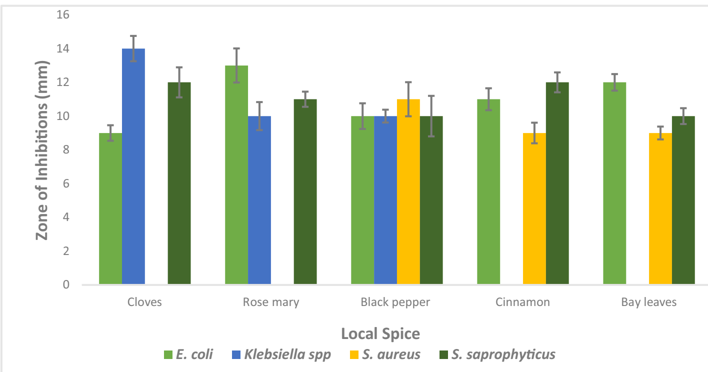

Fig 1: Zone of Inhibition of extracts using Disc Diffusion Method

Figure 1 presents the antibacterial activity of five different spices—Cloves, Rosemary, Black Pepper, Cinnamon, and Bay Leaves—against four bacterial species: *Escherichia coli* (*E. coli*), *Klebsiella spp.*, *Staphylococcus aureus* (*S. aureus*), and *Staphylococcus saprophyticus* (*S. saprophyticus*). The values are expressed as mean inhibition zones in millimeters (mm) with their corresponding standard deviations.

Cloves exhibited strong antibacterial activity against *Klebsiella* spp. (14 ± 0.75 mm) and *S. saprophyticus* (12 ± 0.89 mm), moderate activity against *E. coli* (9 ± 0.46 mm), but no effect on *S. aureus*. Rosemary showed the highest inhibition against E. coli (13 ± 1.01 mm) and also affected *S. saprophyticus* (11 ± 0.45 mm) and *Klebsiella* spp. (10 ± 0.83 mm), but had no effect on S. aureus. Black Pepper was effective against all four bacteria, with inhibition zones ranging from *E. coli* (10 ± 0.76 mm), *Klebsiella* spp. (10 ± 0.38 mm), *S. aureus* (11 ± 1.01 mm), to *S. saprophyticus* (10 ± 1.2 mm). Cinnamon had moderate effectiveness against *E. coli* (11 ± 0.65 mm), *S. saprophyticus* (12 ± 0.50 mm), and *S. aureus* (9 ± 0.61 mm), but showed no inhibition of *Klebsiella spp.* Bay Leaves exhibited antibacterial activity against *E. coli* (12 ± 0.49 mm), *S. aureus* (9 ± 0.38 mm), and *S. saprophyticus* (10 ± 0.47 mm), but were ineffective against *Klebsiella spp*. Statistical analysis showed no significant difference in the zones of inhibition against the isolates

