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Antimicrobial properties of some synthetic food spices commonly used in Nigeria

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Abstract
This study focuses on evaluating the antimicrobial properties of various synthetic spice blends against foodborne pathogens and spoilage microorganisms. This study evaluates the antimicrobial activity of various spice extracts against different microorganisms using the well-in agar and disc diffusion methods. The spice extracts tested include Kitchen Glory, Spicity, Paramount All Purpose Seasoning, Big Mama Spice, and Larsor Chicken Spice. The microorganisms assessed were *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Escherichia coli*. In the disc diffusion method, Kitchen Glory demonstrated the highest inhibition against *Escherichia coli* (14.87±0.24 mm). Spicity showed the highest activity against *Staphylococcus aureus* (14.47±0.64 mm). Paramount All Purpose Seasoning had the highest inhibition against *Staphylococcus saprophyticus* (17.52±0.34 mm). Big Mama Spice displayed the highest inhibition against *Staphylococcus saprophyticus* (19.58±0.78 mm) but was least effective against *Escherichia coli* (7.64±0.47 mm). Larsor Chicken Spice showed moderate activity against all tested microorganisms. However, statistical analysis indicated no significant differences in the zones of inhibition among the extracts against the isolates ($p > 0.05$). In the well in agar diffusion method, Kitchen Glory exhibited the highest zone of inhibition against *Staphylococcus aureus* (28.64±0.34 mm), while Spicity showed significant activity only against *Klebsiella pneumoniae* (20.75±0.78 mm). Paramount All Purpose Seasoning was effective against all tested microorganisms, with the highest activity against *Staphylococcus aureus* (22.43±0.87 mm). Big Mama Spice was inactive against *Klebsiella pneumoniae* but showed good activity against the other microorganisms. Larsor Chicken Spice displayed activity against *Klebsiella pneumoniae* (15.61±0.16 mm) and *Staphylococcus aureus* (20.89±0.87 mm). However, statistical analysis showed no significant differences in the zones of inhibition among the extracts against the isolates ($p > 0.05$). This study indicates that these spice extracts have varying antimicrobial efficacies against different microorganisms, with some spices demonstrating substantial potential as antimicrobial agents. These findings suggest the potential use of certain spice extracts in developing alternative antimicrobial agents.

Keywords: Antimicrobial, extracts, microorganisms, spices, zone of inhibition

Introduction
Food safety is a critical issue with severe health and economic consequences due to foodborne illnesses caused by harmful microorganisms (CDC, 2018; Scharff 2018) [4, 14]. Natural antimicrobial agents found in spices and herbs have been used for centuries in traditional food preservation techniques across cultures (Gyawali & Ibrahim, 2014; Srinivasan, 2014) [4]. Synthetic blends, designed to mimic natural spice flavours, have gained popularity but their antimicrobial properties are poorly understood (Singsri, 2018; Balouiri *et al.*, 2016) [17, 2]. Historically, spices served as enhancers of the sensory qualities of food. For instance, Turmeric and pepper possesses the ability to modify certain food properties such as the taste, aroma, appearance as well as its colour while providing various nutritional benefits. Additionally, ginger, cinnamon and nutmeg are recognized for their digestive aid properties and are considered advantageous for soothing sore throats and the spleen (Rabiu *et al.*, 2023) [7]. The antimicrobial and antioxidant properties present in natural products, whether in the form of herbs, spices, or essential oils, can exhibit variations, with their efficacy not being consistent across all plant species. Furthermore, this efficacy can differ even within the same plant species, depending on the environmental and agricultural conditions in which they are cultivated (Adamu *et al.*, 2023) [1].

