UJMR, Conference Special Issue Vol. 10 No. 3. June, 2025, pp. 367 - 379

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.25103.037

Received: 12 April 2025 **Accepted:** 14 June 2025

Assessment of Antibacterial Potential of *Cochlospermum tinctorium* against Antibiotic-Resistant Bacteria Isolated from Raw Chicken Meat

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Abstract

***The antibacterial activity of Cochlospermum tinctorium was determined in this study against Salmonella sp., Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus, which were all antibiotic-resistant bacteria isolated from fresh chicken meat. The roots of Cochlospermum tinctorium were processed, and extraction was done by maceration. To determine the isolates' patterns of resistance and susceptibility to the antibiotics, antibiotic sensitivity testing was performed, while the disk diffusion method on Mueller Hinton Agar was used to assess the plant's antibacterial activity. The minimum Inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) were determined according to standard protocols. All statistical analyses were performed using R. The results showed all the bacterial isolates exhibited resistance to a number of widely used antibiotics: Septrin, Amoxicillin, Rocephin, Streptomycin, Sparfloxacin, Augmentin, Chloramphenicol, Ampicolox, Erythromycin. The phytochemical screening reveals the presence of alkaloids, tannins, cardiac glycosides, flavonoids, and steroids. Phytochemical screening revealed the presence of** *alkaloids, tannins, cardiac glycosides, flavonoids, and steroids.* **These compounds are known for their antimicrobial properties, suggesting that the extract contains bioactive substances that may contribute to its antibacterial potential. At a high concentration of 500 mg/mL, the extract of Cochlospermum tinctorium was effective in inhibiting all the isolates, with Staphylococcus aureus and Salmonella showing the highest zone of inhibition of 24.00mm and 23.00mm, respectively. The lowest inhibition was observed at 62.5mg/mL with Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus exhibiting the lowest inhibition at 4.00 mm, 6.00 mm, and 7.00 mm, respectively. The Minimum Inhibitory concentration (MIC) ranged from 62.5 to 31.25mg/mL for Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Salmonella, while the minimum bacteriocidal concentration (MBC) was between 125 and 62.5mg/mL.** *Statistically, it shows no significant difference* **in the** *mean zone of inhibition of the plant extract against the bacterial isolates* **(F:0.22, F-crit: 3.24, P-value: 0.881, P>0.05). Indicating the extract may have a** *broad but uniform antibacterial effect.* **Further studies are recommended to explore its spectrum of activity, to identify the lead bioactive metabolite responsible for the antibacterial activity and its toxicological effect in biological organisms.**

Keywords: Cochlospermum tinctorium, Antibiotic Resistance, Antibacterial, Phytochemical Screening, Minimum Inhibitory concentration

INTRODUCTION

The development of the human population, rising wages, and shifts in consumer tastes (more protein in the diet) have all contributed to an increase in the use of animal products (Henchion *et al.*, 2017). Because of the strong demand for animal goods, animal output increased significantly (Dhama *et al.*, 2013). In Nigeria, poultry production accounts for 33% of the total available animal protein source (FAOSTAT, 2021; FAO, 2022). This has far-reaching implications for household eating habits, animal-sourced food consumption, the food chain, and food safety (Bamidele *et al.*, 2022). Poultry farming has been recognised as a hotspot for the development of antimicrobial resistance (AMR), as well as the transfer of drug-resistant microorganisms between food-producing animals and people (Van Boeckel *et al.*, 2015; Roth *et al.*, 2019; Gupta *et al.*, 2021; Zalewska

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

et al., 2022). This is due to the high and chronic use of antibiotics, especially at sub-therapeutic levels, in commercial poultry (Bamidele *et al.*, 2022). The indiscriminate use of antibiotics, both for therapeutic and non-therapeutic purposes (improved feeding, growth promoter), in poultry production systems presents a public health threat to humans (Oluwasile *et al.*, 2014; Sanderson *et al.*, 2016; Mehdi *et al.*, 2018; Hedman *et al.*, 2020). This threat is heightened by the increased demand for animal protein owing to the growing population and economic growth (Tilman *et al.*, 2011; Hedman *et al.*, 2020)