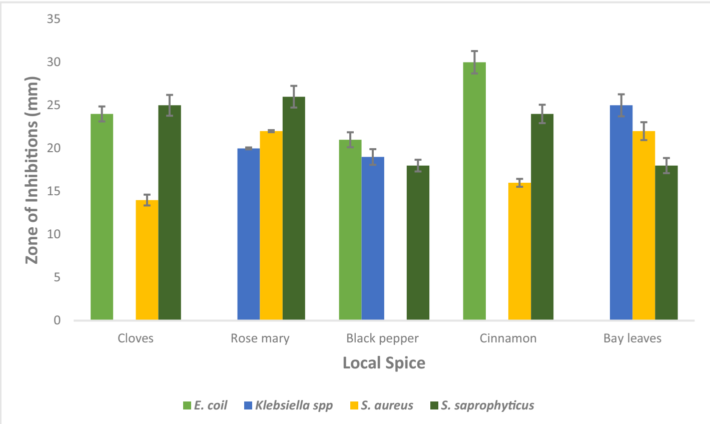

Fig 2: Zone of Inhibition of extracts using Agar Diffusion Method

Figure 2 presents the antibacterial activity of five different spices—Cloves, Rosemary, Black Pepper, Cinnamon, and Bay Leaves—against four bacterial species. Cloves inhibited *E. coli* (24 ± 0.87 mm), *S. aureus* (14 ± 0.63 mm), and *S. saprophyticus* (25 ± 1.2 mm), but had no effect on *Klebsiella* spp.; rosemary was effective against *Klebsiella* spp. (20.14 ± 0.11 mm), *S. aureus* (22.16 ± 0.12 mm), and *S. saprophyticus* (26.51 ± 1.26 mm), but not *E. coli*; black pepper showed activity against *E. coli* (21.65 ± 0.87 mm), *Klebsiella* spp. (19.16 ± 0.19 mm), and *S. saprophyticus* (18.32 ± 0.68 mm), but not *S. aureus*; cinnamon demonstrated strong inhibition of *E. coli* (30.16 ± 1.30 mm), *S. aureus* (16.31 ± 0.46 mm), and *S. saprophyticus* (24.43 ± 1.07 mm), but was ineffective against *Klebsiella* spp.; and bay leaves inhibited *Klebsiella* spp. (25.26 ± 1.28 mm), *S. aureus* (22.53 ± 1.04 mm), and *S. saprophyticus* (18.47 ± 0.88 mm), but had no effect on *E. coli*. Statistical analysis showed no significant difference in the zones of inhibition. Statistical analysis showed no significant difference in the zones of inhibition against the isolates

Discussion

The antibacterial properties of natural spices have garnered significant attention in recent years due to increasing antibiotic resistance among pathogenic bacteria. The present study examines the efficacy of five commonly used spices—Cloves (*Syzygium aromaticum*), Rosemary (*Rosmarinus officinalis*), Black Pepper (*Piper nigrum*), Cinnamon (*Cinnamomum verum*), and Bay Leaves (*Laurus nobilis*)—against four bacterial species: *Escherichia coli* (E. coli), *Klebsiella spp.*, *Staphylococcus aureus* (S. aureus), and *Staphylococcus saprophyticus* (S. saprophyticus).

Cloves exhibited significant antibacterial activity against *E. coli* (24 ± 0.87 mm), *S. aureus* (14 ± 0.63 mm), and *S. saprophyticus* (25 ± 1.2 mm), but showed no effect on *Klebsiella spp.* This finding is consistent with the literature, where Cloves are often cited for their potent antibacterial properties due to their high eugenol content, a compound known for its broad-spectrum antimicrobial effects[5]. The significant inhibition zones observed for *E. coli* and *S. saprophyticus* are particularly noteworthy, suggesting that Cloves could be an effective treatment option for infections caused by these bacteria.However, the absence of activity against *Klebsiella* species indicates that theeffectiveness of this spice can be restricted to particular bacterial strains, possibly as a result of variations in the composition of bacterial cell walls or the existence of efflux pumps that release antimicrobial agents. Foodborne microbes continue to provide serious risks to human health [6].

Rosemary displayed strong inhibitory effects against *Klebsiella spp.* (20.14 ± 0.11 mm), *S. aureus* (22.16 ± 0.12 mm), and *S. saprophyticus* (26.51 ± 1.26 mm), but was ineffective against *E. coli*. This aligns with other studies that have demonstrated the antimicrobial activity of Rosemary, attributed largely to its phenolic compounds such as rosmarinic acid, carnosic acid, and carnosol, which have been shown to disrupt bacterial cell membranes and inhibit biofilm formation[7]. The particularly strong activity against *S. saprophyticus* indicates Rosemary's potential in treating urinary tract infections, which are commonly caused by this bacterium. The absence of inhibition against *E. coli* suggests a selective antibacterial action, possibly due to the outer membrane of *E. coli*, which can act as a barrier to certain antimicrobial compounds [8].Black Pepper showed antibacterial activity against *E. coli* (21.65 ± 0.87 mm), *Klebsiella spp.* (19.16 ± 0.19 mm), and *S. saprophyticus* (18.32 ± 0.68 mm), but did not inhibit *S. aureus*. The efficacy of Black Pepper against multiple bacterial species is well-documented, with piperine being the primary bioactive compound responsible for its antimicrobial effects [9]. Piperine is known to disrupt bacterial cell wall integrity and interfere with enzymatic functions, leading to cell death. The variation in inhibition zones among the tested bacteria suggests that Black Pepper may be more effective against Gram-negative bacteria like *E. coli* and *Klebsiella spp.*than against Gram-positive bacteria such as *S. aureus*. This may be due to differences in the cell wall structures of these bacteria, with Gram-positive bacteria having a thicker peptidoglycan layer that could impede the penetration of antimicrobial agents [10]. Cinnamon exhibited the highest antibacterial activity among the tested spices, with significant inhibition of *E. coli* (30.16 ± 1.30 mm), *S. aureus* (16.31 ± 0.46 mm), and *S. saprophyticus* (24.43 ± 1.07 mm), but was ineffective against *Klebsiella spp.* The potent antibacterial action of Cinnamon is attributed to its high content of cinnamaldehyde, which has been shown to disrupt bacterial cell membranes and inhibit the synthesis of essential proteins [11]. The strong activity against *E. coli* is particularly noteworthy, as this bacterium is a common cause of foodborne illnesses. The findings are consistent with other studies that have reported Cinnamon's effectiveness against a broad spectrum of bacteria, although the lack of activity against *Klebsiella spp.* suggests that the bacterium may possess resistance mechanisms such as efflux pumps or enzymatic degradation of cinnamaldehyde [12].Bay Leaves showed antibacterial activity against *Klebsiella spp.* (25.26 ± 1.28 mm), *S. aureus* (22.53 ± 1.04 mm), and *S. saprophyticus* (18.47 ± 0.88 mm), but were ineffective against *E. coli*.