This study evaluates the antimicrobial properties of several synthetic spices used in Nigeria which includes: LARSOR Chicken: Commonly used in chicken dishes; little is known about its antimicrobial properties. Kitchen Glory: Versatile seasoning for various dishes; limited study on its antimicrobial potential. Big Mama: Used in meat and vegetable dishes; lacks thorough investigation on its antimicrobial role. Paramount Spices: Promises a unique flavour profile; limited research on its antimicrobial impact. Spicity: Marketed for a wide range of dishes; its antimicrobial properties need further study. Understanding their antimicrobial effects can assess their suitability for food preparation, preservation, enhancing food safety and extending shelf life (Martins *et al.*, 2018) [10].

Understanding the antimicrobial effects of synthetic spices can help in making informed decisions about their use in enhancing food safety and developing sustainable food preservation strategies, ultimately leading to improved food safety practices and reduced foodborne illness rates (Gyawali & Ibrahim, 2014) [4].

Identifying potent antimicrobial synthetic blends could lead to their use as natural preservatives, reducing synthetic additives and improving food quality and safety. The findings may inform the development of optimized antimicrobial synthetic spice blends for more effective and sustainable food preservation strategies.

In essence, this study aims to evaluate the potential antimicrobial properties of popular synthetic spice blends, which could have significant implications for enhancing food safety, quality, and shelf life through natural means.

Materials and Method

Collection of spices

Commonly used Nigeria-made spices: Larsor chicken, Spicity, Paramount all purpose seasoning, Big Mama and Kitchen Glory were gotten from the market. Expiry dates of each were observed.

Preparation of spice extracts

Coarsely powdered material of the spices was extracted for 8 hours with distilled water in a conical flask and then the extract was filtered and used for this study as an aqueous extract.

Test microorganisms: Pure cultures *of Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Staphylococcus saprophyticus* were obtained from the Microbiology laboratory of Federal University of Technology Owerri (FUTO), Imo State, Nigeria. These were re-identified and stored at 4°C on the appropriate agar slants.

Antimicrobial activity

The antimicrobial activites of the extracts were carried out by well in the agar diffusion method and Disc diffusion method.

Well-in-agar diffusion assay

This was determined using the method described by Kim *et al.* (1995) [9]. 17 mL of Mueller–Hinton Agar (MHA) was poured into sterile petri dishes and allowed to solidify. Using sterile cotton swabs, the cultures of bacteria as prepared were swabbed uniformly on the surface of the plates and allowed to dry for 5 min. All plates were labeled properly and wells of 5.0 mm in diameter were made in the seeded agar plates using a sterile cork-borer, sufficiently separated from each other to avoid overlap of growth inhibition zones. 0.01 ml of the different concentrations of the Spice Extract was dispensed into the wells. 5 plates were used for each spice extract. The MHA plates were incubated at 37 °C for 24 h. The zones of clearance produced around the wells after incubation were observed and measured (diameter of agar of the well not included) and recorded as the diameter of the zones of growth inhibition produced by the Spice Extract. This was carried out Five times.

Disc diffusion assay

Disc diffusion assay as described by Hudzicki (2009) [6] was used. Filter paper No. 1 was perforated to get paper discs of 5 mm diameter. These were sterilized at 110 °C for 30 min, dipped with the concentration of the extract (20 - 100%) and placed on the surface of the Mueller-Hinton agar plates inoculated with test bacteria. A negative control was set up with 0.1 mL of water, the resulting plates were incubated at 37 °C for 24 h. The zones of inhibition were measured using meter rule to the nearest millimeter (mm).