The overuse of antimicrobial drugs in the chicken industry is a major contributor to the steady development and rising prevalence of multidrug-resistant bacteria. Antibiotic resistance arises when microorganisms survive in the presence of an antibiotic that would ordinarily impede their growth (Fadare *et al.*, 2019). To promote meat output, the chicken industry uses several antibiotics to increase feed conversion, growth promotion, and disease prevention. Antimicrobial resistance is a significant global public health matter that poses a threat to both human and animal populations (Cagnoli *et al.*, 2024). The phenomenon of antibiotic resistance cannot be overemphasised (Bashir *et al.*, 2021). Antibiotic resistance kills at least 700,000 people every year. World Health Organisation predicts that this number could rise to 10 million by 2050, highlighting a health concern not of secondary importance (de Kraker *et al.*, 2016). It is one of the most concerning dangers to world health, and novel anti-infective drugs are required to combat antibiotic resistance (Thabit *et al.* 2015). As a result, effective alternative medications for controlling infectious diseases and limiting the development of antibiotic-resistant bacteria must be identified. Furthermore, new mechanisms of action for these innovative anti-infective drugs are required (Schröder *et al.*, 2017). Plants are thought to contain a variety of Phytochemicals, which could serve as a source of novel anti-infective drugs (Gonfa *et al.*, 2023). Because of their diverse and broad biological applications, plant-based phytochemicals and nanoparticles are currently the focus of research on illness treatment (Ribeiro *et al.*, 2018). Secondary metabolites include alkaloids, glycosides, flavonoids, proanthocyanidins, tannins, terpenoids, phenylpropanoids, resins, lignans, furocoumarins, naphthodianthrones, proteins, and peptides (Senthilkumar *et al.*, 2018). Given the predominant uses of medicinal plants in traditional medicine, there is an upsurge in research to investigate the active medicinal compounds, efficacy, and safety of such plants (Tekuri *et al.*, 2019)

The plant *Cochlospermum tinctorium* is a sub-shrub that belongs to the family Cochlospermaceae, it has long been utilised in traditional medicine in many African countries, including Ivory Coast, Ghana, Cameroon, Nigeria, Gambia, Guinea, Senegal, and Burkina Faso. And it is locally called Oja Ikoko or Sewutu (Yoruba), Obazi or Abanzi (Igbo), and Rawaya or Kyamba (Hausa) languages of Nigeria (Akinloye *et al.*, 2012; Aguilar-Guadarrama and Rios, 2018; Jodi and Sani, 2023). The leaves of *Cochlospermum tinctorium* are used to treat diarrhoea (Magaji *et al.*, 2010), abscesses, and boils, while the blossoms can treat constipation (Johnson-Fulton and Waston, 2018). The roots are decocted or infused with other herbs to cure malaria, urethral discharges, orchitis, and fever (Musa, 2012), abscesses and boils, while the flowers are used against constipation (Johnson-Fulton and Watson, 2018). The root decoction or infusion is used in combination with other herbs to treat malaria, urethral discharges, orchitis, and fever (Musa, 2012). *Cochlospermum tinctorium* roots have been used in traditional medicine to treat viral disorders, diabetes, epilepsy, pain, inflammation, conjunctivitis, leprosy, and testicular irritation (Johnson-Fulton and Waston, 2018). *Cochlospermum tinctorium* rhizomes are used as a decoction to treat fever, hepatitis, abdominal pain, and bilharzia (Ndouyang *et al.*, 2018). *Cochlospermum tinctorium* powder has enormous potential to be used in the field of pharmacology because it has antimicrobial activity against antibiotic-resistant food-borne pathogens, and thus could be exploited as an alternative antimicrobial drug for the treatment of diseases caused by those pathogens (Abdulaziz *et al.*, 2019). Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines (Keita *et al.*, 2022). Since the 1960s, antibiotic resistance has gradually increased, while the number of new antibiotic classes brought to market has decreased (Abdullah *et al.*, 2023), to combat antimicrobial resistance, there is a need for an alternative source of antimicrobial agent for effective treatment to overcome the problem of resistance to the currently available antibiotics. Therefore, this research aimed to assess the antibacterial

potential of *Cochlospermum tinctorium* root extract against antibiotic-resistant bacterial isolates from chicken meat

MATERIAL AND METHODS

Collection of Plant Material

The *Cochlospermum tinctorium* roots were collected from Kankara Town (11.93114°N 7.41115°E), Kankara Local Government Area of Katsina State. The plant material was then taken to the Biology Department, Umaru Musa Yar`adua University, Katsina. The roots were then taken to the Microbiology laboratory of Umaru Musa Yar'adua University, Katsina. The root was air-dried and milled to powder using a mortar and pestle and sieved to obtain fine powder and stored at room temperature with plastic packaging until use.

