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Assessment of the antimicrobial activity of wonderful kola (*Buchholzia coriacea*) on *Salmonella* species isolated from seafoods

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ABSTRACT

The increasing failure of chemotherapeutic agents and the growing incidence of antibiotic resistance among pathogens have prompted the screening of medicinal plants for their antimicrobial potential. This study aimed to determine the efficacy of ethanol and aqueous seed extracts of *Buchholzia coriacea* (wonderful kola) against *Salmonella* species isolated from seafood (clam and prawn) using the agar well diffusion method. Wonderful kola seeds were purchased from Mile 3 Market, and pure cultures of *Salmonella* spp. were isolated from clam and prawn samples using standard microbiological techniques at the Microbiology Laboratory, Rivers State University. The results showed that ethanol yielded a higher quantity of extract (14.37 g) compared to the aqueous solvent (3.88 g). The percentage yields were 28.74% and 7.76% for the ethanol and aqueous extracts, respectively. The minimum inhibitory concentration (MIC) of both aqueous and ethanol extracts for the two isolates (ISO 1 and ISO 2) was 50%. The minimum bactericidal concentration (MBC) of the aqueous extract for both isolates was also 50%, whereas for the ethanol extract, the MBC was 25% for ISO 1 and 50% for ISO 2. Measurable zones of inhibition were observed for both extracts. The aqueous extract produced inhibition zones ranging from 11–15 mm for ISO 1 and 11–17 mm for ISO 2, while the ethanol extract produced zones ranging from 13–17 mm for ISO 1 and 11–14 mm for ISO 2. Although the extracts demonstrated antimicrobial activity against the isolates, the consumption or therapeutic use of plant extracts should be supported by comprehensive research evidence and professional medical advice.

Keywords: Antimicrobial activity, wonderful kola, *Salmonella* sp., seafoods.

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INTRODUCTION

In the realm of traditional medicine and herbal remedies, wonderful kola, scientifically known as *Buchholzia coriacea*, stands out as a potent and versatile botanical (Oyetayo et al., 2010). Native to West Africa, particularly Nigeria and Ghana, this small, bitter-tasting nut has garnered attention for its numerous health benefits and notable antimicrobial properties. Historically, indigenous cultures have revered wonderful kola for its medicinal value, using it to treat various ailments and promote overall well-being (Oyetayo et al., 2010).

The use of wonderful kola in traditional healing practices dates back centuries, underscoring its cultural and historical significance (Oyetayo et al., 2010). Known locally by names such as “Adu-Ogbọn,” this botanical has attracted the interest of researchers and health practitioners due to its reported therapeutic potential. Its rich phytochemical composition includes bioactive compounds such as flavonoids, alkaloids, tannins, and saponins (Akinmoladun et al., 2007). These compounds are associated with a range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and aphrodisiac effects. Consequently, wonderful kola has traditionally been used to manage gastrointestinal disorders, respiratory conditions, sexual dysfunction, and other health challenges (Owolabi et al., 2016).

Although the traditional applications of wonderful kola are well documented through ethnobotanical studies and anecdotal evidence, scientific investigations have increasingly sought to validate its medicinal properties and explore its relevance in modern healthcare. Recent studies have highlighted its potential roles in promoting digestive health, reducing inflammation, mitigating oxidative stress, enhancing sexual function, and supporting respiratory health (Oyedapo et al., 2004; Owolabi et al., 2019).

In addition to these therapeutic claims, wonderful kola has gained attention for its antimicrobial activity, which may be valuable in addressing infectious diseases and the growing global concern of antibiotic resistance. Previous studies have demonstrated its inhibitory effects against a variety of microorganisms, including bacteria, fungi, and viruses (Oloyede et al., 2015; Ajayi et al., 2018). The antimicrobial activity of wonderful kola is attributed to its bioactive constituents, which interfere with microbial growth and proliferation through diverse mechanisms.

Salmonella infections remain a major public health concern worldwide, contributing to illnesses such as gastroenteritis, typhoid fever, and other foodborne diseases (Grassl et al., 2008). The rising prevalence of antibiotic-resistant *Salmonella* strains has further complicated the management of these infections, underscoring the need to explore alternative antimicrobial agents. Although *Buchholzia coriacea* is widely used in traditional medicine for its antimicrobial properties, scientific evidence supporting its efficacy against specific pathogens, particularly *Salmonella* species, remains limited. This gap in knowledge highlights the need for systematic investigation. Therefore, this study aims to evaluate the antimicrobial activity of *Buchholzia coriacea* seed extracts against *Salmonella* spp. isolated from seafood (clam and prawn).