Results and Discussions

Results

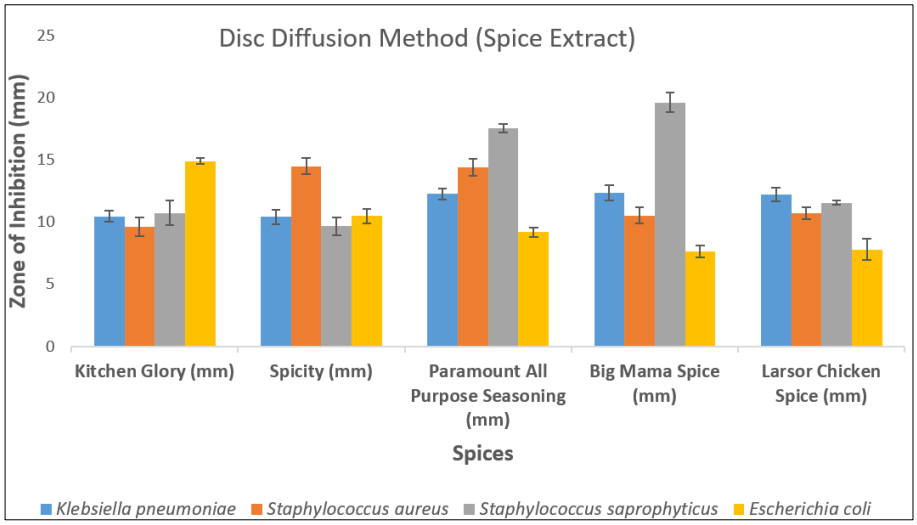

Fig 1: Zone of Inhibition of Spice Extract Using Disc Diffusion Method

Figure 1 shows the Zone of Inhibition of Spice extract using The Disc Diffusion method. For *Klebsiella pneumoniae*, kitchen glory extract exhibited an inhibition zone of approximately 10.45±0.45 millimeters, while Spicity showed a similar zone of inhibition at around 10.39±0.56 millimeters. Paramount All Purpose Seasoning and Big Mama Spice demonstrated larger inhibition zones, measuring about 12.25±0.45 millimeters and 12.34±0.62 millimeters, respectively. Larsor Chicken Spice also displayed a substantial inhibition zone of approximately 12.19±0.56 millimeters. *Staphylococcus aureus* showed varying zones of inhibition among the extracts. Spicity exhibited the largest inhibition zone, measuring around 14.47±0.64 millimeters, followed closely by Paramount All Purpose Seasoning at approximately 14.39±0.67 millimeters. Big Mama Spice displayed a moderate zone of inhibition of about 10.51±0.67 millimeters, while kitchen glory and Larsor Chicken Spice showed inhibition zones of 9.62±0.76 millimeters and 10.72±0.48 millimeters, respectively. In the case of *Staphylococcus saprophyticus*, Paramount All Purpose Seasoning and Big Mama Spice extracts displayed the most significant inhibition zones, measuring approximately 17.52±0.34 millimeters and 19.58±0.78 millimeters, respectively. Larsor Chicken Spice also exhibited a substantial inhibition zone, around 11.54±0.18 millimeters. However, kitchen glory and Spicity extracts showed smaller inhibition zones of 10.71±1.00 millimeters and 9.651±0.72 millimeters, respectively. For *Escherichia coli*, kitchen glory extract demonstrated the largest inhibition zone, measuring approximately 14.87±0.24 millimeters. Spicity exhibited a moderate inhibition zone of about 10.46±0.60 millimeters, while Paramount All Purpose Seasoning and Big Mama Spice extracts showed smaller inhibition zones of approximately 9.18±0.38 millimeters and 7.64±0.47 millimeters, respectively. Larsor Chicken Spice displayed an inhibition zone of around 7.78±0.87 millimeters. Statistical analysis shows no significant difference ($p>0.05$) in the zone of inhibitions of the extracts against isolates.

