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Research Article

Phytochemical potential and antibacterial activity of a traditional herbal tea on the *in vitro* growth of multidrug-resistant avian *E. coli* strains in the Korhogo region

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Abstract : Antibiotic resistance is a global public health problem affecting both humans and animals. In poultry farming, the excessive and inappropriate use of antibiotics has led to the emergence of multi-resistant bacteria such as *E. coli*. In this context, the exploration of effective, and accessible therapeutic alternatives, particularly phytomedicines, is essential. This study aims to evaluate the effect of a traditional herbal tea on the *in vitro* growth of avian strains through phytochemical and antibacterial analysis in the Korhogo region. The phytochemical screening of the aqueous herbal tea extract was performed using colorimetric and precipitation tests in tubes. The susceptibility of avian *E. coli* strains to the extract and their antibacterial parameters (MIC and MBC) were determined using the punched-well method in Mueller-Hinton agar and the liquid dilution method, respectively. Phytochemical analysis allowed for the detection of pharmacological and antimicrobial properties. The antimicrobial tests

showed that all *E. coli* strains were susceptible to the herbal tea extract, with inhibition zone diameters ranging from 10.5 ± 0.70 mm to 14.5 ± 0.70 mm. The MIC and MBC ranged from 12.5 to 25 mg/ mL and 12.5 to 100 mg/ mL, respectively, with the lowest values for the reference strain. Therefore, the herbal tea possessed bactericidal properties against all strains of *E. coli*. except for two strains where the effect was bacteriostatic. Ultimately, herbal tea with its antibacterial properties could be a promising therapeutic alternative to antibiotics for poultry farmers to combat avian colibacillosis.

Key words: Antibiotic resistance, Avian *E. coli*, Herbal tea, Antibacterial properties

I.INTRODUCTION

In Côte d'Ivoire, the poultry sector is experiencing rapid growth, driven by increasing demand for animal protein. In 2024, the Ivorian poultry sector generated an estimated turnover of 380 billion CFA francs, representing one of the main components of the livestock sub-sector ^[1]. Local production meets nearly 96% of national demand, significantly limiting imports ^[2]. Livestock farming as a whole contributes approximately 2.5% to the national GDP, and poultry plays a strategic role ^[3]. It therefore constitutes a lever for professional integration, particularly for young people and women. However, the sector faces several major challenges, including avian diseases (Newcastle disease, avian influenza) which cause significant losses ^[4], while the high cost of feed (70% of expenses) reduces profitability ^[5]. Furthermore, there is a lack of technical training (only 30% of farmers apply best practices) ^[6] and limited access to credit (less than 20% of poultry farmers financed) ^[7] exacerbate the situation.

Furthermore, all these difficulties contribute to poor rearing conditions, exposing poultry to various diseases (parasitic and bacterial). Among the bacteria of major concern, *Escherichia coli* occupies a central place due to its ability to colonize various hosts, cause enteric and extra-intestinal infections, and, above all, develop antibiotic resistance mechanisms ^[8]. In the poultry sector, avian strains of *E. coli*, often grouped under the term Avian Pathogenic *Escherichia coli* (APEC) is one of the most problematic pathogens, responsible for colibacillosis leading to significant economic losses ^[9] due to increased mortality, and a public health risk due to the potential transfer of resistance genes to human bacteria ^[10]. Faced with this growing threat, the search for new therapeutic alternatives that are safe, effective, and accessible has become a priority.

Traditional pharmacopoeia plays an important role in this quest because it offers a variety of medicinal plants used to treat various human and animal diseases. Indeed, numerous ethnobotanical, phytochemical and antimicrobial studies have been conducted on herbal teas and decoctions ^[11, 12,13] in most developing countries and particularly in Côte d'Ivoire with the aim of finding new active ingredients with a broad spectrum of biological activity against these multi-resistant germs ^[14, 15, 16, 17]. However, despite their frequent use, scientific data validating their phytochemical and antibacterial potential, particularly against multidrug-resistant avian strains, remain limited. Therefore, the present study rightly aims to evaluate the phytochemical potential and antibacterial activity of a traditional herbal tea (produced and marketed under the name “Ma pensée est finie” commonly known as “Edjanoukôko” in Côte d'Ivoire) on the *in vitro* growth of multidrug-resistant avian *E. coli* strains in the Korhogo region.