The antimicrobial properties of Bay Leaves are primarily due to their content of essential oils, particularly eucalyptol, which has been shown to possess strong antibacterial effects [13]. The significant inhibition of *Klebsiella spp.* suggests that Bay Leaves could be particularly useful in treating infections caused by this bacterium, which is known for its multidrug resistance. The lack of activity against *E. coli* is somewhat surprising, given the broad-spectrum activity of eucalyptol, and suggests that further investigation is needed to understand the mechanisms underlying this selective antibacterial action [14]

The findings of this study are largely consistent with existing literature on the antimicrobial properties of spices. For instance, Cloves have been widely recognized for their potent antibacterial activity, particularly against Gram-negative bacteria like *E. coli* (Cortés-Rojas et al., 2014). Similarly, the strong activity of Cinnamon against a broad range of bacteria, including *E. coli* and *S. aureus*, has been well-documented, with studies highlighting the role of cinnamaldehyde as a key antimicrobial agent [11].

Rosemary's selective antibacterial activity, particularly its effectiveness against *S. saprophyticus* and *Klebsiella spp.*, aligns with other studies that have emphasized the role of phenolic compounds in its antimicrobial action [7]. The differential activity of Black Pepper against Gram-negative and Gram-positive bacteria is also supported by previous research, which has noted the challenges associated with targeting Gram-positive bacteria due to their thicker peptidoglycan layer [9].

The significant inhibition of *Klebsiella spp.* by Bay Leaves is particularly noteworthy, as this bacterium is known for its resistance to multiple antibiotics. This finding is consistent with studies that have demonstrated the efficacy of essential oils, such as eucalyptol, in combating multidrug-resistant bacteria [13]. However, the lack of activity against *E. coli* observed in this study contrasts with some reports that have documented the broad-spectrum activity of Bay Leaves, suggesting the need for further investigation [14].

However, it is important to note that several factors, including the method of extraction, the concentration of active compounds, and the type of microorganism tested can influence the antimicrobial effectiveness of spices. Therefore, further research is warranted to optimize the conditions for the use of these spices in food preservation. By harnessing the antimicrobial properties of these spices, it may be possible to reduce the incidence of foodborne illnesses and meet the growing consumer demand for natural and safe food products.

The results from the study have shown that the extracts from the spices can be used as antimicrobial therapy for serious infections. This implies that by consumption of food cooked with such natural spices, some level of treatment in the infections of an immunocompromised individual may also be achieved.

COMPETING INTERESTS: The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS: Udensi J. U. conceptualized the study, supervised the project, and contributed to the design and execution of experiments. Akanazu, C. O. assisted in data collection, laboratory analysis, and statistical evaluation. Anyanwu C. O. contributed to the literature review, methodology development, and manuscript drafting. Anyanwu E. O. supported data interpretation and assisted with laboratory setup and technical validation. Onyima, E. C. participated in the analysis of findings, preparation of tables and figures, and editing of the final manuscript.

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