MATERIALS AND METHODS

Study area

The study was conducted in Port Harcourt City Local Government Area, Rivers State, Nigeria.

Sample collection

Wonderful kola (*Buchholzia coriacea*) seeds were purchased from Mile 3 Market and immediately transported to the Microbiology Laboratory, Rivers State University, for analysis. The seeds were chopped into smaller pieces using a sterile knife, air-dried at room temperature for several hours, and ground into powder using a sterilized mortar and pestle (washed with detergent and rinsed with 95% ethanol). The powdered samples were wrapped in clean aluminum foil, labeled appropriately, and stored until extraction.

Collection of bacterial isolates

Pure cultures of *Salmonella* spp. previously isolated from seafood samples (clam and prawn) were obtained from the Microbiology Laboratory, Rivers State University.

Extraction method

Extraction was carried out using distilled water and 70% ethanol as solvents. Fifty grams (50 g) of the powdered sample was weighed and dissolved in 200 mL of each solvent in separate sterile bottles. The mixtures were shaken vigorously for 30 minutes and allowed to stand at room temperature for 24 hours. Thereafter, each mixture was filtered using a clean funnel and sterile cotton wool into a 50 mL beaker to remove plant debris. The filtrates were concentrated in a water bath at 45°C for 48 hours. The resulting semi-solid extracts were collected, weighed, labeled, and stored at room temperature until required (Ejikeugwu et al., 2015).

Preparation of stock solutions

Fourteen EDTA bottles were labeled according to solvent type and concentration. Seven bottles were labeled for the ethanol extract (100%, 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.65%), and seven for the aqueous extract at corresponding concentrations.

For the 100% concentration, 2 g of the semi-solid extract was dissolved in 2.5 mL of distilled water and 2.5 mL of dimethyl sulfoxide (DMSO). The bottles were sealed and shaken thoroughly to ensure complete dissolution. For subsequent dilutions, 2.5 mL of distilled water was added to each of the remaining bottles. Serial dilution was performed by transferring 2.5 mL from the 100% concentration into the 50% bottle and continuing this process sequentially to obtain the remaining concentrations (Ogbonna et al., 2022).

Preparation of bacterial inoculum

A loopful of each pure culture was inoculated into sterile normal saline and adjusted to turbidity comparable to a standard inoculum. The suspensions were covered and used as required.

Determination of Minimum Inhibitory Concentration (MIC)

Twenty-eight test tubes were prepared and labeled according to isolate (ISO 1 and ISO 2), extract type (ethanol and aqueous), and concentration (100%–1.65%). Each tube contained 2 mL of nutrient broth, 1 mL of the appropriate extract concentration, and 0.1 mL of the standardized bacterial inoculum. The tubes were plugged with cotton wool and incubated at 37°C for 24 hours. Following incubation, tubes showing clear broth (no turbidity) were recorded as exhibiting inhibition (negative for growth), while turbid tubes were recorded as positive for bacterial growth (Ejikeugwu et al., 2015).

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined from the MIC assay. Nutrient agar plates were prepared, and the test organisms were evenly spread using sterile swab sticks. Agar wells (approximately 5 mm in diameter) were created using a sterile cork borer. Extracts of varying concentrations were introduced into the wells using sterile syringes. The plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition were measured in millimeters (mm) and recorded for each isolate and concentration (Valgas et al., 2007).

Calculation of Activity Index

$$\text{Activity Index (AI)} = \frac{\text{zone of Inhibition by extract}}{Zone\ of\ Inhibition\ by\ a\ standard\ antimicrobial\ agent}$$

Percentage Activity Index (%) = AI × 100

The standard antimicrobial agent used was tetracycline. The standard zones of inhibition for antibacterial susceptibility were compared with reference values (Table 1) to determine whether the test organisms were sensitive, intermediate, or resistant to the antimicrobial agent tested.

Table 1. Standard zone of inhibition of tetracycline according to Talaro (2008) and Cheesbrough (2010).

		S	I	R
Gram negative enteric bacteria	30µg	≥17mm	13-16mm	≤12mm

S = Sensitive ; **I** = Intermediate; **R** = Resistant.

RESULTS

The percentage activity index of wonderful kola (*Buchholzia coriacea*) seed extracts against the test isolates is presented in Table 2. The percentage activity index was calculated as the ratio of the observed zone of inhibition produced by the extract to that of the standard antimicrobial agent (tetracycline), multiplied by 100.