Extraction of Plant Material

The extract used for phytochemical screening and antimicrobial analysis was prepared as described from previous work (Jodi and Sani, 2023). The adopted method of extraction was the maceration method, where 45grams of the plant root powder were macerated in distilled water for 72 hours with frequent shaking. Following 72 hours, the mixture was filtered first using muslin cloth, followed by re-filtration using Whatman no. 1 filter paper. Immediately after the filtration, the liquid was concentrated on a plate burner at 100 °C for hours to obtain solid crude extract, which will then be stored at 40 °C in the refrigerator for storage and preservation.

Phytochemical Screening of *Cochlospermum tinctorium* Root Extract

Phytochemical screenings for tannins, saponins, alkaloids, flavonoids, and steroids, were performed as described in previous works (Santhi and Sengottuvel, 2016; Jodi and Sani, 2022).

Test for Alkaloids

Two drops of Mayer's reagent were added to 0.5g of aqueous extract in a test tube. Appearance of yellow-creamy precipitate indicates the presence of alkaloids.

Test for Saponins

Cochlosperum tinctorium 100 mg extract was added to 2 mL 25% H2SO4, then autoclaved for 120 minutes at 100 °C. The extract was extracted with ether and dried. 1 mL aquadest is added, then vortexed for 5 minutes.

Test for Cardiac Glycosides

Half a gram (0.5g) of *Cochlospermum tinctorium* extract was weighed using a top-loading balance and dissolved in 2ml glacial acetic acid in a test tube. Three drops of ferric chloride solution were added, followed by adding 1ml of concentrated H_2SO_4. A brown at the interface indicates the presence of deoxysugars, a characteristic of cardenoloids.

Test for Steroids

Two millilitres (2ml) of acetic anhydride to 0.5g of *Cochlospermum tinctorium* root extract and 2ml of H_2SO_4 Color changes from violet to blue, which indicates the presence of steroid.

Tannins

About 0.5ml of the extract was dissolved in 10ml of distilled water and then filtered. A few drops of iron chloride ($FeCl_3$) solution were added to the filtrate. Formation of blue-black precipitation indicated the presence of hydrolysable tannin, and green precipitation indicated the presence of condensed tannin.

Flavonoids

A few drops of lead acetate solution were used to test the extracts. Flavonoids area yellow precipitate

Bacterial characterisation

The chicken meat sample was sourced from local vendors in Katsina Metropolis. The samples were enriched for 24hours at 37°C, serially diluted and plated on Nutrient Agar. The inoculated plates were incubated at 37°C for 24 hours. Morphologically distinct colonies were sub-cultured for identification and characterisation after the required incubation period of 24 hours. The Gram reaction, colony morphology, and biochemical tests: catalase, oxidase, urease, coagulase, indole, methyl red, Voges - Proskauer, and Simon citrate tests were carried out to identify the bacterial isolates as described by previous works (Omemu, *et al.*, 2018; Aun and Oo, 2020; Islam *et al.*, 2020; Hassen *et al.*, 2022)

Citrate Utilisation Test

Bacterial colonies from fresh plates (18-24 hours old) were inoculated onto a slope of Simmons citrate agar, and incubated overnight at 37°C. A

change in media from green to blue indicated a favourable reaction, implying that the organism can use citrate as its only source of carbon and energy.

Motility test

The medium contains 10g of gelatin, 5.0g of sodium chloride, 3g of beef extract, and 4.0g of 1L of distilled water. The bacterial strains were injected ¾ of the way down the stabbing media tube. During growth, motile bacteria migrate away from the line of inoculation to generate a dense turbidity in the surrounding media, while nonmotile bacteria grow only along the line of inoculation.

Coagulase test

The tube method was employed, using a nutritional broth and 1 ml of plasma. Two test tubes were prepared and labelled as "test" and "positive control." One colony of the test bacterium was added to the "test" tube, while one colony of Staphylococcus aureus was added to the positive control tube. Both tubes were initially incubated for 4 hours, then for 24 hours. A coagulase-positive result was indicated by the presence of gel-like clumps at the bottom of the tube, while the absence of clumping indicated a negative result.