2.MATERIALS AND METHODS

2.1Material

2.1.1. Plant material: The plant material was constituted a brown herbal tea sold in Abidjan, Abengourou, Soubré, Bouaké, Bondoukou, Korhogo, etc., by a traditional healer for its anti-

hemorrhoidal, antitussive, energizing, analgesic, aphrodisiac, antimicrobial, etc. properties. According to the manufacturer, this herbal tea is an aqueous decoction obtained from the barks of medicinal plants with multiple therapeutic virtues, combined with spices such as ginger (*Zingiber officinale*), peanut (*Arachis hypogaea*), African long pepper (*Xylopia aethiopica*; *annonaceae*) and traditional potash. For reasons of professional secrecy, the scientific names of the medicinal plants used by the manufacturer could not be obtained. The herbal tea is shown in **Figure 1**.



Figure 1: Photo of the herbal tea

2.1.2. Bacterial strains: In this study, fifteen (15) resistant strains of avian *Escherichia coli* and one reference strain (ATCC-25922) were used. The *E. coli* strains and the reference strain were provided by the National Laboratory for Agricultural Development Support (LANADA) in Korhogo and the Pasteur Institute of Côte d'Ivoire, respectively. These strains originated from farms in the following localities: Tioro, Résidentiel, Makalé, Kafoule, Kafigué, Karakoro, Lakpolo, Quartier 14, and Mbengué. They were stored at -20°C in the LANADA bacteriology unit for microbiological testing. **Table I** presents all the microbiological characteristics of the different *E. coli* strains tested and their antibiograms.

Table 1: Microbiological characteristics of the different *E. coli* strains tested and their antibiograms.

Escherichia coli strains	Registration date of E. coli	Antibiotics (EUCAST,2024)			
		Resistant	Dosage	Sensitive	Dosage
1	16/05/2023	Erythromycin	15 µg	Ceftriaxone	30 µg
2	27/06/2023	Tetracycline	30 µg	Cefoxitin	30 µg
3	13/08/2023	Colistin	10 µg	gentamicin	10 µg
4	20/10/2023	Amoxicillin+ clavulanic	20/10 µg		
5	22/11/2023	(AMC)	30 µg		
6	19/02/2024	Oxytetracycline	10 µg		
7	08/04/2024	Streptomycin	30 µg		
8	02/05/2024	Doxycycline			
9	22/05/2024				
10	22/05/2024				
11	22/05/2024				

12	28/06/2024				
13	14/06/2024				
14	21/06/2024				
15	04/07 :2024				
Culture medium	Chromogenic	Chromogenic		Chromogenic	

NB: The same resistant and sensitive antibiotics were repeated for the other fourteen (14) strains and the same dosages were also used.

2.2. Methods:

2.2.1. Preparation of the dry extract of the herbal tea : The method described by ^[18], with some modifications, was used for the preparation of the dry extract of the herbal tea. Thus, 500 mL of the herbal tea was first squeezed through a white cloth to remove any impurities. Then, after double filtration through absorbent cotton and once through Whatman paper (3 mm), the resulting filtrate was—The concentrate was placed in an oven until all the water had evaporated. The resulting dry extract was weighed and stored in a sterile vial for various phytochemical and antibacterial tests.

2.2.2. Phytochemical screening of the herbal tea:The phytochemical screening of the aqueous extract of the herbal tea was carried out using colorimetric and precipitation tests in tubes, as described by ^[19]. The chemical groups sought were: alkaloids, saponins, flavonoids, polyphenols, sterols, catecholic and gallic tannins, anthocyanins, and leucoanthocyanins.

2.2.3. Assay of phenolic compounds in herbal tea: The total polyphenol, total flavonoid, and total tannin content of the herbal tea were determined according to the methods described by ^[20, 21, 22] respectively, with a slight modification. Gallic acid, quercetin, and tannic acid are used as standards for quantifying total polyphenol, flavonoid, and tannin content, respectively. Results are expressed in micrograms of gallic acid, quercetin, or tannic acid equivalent per gram of dry extract ($\mu\text{g EAG/g}$ dry extract). The tests were performed in triplicate.