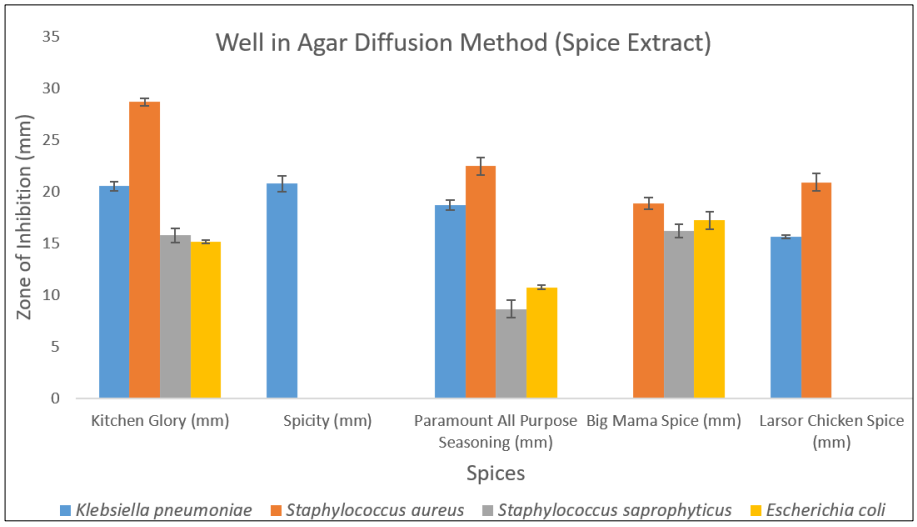

Fig 2: Zone of Inhibition of extracts using Well in Agar Diffusion Method

The antimicrobial activity of different spice extracts was evaluated against various microorganisms using the well-in agar diffusion method. The activity of the extracts against *Klebsiella pneumonia* showed that Spicity exhibited the highest zone of inhibition (20.75±0.78 mm), followed by Kitchen Glory (20.51±0.46 mm) and Paramount All Purpose Seasoning (18.65±0.49 mm). Big Mama Spice did not show any inhibitory activity, while Larsor Chicken Spice demonstrated a zone of inhibition of 15.61±0.16 mm. For *Staphylococcus aureus*, Kitchen Glory exhibited the largest zone of inhibition (28.64±0.34 mm), followed by Larsor Chicken Spice (20.89±0.87 mm) and Paramount All Purpose Seasoning (22.43±0.87 mm). Both Spicity and Big Mama Spice did not show any inhibitory activity against this microorganism. In the case of *Staphylococcus saprophyticus*, Kitchen Glory (15.75±0.69 mm) and Big Mama Spice (16.17±0.64 mm) were the only spice extracts that exhibited inhibitory activity, while Spicity, Paramount All Purpose Seasoning, and Larsor Chicken Spice did not show any zone of inhibition.

Against *Escherichia coli*, Big Mama Spice demonstrated the highest zone of inhibition (17.19±0.87 mm), followed by Kitchen Glory (15.13±0.16 mm) and Paramount All Purpose Seasoning (10.73±0.19 mm). Both Spicity and Larsor Chicken Spice did not exhibit any inhibitory activity against this microorganism. Statistical analysis shows no significant difference ($p>0.05$) in the zone of inhibitions of the extracts against isolates.

Discussion

According to Ibrahim *et al.* (2023) [7], spices demonstrate a wide-ranging spectrum of activity against diverse microorganisms, encompassing both Dimorphic fungi as well as Gram-positive and Gram-negative bacterium. The disc diffusion method is a widely used technique for determining the susceptibility of microorganisms to antimicrobial agents, including plant extracts (Balouiri *et al.*, 2016) [2]. Synthetic spices represent a fascinating intersection of food science, flavour technology, and microbiology. Brands like LARSOR, Kitchen Glory, Big Mama, Paramount, and Spicity showcase the diverse applications of these lab-created seasonings, from gourmet cooking to large-scale food production. The results indicate that the spice extracts exhibited varying degrees of antimicrobial activity against the tested bacterial strains, as evidenced by the different zones of inhibition observed.