Indole test

The test isolates were inoculated into a bijou bottle containing 3 ml of sterile tryptone water and incubated at 35-37°C for 48 hours. To test for Indole, add 0.5 ml (5 drops) of Kovac's reagent (isoamyl alcohol, para-dimethyl aminobenzaldehyde, and strong hydrochloric acid) and gently shake. A red colour in the surface layer within 10 minutes indicated a favourable reaction, but a yellow colour suggested a negative reaction

Methyl red test

The isolates were inoculated into glucose phosphate broth, which included glucose and phosphate buffer, and cultured at 37°C for 48 hours. The pH of the media was determined by adding five drops of methyl red reagent. The methyl red reagent was dispersed by gently rolling the tube between the palms. The appearance of red colour was interpreted as positive, and yellow as negative.

Voges-Proskauer test

The isolates were injected into glucose phosphate broth and incubated for 48 hours. The soup was mixed with 0.6 ml of 5% ethanol solution of alpha-naphthol. The tube was allowed to stand for 15 minutes. The hue cherry red was considered favourable, whereas no colour change signalled a negative.

Urease test

The test organism was inoculated heavily in a bijou bottle containing 3 mL sterile urea broth and incubated at 35°C for up to 7 days. A colour change from yellow to rose pink was taken as positive. Organisms positive to this test hydrolyse urea to produce ammonium ions with a subsequent change in pH to alkaline (reaching 8.1) from an initial pH of 6.8.

Triple Sugar Iron (TSI) agar

The bacteria colony was stabbed through the centre of the TSI agar with a sterile needle and then streaked on the surface of the agar slant. The culture was incubated at 37 °C for 24 h. The change in colour of the agar slant after incubation revealed whether there was the production of hydrogen sulfide (H2S), and carbohydrate fermentation occurred

Oxidase test

The tested single colony bacteria were smeared on filter paper that had been saturated with 1% (v/v) oxidase reagent and checked for purple production. The positive test result was reported as a purple hue development within ten seconds.

Catalase Test

Catalase tests were performed by putting one or two drops of 3% (v/v) hydrogen peroxide solution on a glass slide containing pure bacteria culture. A positive catalase test result was determined by the production of gas bubbles within 10 seconds of the combination.

Antibiotic Sensitivity Test

The disk-diffusion technique was used to assess the isolates' sensitivity to the antibiotics as described by CLSI (2021). A 0.5 McFarland standard was prepared, and the desired colony was picked and introduced into a sterile normal saline; it was shaken thoroughly until its turbidity matched that of the McFarland standard. The Mueller-Hinton agar medium was

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streaked with the standardised inocula using a cotton swab stick. The antibiotic disks; (10µg), Pefloxacin (10µg), Ampliclox (30µg), Zinnacef (20µg), Amoxicillin (30µg), Rocephin (25µg), Ciprofloxacin (10µg), Streptomycin (30µg), Septrin (30µg), and Erythromycin (10µg), Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Tarivid (10µg) were impregnated on the surface of the seeded plates. Following a 24-hour incubation period at 37°C, the diameter of the clear zone of inhibition was measured in millimetres and interpreted using the CLSI protocol (2021).

Antibacterial activity of the plant extracts using the disk diffusion method

The sterile disks were made in different concentrations of the plant extract: 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml. The disks were dispensed on the surface of the seeded plates using sterile forceps in the proper arrangement. Following a 24-hour incubation period at 37°C, the diameter of the clear zone of inhibition was measured (Magashi *et al.*, 2018). The Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) were conducted as described by previous work (Bashir *et al.*, 2022). The diameter of the zone of inhibition was measured in millimetres and statistically analysed using R to determine significant differences in the mean zone of inhibition of the plant extract against the four bacterial isolates

RESULTS

Phytochemical Composition of the Plant Aqueous Extract (*Cochlospermum tinctorium*)

The results of the phytochemical screening showed that the roots of *Cochlospermum tinctorium* contained phenolic compounds; tannins, Cardiac glycosides, flavonoids, Steroids, alkaloids, but no saponins (Table 1).

Table 1: Phytochemical Composition of the Extract

Phytochemical	Inference/Result
Tannins	+
Cardiac glycosides	+
Saponins	-
Alkaloids	+
Flavonoids	+
Steroids	+

Key: + = Positive - = Negative

Morphology, Cultural and biochemical characteristics of the test isolates

The morphological and biochemical characterisation of the bacterial isolates is presented in Table 2. Biochemical reaction of the test bacteria was confirmed by comparing the reaction with that in Bergey's Manual of Determinative bacteriological, 2nd edition. The confirmed organisms are *Staphylococcus aureus, Escherichia coli, Salmonella* sp, and *Pseudomonas aeruginosa*.