2.2.4. Evaluation of the antibacterial activity of the aqueous extract of the herbal tea:

2.2.4.1. Preparation of the bacterial inoculum: The inoculum of each bacterial strain was prepared by homogenizing two young colonies aged 18 to 24 hours in 10 mL of Mueller-Hinton broth and then incubating them for 3 hours at 37 °C. Following incubation, 0.1 mL of the broth was added to 10 mL of sterile distilled water to obtain an inoculum estimated at 5×10^6 CFU / mL with a turbidity of 0.5 MacFarland.

2.2.4.2. Determination of the sensitivity of bacterial strains to the herbal tea extract: The punched-out well method in agar A Petri dish was used to test the sensitivity of the strains to the dry extract of the herbal tea. Mueller-Hinton agar was first flood-inoculated with the previously prepared inoculum. After aspirating the excess liquid using a sterile Pasteur pipette and drying in an oven for 15 to 30 minutes at 37°C, 6 mm diameter wells were made in the agar. These wells, separated by at least 20 mm, were filled with 80 μL of the dry extract of the herbal tea at 200 mg/ mL ^[23]. Concurrently, gentamicin was used as the standard positive control antibiotic. After 45 min of pre-diffusion, the mixture was incubated at 37°C for 18 hours. The effect of the phytomedicine on the studied strain was assessed by measuring the

diameter of the growth inhibition zone around the well [24]. This test was performed in duplicate for the extract and gentamicin, and their activity was evaluated according to the [24].

2.2.4.3. Determination of the antibacterial parameters of the herbal tea extract:

2.2.4.3.1. Preparation of the concentration range of the herbal tea extract: A range of concentrations of the aqueous extract of the herbal tea, ranging from 200 to 1,562 mg/ mL. The solution was prepared using the double dilution method in test tubes [25]. One hundred and ten milligrams of fine powder of the herbal extract were mixed with 5 mL of distilled water to create an initial concentration of C1 = 200 mg/ mL. Then, 5 mL of this solution were added to 5 mL of distilled water to obtain a concentration of C2 = 100 mg/ mL. Using this technique, the following concentrations were prepared: C3 = 50 mg/ mL; C4 = 25 mg/ mL; C5 = 12.5 mg/ mL; C6 = 6.25 mg/ mL; C7 = 3.125 mg/ mL; C8 = 1.562 mg/ mL. The contents of the prepared tubes were sterilized at 121°C for 15 minutes in an autoclave.

2.2.4.3.2. Determination of the minimum inhibitory concentration of the herbal tea extract: The minimum inhibitory concentration (MIC) was determined by adding 1 mL of each strain's inoculum to 1 mL of the dry extract of the herbal tea in eight (8) hemolysis tubes. Two (2) additional tubes were used: one containing only 1 mL of the aqueous extract (growth control) and the other containing 1 mL of the inoculum (sterility control). After homogenization, the tubes were incubated at 37°C for 18 to 24 hours. After incubation, observation of the tubes revealed the MIC corresponding to the lowest concentration that did not result in visible growth of the tested bacteria. This is indicated by the absence of cloudiness in the tube(s) in question.

2.2.4.3.3. Determination of the minimum bactericidal concentration of the herbal tea extract: To determine the minimum bactericidal concentration (CMB), two Petri dishes, A and B, each containing Mueller-Hinton agar, were used. Dish A was streaked 5 cm apart with 0.1 mL of the contents of each tube at a concentration greater than or equal to the MIC, using a sterile calibrated loop. Simultaneously, dilutions from the stock suspension (10^{-4}) were prepared up to a 10^{-4} dilution. These dilutions and the stock suspension were then streaked into dish B. Both dishes were then incubated at 37 °C for 18 to 24 hours. The MBC was obtained by comparing the different colonies in plate A to those of the 10^{-4} dilution in Petri dish B. The MBC corresponds to the concentration of the phytomedicinal extract with a number of colonies in plate A less than or equal to that of the 10^{-4} dilution in plate B. This MBC is the lowest concentration that allows at most 0.01% of the bacteria from the initial suspension to survive for 24 hours. Finally, the MBC/MIC ratio was calculated to determine the antibacterial potency of the extract [26].