For the ethanol extract against Gram-negative enteric bacteria, the percentage activity index was 100% for ISO 1 (17/17 × 100) and 82.3% for ISO 2 (14/17 × 100).

For the aqueous extract, the percentage activity index was 88.2% for ISO 1 (15/17 × 100) and 100% for ISO 2 (17/17 × 100). Figure 1 and 2 showed the zone of inhibition of various isolates at different concentrations for the aqueous and ethanol extracts.

Percentage yield of extracts

Following evaporation and concentration of the extracts, the weights of the semi-solid extracts were determined to be 3.9 g for the aqueous extract and 14.4 g for the ethanol extract. The corresponding percentage yields were 7.8% for the aqueous extract and 28.74% for the ethanol extract (Table 3).

Percentage yield of extract was calculated as:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract}}{Weight\ of \frac{pulverized}{macerated} sample\ used}\ x\ 100$$

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial activity of wonderful kola (*Buchholzia coriacea*) was further evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for each extract and isolate. The MIC results (Table 4) showed that both aqueous and ethanol extracts exhibited a minimum inhibitory concentration of 50% against ISO 1 and ISO 2.

The MBC results (Table 5) indicated that both isolates

Table 2. Percentage activity index of different extracts of wonderful kola on bacterial isolates (for ethanol and aqueous extract respectively).

Ethanol	Concentration (%)	Zone(mm)	% Activity	Interpretation
ISO 1	100	17	100	S
	50	15	88.2	I
	25	15	88.2	I
	12.5	13	76.4	I
ISO 2	100	14	82.3	I
	50	14	82.3	I
	25	12	70.6	R
	12.5	10	58.8	R
Aqueous Extract				
ISO 1	100	15	88.2	I
	50	13	76.4	I
	25	13	76.4	I
	12.5	11	64.7	R
ISO 2	100	17	100	S
	50	14	82.3	I
	25	12	70.6	R
	12.5	11	64.7	R

S = Sensitive ; **I** = Intermediate; **R** = Resistant.

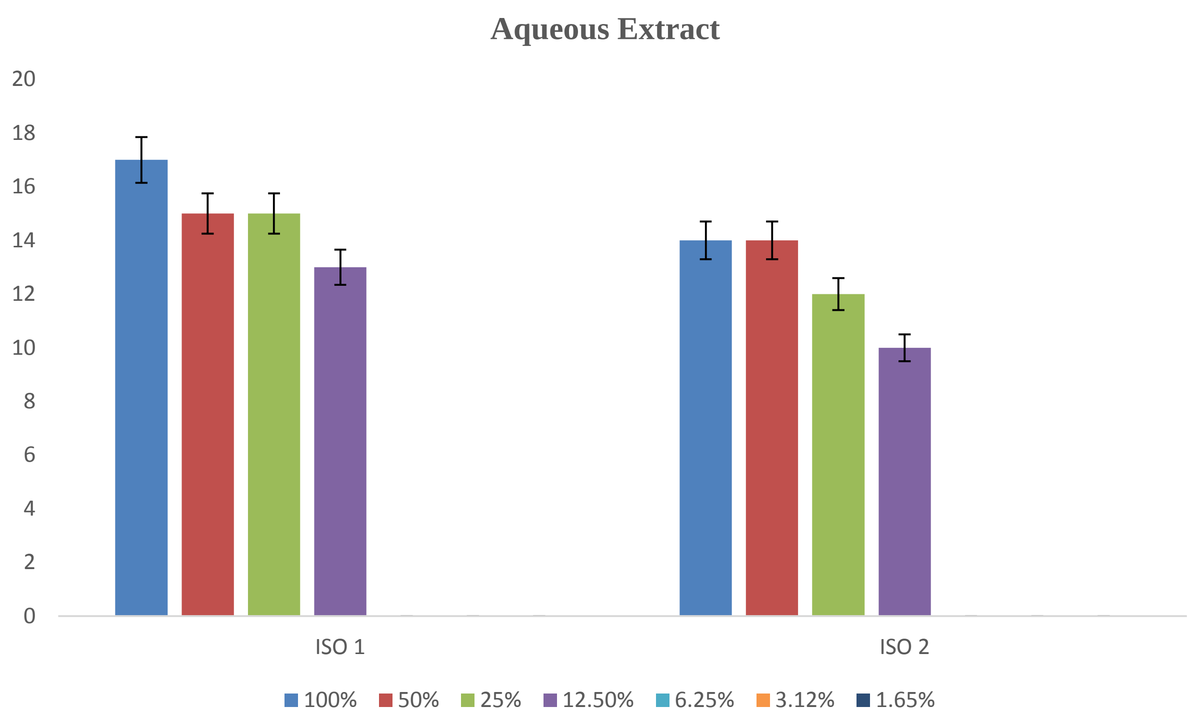

Figure 1. Zone of inhibition of various isolates at different concentrations.