Table 2: Morphological and Biochemical characteristics of the bacterial isolates

Characteristics	*S. aureus*	*E. coli*	*Salmonella sp*	*P. aeruginosa*
Gram	+ve	-ve	-ve	-ve
Shape	cocci	rods	rods	rods
Citrate (Cit)	+	-	+	+
Motility (Mot)	-	+	+	A
Indole (Ind)	-	+	-	-
Coagulase (Coa)	+	-	-	-
Urease (Urea)	+	-	-	+
Methyl Red (MR)	-	+	-	-
Voges-Proskauer (VP)	+	-	-	A
Triple Sugar Iron (TSI)		A/A	K/A	K/A
Catalase (Cat)	+	+	-	+
Oxidase (Oxd)	-	-	-	+
H_2S	-	-	+	-

Key: +ve = Positive, -ve =Negative, Ind = Indole, MR = Methyl red, VP = Voges-Prokeur, Cat = Catalase, H_2S = Hydrogen sulphide, Mot = Motility, Coa = Coagulase, Oxd =Oxidase

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Antibiotic sensitivity patterns of the bacterial isolates

The results of the antibiotic susceptibility test of the isolated bacteria are shown in Table 3; most of the isolates demonstrated varying degrees of susceptibility and resistance to the tested antibiotics. Pefloxacin and Ciprofloxacin exhibited antibacterial activity against all isolates: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella sp, while all the isolates were resistant to Amoxicillin. All the tested isolates were resistant to Amoxicillin. Escherichia coli is resistant to Septrin, Augmentin, and Amoxicillin, but Salmonella is resistant to Septrin, Chloramphenicol, Sparfloxacin, and Amoxicillin.

Table 3. Antibiotic sensitivity pattern of the bacterial Isolates

	Antibiotics/ Zone of Inhibition(mm)			
Antibiotic	***S. aureus***	***E. coli***	***Salmonella sp***	***P. aeruginosa***
APX	8 ± 0.30	-	-	-
Z	17 ± 0.50	-	-	-
OFX	-	11 ± 0.10	17 ± 0.40	15 ± 0.50
E	7 ± 0.40	-	-	-
ST	4 ± 0.10	16 ± 0.10	20 ± 0.00	8 ± 0.00
R	20 ± 0.20	-	-	-
SXT	22 ± 0.50	5 ± 0.50	7 ± 0.50	19 ± 0.50
CH	-	8 ± 0.50	7 ± 0.60	17 ± 0.30
SP	-	20 ± 1.00	5 ± 0.50	15 ± 1.50
CPX	19 ± 0.50	18 ± 0.80	22 ± 0.50	20 ± 1.00
AM	7 ± 0.70	6 ± 0.50	8 ± 0.00	6 ± 0.50
AU	-	7 ± 0.00	19 ± 0.20	18 ± 1.00
CN	8 ± 0.00	17 ± 1.00	18 ± 1.80	18 ± 0.10
PEF	19 ± 0.50	19 ± 0.10	17 ± 1.20	21 ± 0.50

Key: R=Resistance, S =Sensitive, I =Intermediate, SXT =Septrin, OFX =Tarivid, Z =Zinnacef, SP =Sparfloxacin, CH =Chloramphenicol, PEF=Pefloxacin, CN =Gentamycin, AM =Amoxicillin, AU =Augmentin, CPX =Ciprofloxacin, ST=Streptomycin, APX =Ampiclox, E = Erythromycin, CN =Gentamycin