2.3. Statistical analysis:

The results were analyzed using GraphPad Prism 8.0 software (Microsoft USA). Differences between means were determined using variance analysis with multiple ANOVA coupled with Tukey 's test at a significance level of 5% ($p < 0.05$ was considered significant). The results are expressed as means with the standard error.

3. RESULTS AND DISCUSSION

III.1-Results

III.1.1-Phytochemical profile of the herbal tea

Qualitative phytochemical tests performed on the aqueous extract of the herbal tea showed a high presence of secondary metabolites. In particular, the extract contains flavonoids, total polyphenols, leucoanthocyanins, anthocyanins, and catechins. Conversely, the phytochemical tests revealed the absence of alkaloids, gallic tannins, terpenes, sterols, and saponins in this extract. These results are recorded in **Table 2**.

Table 2: Phytochemical composition of the herbal tea

Phytochemical compounds detected	Herbal tea
Polyphenols	+
Flavonoids	+
Alkaloids	-
Saponins	-
Catechetical tannins	+
Gallic tannins	-
Anthocyanins	+
Terpenes and sterols	-
Leucoanthocyanes	+
Quinones	-

+: Presence; -: Absence

3.1.2. Phenolic compound content of the herbal tea: The dry extract of the herbal tea yielded total polyphenol, flavonoid and tannin contents of 63.33 ± 0.23 mg EAG/g; 212.66 ± 0.21 mg EQ/g and 18.26 ± 0.31 mg EAT/g of dry extract, respectively (**Figure 2**). The highest content was measured in the hydroalcoholic extract.

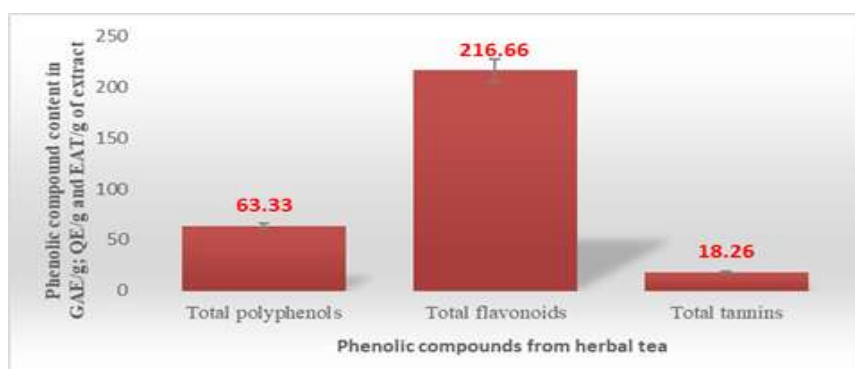


Figure 2: Phenolic compound content of the dry extract of the herbal tea

3.1.2. Sensitivity of strains to the herbal tea extract: *E. coli* strains to the herbal tea extract and to gentamicin was determined using the diameters of the inhibition zones presented in **Table 3** and **Figure 2**. The results showed variation in the inhibition zone for all strains with both the herbal tea extract and the reference antibiotic. The diameters of the inhibition zones ranged from 10.5 ± 0.70 mm to 14.5 ± 0.70 mm, and from 25.5 ± 0.70 mm to 29.5 ± 0.70 mm, respectively. However, gentamicin proved more effective against the tested reference strain. The herbal tea extract was active against all strains, as its inhibition zone was greater than or equal to 10 mm.

3.1.3. Determination of the antibacterial parameters of the aqueous extract of the herbal tea: The antibacterial properties of the herbal tea extract against different strains were evaluated by determining the antimicrobial parameters (MIC and MBC) and are illustrated in **Figure 3** and **Table 4**. The MIC observed for all *E. coli* strains ranged from 12.5 to 25 mg/ mL. The MBC fluctuated between 12.5 and 100 mg/ mL. The reference *E. coli* strain, with an MBC of 12.5 mg/ mL, exhibited the lowest MBC value. The susceptibility testing of the *E. coli* strains revealed that the herbal tea extract demonstrated bactericidal activity against all strains except strains 5 and 7, which showed a bacteriostatic effect.