Table 3. Percentage yield of extracts of plants.

Plant material	Solvent of extraction	Weight of pulverized/macerated sample used (g)	Weight of extracts (g)	Percentage Yield of extracts (%)
Wonderful kola	Ethanol	50.0	14.7	28.74
	Aqueous	50.0	3.9	7.8

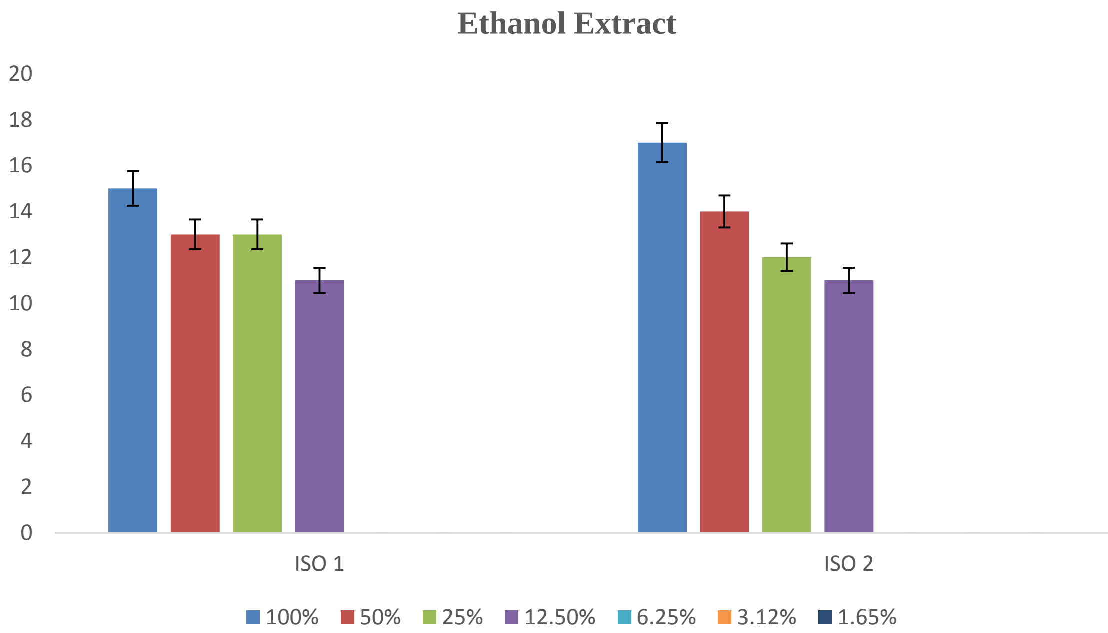

Figure 2. Zones of inhibition of various isolates at different concentrations.

Table 4. Minimum Inhibitory Concentration (MIC) of the different concentrations of aqueous and ethanol extracts.

	100%	50%	25%	12.5%	6.25%	3.12%	1.65%
Aqueous							
ISO 1	-	-	+	+	+	+	+
ISO 2	-	-	+	+	+	+	+
Ethanol							
ISO 1	-	-	+	+	+	+	+
ISO 2	-	-	+	+	+	+	+

KEY: +ve = turbidity in tubes, -ve =clear tubes.

Table 5. Minimum Bactericidal Concentration (MBC) of the different concentrations of aqueous and ethanol extracts.

	100%	50%	25%	12.5%	6.25%	3.12%	1.65%
Aqueous							
ISO 1	-	-	+	+	+	+	+
ISO 2	-	-	+	+	+	+	+
Ethanol							
ISO 1	-	-	-	-	+	+	+
ISO 2	-	-	-	-	+	+	+

KEY: +ve = growth on petri dish, -ve = no growth).

(ISO 1 and ISO 2) had an MBC of 50% for the aqueous extract. In contrast, for the ethanol extract, both isolates exhibited an MBC of 12.5%.