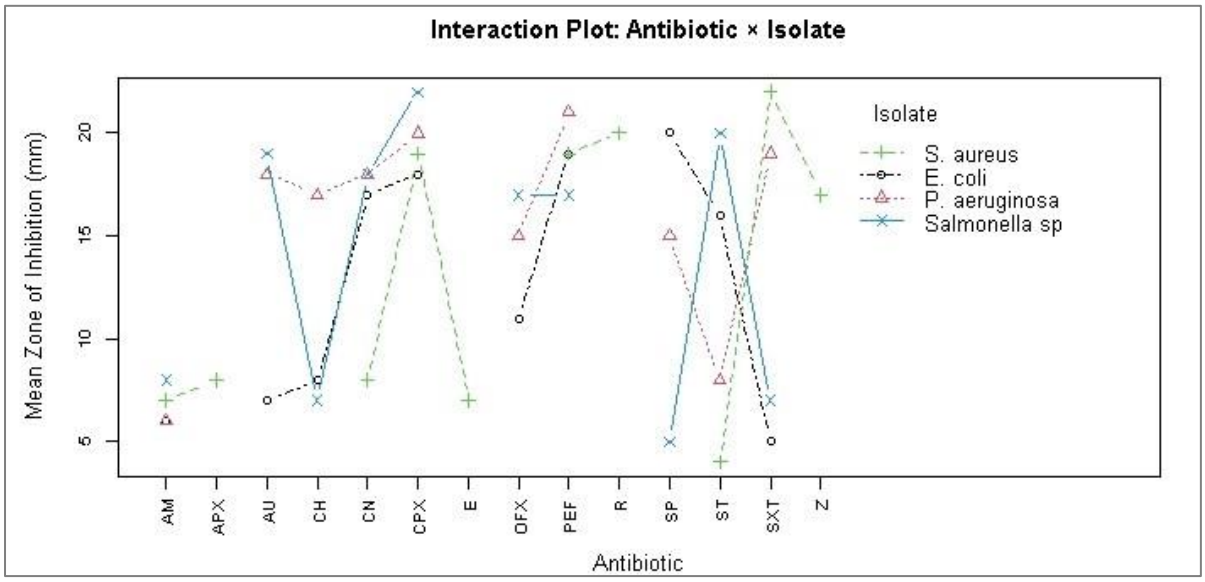

Fig. 1. Interaction plot between the antibiotics and bacteria isolates

Interaction Between Antibiotics and Bacterial Isolates on Zone of Inhibition

The x-axis represents the different antibiotics tested, while separate lines (with distinct colours and point shapes) represent each bacterial isolate (Figure 1). The plot shows the average zone of inhibition for each antibiotic-isolate combination, helping to assess whether there is an interaction effect—that is, whether the effect of antibiotics on inhibition zones differs depending on the bacterial isolate.

A two-way ANOVA was conducted using R to evaluate the effects of different antibiotics and bacterial isolates on the zone of inhibition. The results showed that there were no statistically significant differences in the zone of inhibition among the antibiotics ($F(13, 23) = 1.609$, $p = 0.154$, F-crit = 2.321) or among the bacterial isolates ($F(3, 23) = 0.619$, $p = 0.610$, F-crit = 3.009).

Multiple Antibiotics Resistance Index (MARI) of the bacterial isolates

The result for the multiple antibiotic resistance index for the bacteria isolates is presented in Table 4. The study revealed the resistance of some antibiotics tested against the bacterial isolate, where *Staphylococcus aureus* shows the highest resistance with MARI (0.5), followed by *Salmonella* (0.40), *Escherichia coli* (0.30) and *Pseudomonas aeruginosa* with the lowest resistance of Mari index (0.10).

Table 4: Multiple Antibiotic Resistance Index (MARI) of the bacterial isolates

Isolates	MARI	Antibiotics Resistant
Staphylococcus aureus	0.50	E, CN, APX, S, AM.
Escherichia coli	0.30	SXT, AU, AM.
Salmonella sp	0.40	SXT, CH, SP, AM.
Pseudomonas aeruginosa	0.10	AM.

Key: SXT = Septrin, AM = Amoxicillin, R = Rocephin, S = Streptomycin, SP=Sparfloxacin, AU= Augmentin, CH= Chloramphenicol APX =Ampicolox, E = Erythromycin

Table 5: Antibacterial Activity of *Cochlospermum tinctorium* Aqueous Extract

Isolate	Zone diameter (mm) Concentrations of the Plant Extract				
	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	Control (CPX)
Staphylococcus aureus	24±0.50	19±1.20	13±0.90	7±0.50	20±0.10
Escherichia coli	19±0.00	13±0.70	8±1.30	4±0.00	22±0.80
Pseudomonas aeruginosa	22±0.20	16±0.80	10±0.00	6±.0.00	20±0.70
Salmonella sp	23±1.00	16±0.00	11±0.50	8±0.10	19±0.30