Table 3: Diameter of the inhibition zones of *E. coli* strains by the aqueous extract of the herbal tea

Bacterial strains	Aqueous extract (100 mg/ mL)	Reference antibiotic (Gentamicin, 30)
	Inhibition diameters (mm)	
<i>E. coli</i> 1	14.50 ± 0.70 ^b	27.50 ± 0.70 ^{abc}
<i>E. coli</i> 2	14.50 ± 0.70 ^b	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 3	14.50 ± 0.70 ^b	25.50 ± 0.70 ^a
<i>E. coli</i> 4	11.50 ± 0.70 ^{ab}	25.50 ± 0.70 ^a
<i>E. coli</i> 5	10.50 ± 0.70 ^a	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 6	12.50 ± 0.70 ^{ab}	28.50 ± 0.70 ^{bc}
<i>E. coli</i> 7	10.50 ± 0.70 ^a	25.50 ± 0.70 ^a
<i>E. coli</i> 8	14.50 ± 0.70 ^b	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 9	14.50 ± 0.70 ^b	27.50 ± 0.70 ^{abc}
<i>E. coli</i> 10	14.50 ± 0.70 ^b	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 11	11.50 ± 0.70 ^{ab}	25.50 ± 0.70 ^a
<i>E. coli</i> 12	12.50 ± 0.70 ^{ab}	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 13	14.50 ± 0.70 ^b	26.50 ± 0.70 ^{ab}
<i>E. coli</i> 14	14.50 ± 0.70 ^b	27.50 ± 0.70 ^{abc}
<i>E. coli</i> 15	13.00 ± 0.70 ^{ab}	28.50 ± 0.70 ^{bc}
<i>E. coli</i> ATCC 25922	11.50 ± 0.70 ^{ab}	29.50 ± 0.70 ^c

NB: There no significant difference between values in the same column that have the same letter as their superscript.

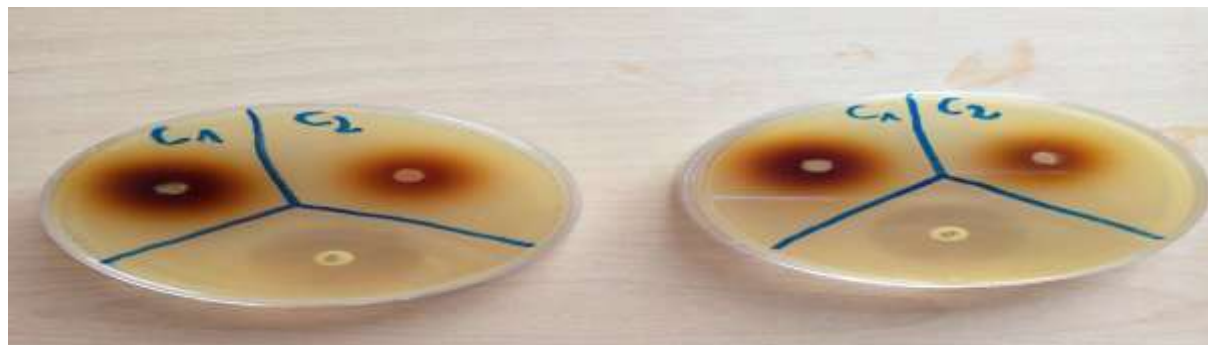


Figure 2: Petri dishes containing the diameters of the zones of inhibition of the strains by the aqueous extract of the herbal tea.