DISCUSSION

In recent years, there has been growing interest in the use of plant-derived materials as alternative therapeutic agents for controlling pathogenic microorganisms. The increasing failure of conventional chemotherapeutic agents and the rising prevalence of antibiotic-resistant pathogens have prompted the screening of medicinal plants such as *Buchholzia coriacea* (wonderful kola) for their antimicrobial potential.

In the present study, the ethanol extraction yielded a higher quantity of extract compared to the aqueous extraction, resulting in differences in extract weight and percentage yield. This finding is consistent with the reports of Ogunjobi and Nnadozie (2004) and Ezeifeka et al. (2004), who suggested that the active constituents of the seeds are more effectively extracted with ethanol than with water. Ethanol is widely recognized as a more efficient extracting solvent due to its polarity, which allows it to dissolve a broader range of bioactive compounds, including phenolics, flavonoids, alkaloids, and terpenoids (Lee et al., 2024).

Both seed extracts demonstrated appreciable antibacterial activity against the two *Salmonella* isolates (ISO 1 and ISO 2). With the ethanol extract, ISO 1 was more susceptible than ISO 2, indicating stronger antibacterial activity against ISO 1. Similarly, the aqueous extract showed greater activity against ISO 1 compared to ISO 2. These differences in susceptibility may be attributed to variations in the intrinsic resistance profiles of the isolates.

The activity index values obtained in this study suggest the presence of intrinsic bioactive constituents in wonderful kola seeds that may be harnessed to combat microbial infections, particularly in regions where access to conventional antibiotics may be limited. This finding supports the traditional use of the plant for therapeutic purposes. The plant has been reported to possess multiple pharmacological properties, including analgesic, amoebicidal, antibacterial, cardiotonic, cholagogue, digestive, emmenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic, and vermifuge effects, as well as activity against jaundice (Anibijuwon & Udeze, 2009).

Both ethanol and aqueous extracts produced measurable zones of inhibition against the test isolates. The ethanol extract produced inhibition zones ranging from 13–17 mm for ISO 1 and 10–14 mm for ISO 2. However, no inhibitory effect was observed at lower concentrations (6.25%–1.65%), indicating concentration-dependent antimicrobial activity. Similarly, the aqueous extract produced inhibition zones ranging from 11–15 mm for ISO 1 and 11–17 mm for ISO 2, with no inhibitory effect at concentrations between 6.25% and 1.65%. These findings are in agreement with Umeokoli et al. (2016), who reported that methanolic extracts of wonderful kola seeds exhibited better antimicrobial performance compared to aqueous extracts.

The minimum inhibitory concentration (MIC) results showed that both aqueous and ethanol extracts had an MIC of 50% against ISO 1 and ISO 2. This observation aligns partially with the findings of Dahake et al. (2009), who reported similar antimicrobial patterns in studies involving plant extracts against bacterial isolates, although variations may arise from differences in extraction methods, plant species, and bacterial strains used.

The minimum bactericidal concentration (MBC) results revealed that the aqueous extract had an MBC of 50% for both isolates, while the ethanol extract demonstrated a lower MBC of 12.5% for both ISO 1 and ISO 2, indicating stronger bactericidal activity. This finding differs from the report of Durand et al. (2015), who observed MBC values ranging from 0.078–1.25 mg/mL for *Cola nitida* bark extract against *Staphylococcus* strains isolated from meat samples. Such discrepancies may be attributed to differences in plant species, plant parts used, extraction methods, phytochemical composition, and the origin or resistance profile of the bacterial strains tested.

CONCLUSION

This study demonstrated that both ethanol and aqueous extracts of *Buchholzia coriacea* possess antimicrobial activity against *Salmonella* isolates obtained from seafood samples. The ethanol extraction yielded a higher percentage of extract and exhibited a higher percentage activity index compared to the aqueous extract, suggesting greater antimicrobial potency.

The findings confirm that wonderful kola seeds contain bioactive constituents capable of inhibiting the growth of *Salmonella* species, although the degree of efficacy varies depending on the type of extract and concentration used. The results further indicate that concentrations below 50% are unlikely to produce significant antibacterial effects under the conditions of this study.

Further research is recommended to evaluate the safety profile and potential toxicological effects of prolonged or excessive use of wonderful kola extracts, particularly through in vivo studies using animal models. Additionally, investigations involving other extraction solvents and standardized concentration measurements (e.g., mg/mL) are necessary to optimize antimicrobial efficacy and establish reliable MIC and MBC values. Public health awareness should also be promoted to discourage the indiscriminate use of plant extracts as alternatives to clinically approved antimicrobial agents without proper scientific validation.

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