Key: - = Presence of growth, CPX = Ciprofloxacin

Table 6: Determination of Minimum Bactericidal Concentration (MBC) and Minimum Bacteriocidal Concentration (MBC) of the Cochlospermum aqueous extract

Isolates	Concentrations of the extracts	
	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	62.5	125
E coli	62.5	125
Pseudomonas aeruginosa	62.5	125
Salmonella enteritidis	31.5	65

Key: MIC = Minimum inhibitory concentration, MBC = Minimum Bacteriocidal concentration

Evaluation of the antibacterial activity of *Cochlospermum tinctorium* aqueous extract

The results for the antibacterial activity of the aqueous extract of *Cochlospermum tinctorium* root powder against antibiotic-resistant bacterial isolates are presented in Table 5. The aqueous extract of *Cochlospermum tinctorium* root powder reveals a maximum zone of inhibition of 24.00mm against *Staphylococcus aureus*, 23.00 mm against *Salmonella* sp and a minimum inhibition of 4.00mm against *Escherichia coli*, 6.00mm against *Pseudomonas aeruginosa* and 7.00mm against *Staphylococcus aureus*. The work showed that at varying doses, the antibacterial properties of the aqueous extracts demonstrated that *Cochlospermum tinctorium* was effective against the diverse test microorganisms.

Minimum Inhibitory concentration and minimum bacteriocidal concentration (MBC) of the *Cochlospermum tinctorium* aqueous extract

The result of the minimum inhibitory concentration (MIC) of *Cochlospermum tinctorium* aqueous extract against antibiotic-resistant bacterial isolates is presented in Table 6. It was observed that the *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* showed MIC at 62.5mg/mL, while *Salmonella* sp showed MIC at 31.25 mg/mL. The result of the minimum bactericidal concentration (MBC) of *Cochlospermum tinctorium* crude aqueous extract against antibiotic-resistant bacterial isolates is presented in Table 6.

DISCUSSION

The Cochlospermum genus is renowned for its richness in secondary metabolites, notably phenolic compounds, flavonoids, lignans, carotenoids, and sterols, which underpin their extensive medicinal applications (Aguilar-Guadarrama and Rios, 2018; de Miranda et al., 2019). These compounds play a crucial role in neutralising free radicals and alleviating oxidative stress, thereby supporting the plant's traditional use in Angolan medicine (Samba et al., 2025). The Phytochemical compounds, including tannins, Cardiac glycosides, flavonoids, Steroids, and alkaloids, but no saponins, were detected in *Cochlospermum tinctorium*, consistent with earlier research by Jodi and Sani (2022), which found that the roots of *Cochlospermum tinctorium* contained tannins, flavonoids, saponins, and alkaloids, but no steroids. According to Tijjani et al. (2009), the methanol rhizome extract of Cochlospermum tinctorium contained flavonoids, cardiac glycosides, and tannins. Ahmed et al. (2011) found saponins, flavonoids, tannins, steroids, cardiac glycosides, and alkaloids in the plant's aqueous methanol extracts of the leaves, roots, and root bark. Etuk et al. (2009) also found that the aqueous root extract of Cochlospermum tinctorium included volatile oils, alkaloids, tannins, cardiac glycosides, saponins, flavonoids, triterpenes, and cyanogenic glycosides.

The bacterial isolates from the chicken meat samples were predominantly gram-negative which is known to cause foodborne disease, which relates to the findings of Ali et al. (2020), who isolated Salmonella and Escherichia coli from broiler meat, and Abd El Tawabs et al. (2015), who isolated E. coli, Salmonella, and S. aureus from chicken meat and meat products. Similar findings were reported by Lamada et al. (2012) and Abdaslam et al. (2014). Hue et al. (2011) discovered the presence of Salmonella and other harmful bacteria in slaughtered chicken flesh, while Klaharn et al. (2022) isolated Staphylococcus sp. from chicken meat, and Jodi and Sani (2022) discovered the presence of Salmonella, Escherichia coli, and Staphylococcus aureus in chicken meat. Thus, the findings of the aforementioned scholars are correlates with this work. The presence of these organisms in raw meat has important public health consequences (Sousa, 2008). This could be due to insufficient cleaning and disinfection of equipment and surfaces, poor personal hygiene, and the usage of unskilled staff.