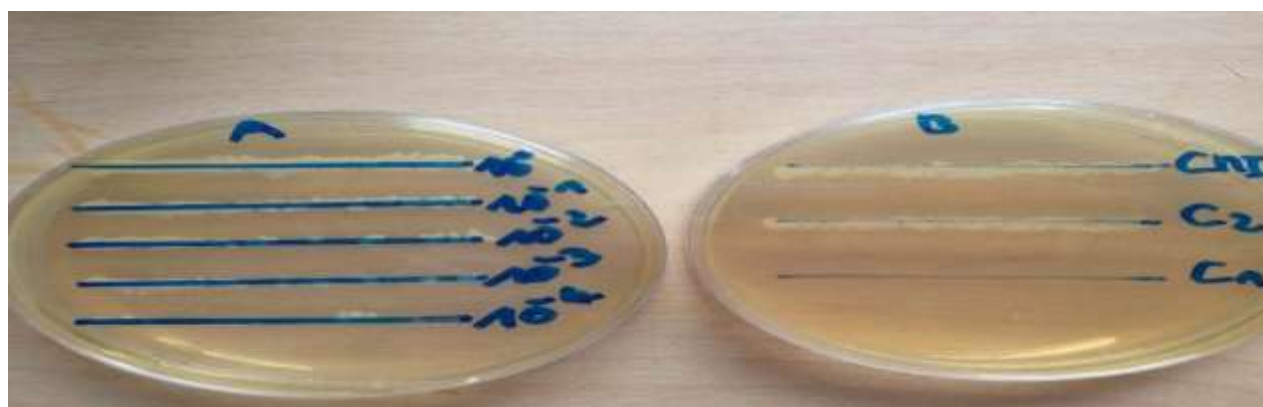


Figure 3: Comparison of boxes A and B for determining the MBC

Table 4: Antibacterial parameters of the aqueous extract of herbal tea

Settings Germs	MIC (mg/ mL)	MBC (mg/ mL)	Antibacterial power (MBC/MIC)
<i>E. coli</i> 1	12.5	12.5	1
<i>E. coli</i> 2	12.5	50	4
<i>E. coli</i> 3	12.5	50	4
<i>E. coli</i> 4	12.5	50	4
<i>E. coli</i> 5	12.5	100	8
<i>E. coli</i> 6	12.5	50	4
<i>E. coli</i> 7	1,562	25	16
<i>E. coli</i> 8	25	100	4
<i>E. coli</i> 9	12.5	50	4
<i>E. coli</i> 10	12.5	50	4
<i>E. coli</i> 11	12.5	50	4
<i>E. coli</i> 12	12.5	50	4
<i>E. coli</i> 13	12.5	50	4

<i>E. coli</i> 14	12.5	50	4
<i>E. coli</i> 15	25	25	1
<i>E. coli</i> ATCC 25922	12.5	12.5	1

3.2. Discussion

The overall objective of this research was to contribute to the fight against resistance in avian *E. coli* strains among poultry farmers in the Korhogo region through the phytochemical and antibacterial evaluation of a local traditional herbal tea. on the in vitro growth of these strains. First, phytochemical sorting of the dry extract of the herbal tea revealed that the extract contains flavonoids, total polyphenols, leucoanthocyanins, anthocyanins, and catechins, representing secondary metabolites produced in plants. Most of these secondary metabolites are phenolic compounds (flavonoids, polyphenols, and tannins) with antioxidant properties, and therefore capable of combating oxidative stress, which is the cause of several infections in both humans and animals, particularly poultry. ^[27] Their presence could justify, on the one hand, their involvement in the biological, pharmacological, and therapeutic activities of the herbal tea, and on the other hand, its use in traditional medicine. ^[28, 29] However, the absence of other chemical groups could be explained not only by the extraction solvent but also by the botanical and geographical origin of the medicinal plants used to prepare the herbal tea. Indeed, only polar compounds are extracted by the water solvent, just as herbal tea is an aqueous decoction, hence the absence of terpenes ^[30]. These results are consistent with those of ^[31], who detected the same compounds in an antidiabetic herbal medicine sold in Korhogo.