The spice extracts, including kitchen glory, Spicity, Paramount All Purpose Seasoning, Big Mama Spice, and

Larsor Chicken Spice, showed moderate to good inhibitory activity against *K. pneumoniae*, with zones of inhibition ranging from approximately 10 to 12 millimeters. This suggests that these spice extracts possess compounds with antimicrobial properties that can inhibit the growth of this pathogenic bacterium. Studies have demonstrated that certain spices contain compounds like eugenol, carvacrol, and cinnamaldehyde, which possess strong antibacterial properties against Gram-negative bacteria like *K. pneumoniae* (Nascimento *et al.*, 2000; Burt, 2004) [11, 3].

Spicity and Paramount All Purpose Seasoning show the largest zones of inhibition against *S. aureus*, suggesting they are particularly effective against S. aureus. This could be due to higher levels of antimicrobial compounds like thymol and cinnamic aldehyde, which have been reported to be effective against Gram-positive bacteria like *S. aureus* (Burt, 2004; Singh *et al*., 2008) [3, 16]. The variation in activity could be attributed to differences in the phytochemical composition and concentrations of bioactive compounds present in the extracts.

Big Mama Spice and Paramount All Purpose Seasoning exhibit significantly larger inhibition zones, indicating their strong antibacterial activity against *S. saprophyticus*. These findings suggest that certain spice extracts, particularly Paramount All Purpose Seasoning and Big Mama Spice, possess potent antimicrobial compounds effective against this bacterial strain. The efficacy of certain spices against *E. coli* can be attributed to the presence of compounds like allicin, capsaicin, and gingerol, which are known for their potent antibacterial properties (Burt, 2004; Shan *et al.*, 2007) [3, 15].

The well-in agar diffusion method provides valuable insights into the antimicrobial activities of different spice extracts against various microorganisms. This method is a widely used technique for evaluating the antimicrobial potential of natural compounds, including plant extracts.

Spicity and Kitchen Glory showed the highest zones of inhibition, indicating strong antibacterial activity against *Klebsiella pneumoniae*. This is consistent with studies that demonstrate the efficacy of certain spice extracts in inhibiting the growth of gram-negative bacteria such as *Klebsiella pneumoniae*. For instance, Shan *et al.* (2007) [15] found that spice extracts possess significant antibacterial properties against a range of pathogens. The effectiveness of Kitchen Glory against *Staphylococcus aureus* is important, as this bacterium is a common cause of infections and is known for its resistance to many antibiotics. Previous research by Burt (2004) [3] supports the strong antibacterial activity of spice extracts, particularly against gram-positive bacteria like *Staphylococcus aureus.* Only Kitchen Glory and Big Mama Spice showed inhibitory activity against *Staphylococcus saprophyticus*. This limited activity might be due to the specific compounds present in these extracts that are effective against this particular microorganism. The variability in antibacterial efficacy among different spices is well-documented, as different spices contain distinct active compounds with varying degrees of effectiveness against different pathogens (Prabuseenivasan *et al.*, 2006) [12]. Big Mama Spice exhibited the highest zone of inhibition against *Escherichia coli*, followed by Kitchen Glory. The strong activity of Big Mama Spice could be attributed to specific bioactive compounds that are particularly effective against gram-negative bacteria. This finding aligns with the results from Iqbal *et al.* (2017) [8], who noted that natural antimicrobials could significantly inhibit the growth of *E. coli.*

The antimicrobial activity of spice extracts can be attributed to the presence of various bioactive compounds, such as phenolic compounds, terpenoids, alkaloids, and essential oils (Yashin *et al.*, 2017) [19]. These compounds can exhibit antimicrobial properties through various mechanisms, including disruption of cell membranes, inhibition of enzymes, and interference with cellular processes (Gyawali & Ibrahim, 2014) [5]. These results suggest that spice extracts can be promising sources of natural antimicrobial agents, with potential applications in food preservation, pharmaceutical industries, and alternative therapeutic approaches.

Conclusion

The study has shown that these spices exhibited antimicrobial properties against some pathogenic microorganisms. However, further research should be carried out to ascertain their actual chemical components and very importantly to determine their carcinogenic properties since they are synthetic in nature.

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