The use of antibiotics in livestock and the resultant residue contribute to high antibiotic resistance levels of S. aureus found in meat products. Previous research has also shown that Salmonella spp. are sensitive to Ciprofloxacin and Gentamicin (Manjunath et al., 2011; Ansari et al., 2014). Staphylococcus aureus was particularly susceptible to gentamycin and ciprofloxacin. Meanwhile, they were highly resistant to erythromycin, chloramphenicol, ampicolox, streptomycin, and amoxicillin. These findings were consistent with those of Datta et al. (2012), Abd El- Salam (2014), and Ezzat et al. (2014). Our findings demonstrated that various antibiotic resistances are common among isolates of E. coli, Salmonella, and Staphylococcus aureus. According to Abd El Tawabs et al. (2015), plants are being considered as an effective alternative to combat the spread of antibiotic-resistant microbes (Rosina et al., 2009).

The multiple antibiotic resistance (MAR) index is a valid and cost-effective tool for tracking the source of bacteria (Adzitey 2015; Davis & Brown 2016). It is a quick and simple procedure (Khan et al., 2015). Bacteria like Escherichia coli and Salmonella sp. can be recognised as high-risk sources of food contamination using MAR indexing (Khan et al., 2015). Indices are bigger than 0.2 if an isolate comes from a source where antibiotics are used extensively and/or in substantial concentrations (Shubra et al., 2014; Mthembu et al., 2019).

Cochlospermum tinctorium plant extract, however, exhibits good inhibition of the test bacterial isolates. At a greater dose of 500 mg/mL, the inhibition activity was found to fall between 24.00 and 19.00 mm, which is quite

near to the inhibition zone of 19 to 22 mm for the control antibiotic (Table 5). Statistically, it shows no significant difference in the mean zone of inhibition of the plant extract against the four bacterial isolates (F:0.22, F-crit: 3.24, P-value: 0.881, P>0.05). Pseudomonas aeruginosa and Staphylococcus aureus are among the pathogens that the plant extracts are effective against, according to Alain et al. (2014). The plant's metabolites would be connected to the antibacterial activity that was seen. It is true that triterpenes and flavonoids have anti-inflammatory (Özçelik et al., 2011), anti-ulcer (Madivoli et al., 2018), and antibacterial (Ayeni, 2018) qualities. The reason for high antibacterial activity on S. aureus could be due to the fact that S. aureus are gram-positive bacteria with an ineffective permeability barrier in their outer peptidoglycan layer may be the cause of their strong antibacterial activity. Nonetheless, the plant's great activity may be explained by the existence of several distinct bioactive chemicals, each of which acted differently and inhibited the growth of bacteria. Although the exact mechanisms of action of plant elements are still unknown, it is evident that the type of solvent utilised has a significant impact on the extracts' efficacy (Jodi and Sani, 2022). It is evident from this observation that the plant extracts' bactericidal and bacteriostatic properties are a result of the non-polar residue they contain (Antarasen and AmlaBatra, 2012). This was closely related to the discovery made by Tijjani et al. (2009) that Cochlospermum tinctorium root powder had substantial antibacterial activity against Escherichia coli (14.30 mm) and Staphylococcus aureus (19.00 mm) at 2000 µg/ml)

The isolates; *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* showed MBC at 125 mg/mL while *Salmonella* sp showed MBC at 62.5 mg/mL. We found that the MIC values in our investigation were less than the MBC values. This suggests that while the plant extracts were bactericidal at higher concentrations, they were bacteriostatic at lower ones. According to earlier research by Okemo et al. and Etuk et al. (2009), organisms would be killed more quickly at larger concentrations of plant extract.

CONCLUSION

The antibiotic-resistant bacterial isolates, *Salmonella* sp., *Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus aureus,* were all susceptible to the antibacterial action of the *Cochlospermum tinctorium* root extract. The plant extract inhibited both the gram-positive and gram-negative bacteria of the test organisms. The plant extract has a wide range of activities. Additionally, none of the test isolates seemed to be totally resistant to the extracts. This may be due to the plant bioactive compounds. Taking into account the imminent and present state of infections' conventional drug resistance, *Cochlospermum tinctorium* appears to be a promising alternative antibacterial agent which have demonstrated a high level of antibacterial activity against *S. aureus, E. coli, Pseudomonas aeruginosa* and *Salmonella spp*. Further investigations should be carried to determine the lead bioactive compound responsible for the antibacterial activity of *Cochlospermum tinctorium* and its toxicological effect in biological organisms.

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