In terms of antibacterial tests, the sensitivity of the different *E. coli* strains tested to the herbal tea extract revealed that the diameters of the inhibition zones varied between 10.5 ± 0.70 mm and 14.5 ± 0.70 mm. These values suggest that all the tested strains were sensitive to the herbal tea extract, in accordance with the work of ^[23]. Indeed, according to these authors, a bacterial strain is considered sensitive to a phytomedicine when the diameter of the generated inhibition zone is equal to or greater than 10 mm. However, the effectiveness of the herbal tea appears to be slightly variable from one strain to another. This variation could be due to differences in the biological characteristics of each strain, such as their ability to resist the antibacterial compounds present in the extract, namely the chemical compounds highlighted in the phytochemical analysis. In comparison, the reference antibiotic, gentamicin, generated inhibition zones ranging from 25.5 ± 0.70 mm to 29.5 ± 0.70 mm, also indicating a general sensitivity of *E. coli* strains. However, the effectiveness of gentamicin was particularly pronounced on the reference strain, where the inhibition diameters were the largest. This confirms the effectiveness of this antibiotic compared to the herbal tea extract, although the latter also exhibits quite interesting antimicrobial potential. These results are consistent with previous work. from several researchers. Indeed, according to ^[32], a bacterial strain is considered sensitive to gentamicin when the diameter of the inhibition zone is equal to or greater than 18 mm. Similarly, according to the study by ^[33], a strain is considered sensitive when the inhibition diameter reaches or exceeds 15 mm.

Regarding the antibacterial parameters MIC and MBC, it should be noted that the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent capable of inhibiting bacterial growth, while the minimum bactericidal concentration (MBC) is the concentration required to kill the bacteria. Plant extracts, such as herbal teas, can have antimicrobial effects, but their effectiveness varies depending on the plant, the bacterial strain, and the concentration of the extract. Studies, such as that of ^[34] showed that these extracts can act with bactericidal or bacteriostatic activity, depending on these factors. The antimicrobial parameters obtained during this study confirmed these observations. Indeed, for *E. coli*, the MIC varied between 12.5 and 25 mg/ mL and the MBC between 12.5 and 100 mg/ mL ,

with a reference strain having an MBC of 12.5 mg/ mL . All strains exhibited bactericidal sensitivity, while the other (5 and 7) strains showed bacteriostatic sensitivity. These data are consistent with those of [26], who indicates that an MBC/MIC ratio less than or equal to 4 indicates bactericidal activity, and greater than 4, bacteriostatic activity. This distinction is crucial for the development of natural antimicrobials. The results obtained are consistent with the work of [35, 36, 37] on tea extracts (*Camellia sinensis*) used as an herbal tea. Indeed, these authors showed that certain phenolic compounds exert a significant bacteriostatic effect against *E. coli* (growth inhibition) and can, at sufficient doses, be bactericidal. Their mechanisms include membrane disruption, enzyme inhibition (including DNA gyrase), and interference with bacterial virulence systems.

Furthermore, the effectiveness of extracts also depends on the extraction method and the bioactive compounds present, such as flavonoids, tannins and alkaloids, but also on extrinsic factors such as climate and harvest time [38, 39, 40, 41,42]. These findings corroborate the results of recent work and highlight the complexity of the antimicrobial efficacy of plant extracts. However, it is interesting to note that although the herbal tea extract induced smaller zones of inhibition than gentamicin, it remains promising as an alternative or complement to traditional antimicrobial treatments, particularly in a context where antibiotic resistance is increasingly concerning. This type of result underscores the importance of continuing research on the use of medicinal plants, which could offer natural solutions for combating bacterial infections, especially in regions where access to antibiotics is limited and resistance is a major problem.

CONCLUSION

This work has highlighted the importance of local phytotherapeutic resources in combating antibiotic resistance in avian *E. coli* strains (*responsible for colibacillosis*) among livestock farmers in the Korhogo region. Phytochemical analysis of the studied herbal tea revealed a richness in bioactive compounds such as total polyphenols, flavonoids, anthocyanins, leucoanthocyanins, and tannins, known for their antimicrobial properties. Test results confirmed the herbal tea's effectiveness against *Escherichia coli* strains, although slightly less than that of gentamicin. However, this potential opens the way to using this herbal tea as an alternative or complement to conventional antibiotics. By promoting traditional medicine and encouraging the responsible use of medicinal plants, this study contributes to animal health, the preservation of local knowledge, and the fight against antibiotic resistance. Furthermore, the studies could not only be extended to other pathogenic strains and confirmed by *in vivo* tests but also contribute to the promotion of the use of local phytotherapeutic resources in regions with limited access to antibiotics, for the control of bacterial infections in poultry farming.

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