Original Research Article

Antibacterial Activities of Aqueous and Hydroethanolic Extracts of *Anogeissus leiocarpus* on the *In Vitro* Growth of two Multiresistant Strains of *Salmonella typhimurium* Isolated from Broilers Chickens

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ABSTRACT

Poultry products have quickly become one of the most consumed sources of protein in the world. However, poultry farmers face enormous economic losses caused by the emergence of multi-resistant bacteria. In addition, the consumption of eggs and poultry meat also causes several infectious diseases within populations as well as poisonings and therefore a public health problem.

Aims: The objective of our study was to evaluate *in vitro* the antibacterial properties of aqueous and 70% hydroethanolic extracts of the leaves of *Anogeissus leiocarpus* on two strains of multiresistant *Salmonella typhimurium* isolated from the faeces of broilers chickens.

Methodology: The agar well method was used to test the susceptibility of bacterial strains while the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the liquid dilution method.

Results: On the two multiresistant *Salmonella typhymurium* germs, the aqueous extract of *Anogeissus leiocarpus* gave zones of inhibition diameter of 13 and 19 mm against 16 and 22 mm for the 70% hydroethanolic extract. Faced with the two strains of *Salmonella typhymurium*, the aqueous and 70% hydroethanolic extracts recorded minimum bactericidal concentrations (MBC) which were 25 mg/mL and 12.5 mg/mL. The results of the phytochemical analysis of the extracts studied reveal the presence of several families of secondary metabolites including phenolic compounds, cardiac glycosides, saponins, alkaloids and then sterols and terpenes. However, these secondary metabolites are distributed differently within the aqueous and 70% hydroethanolic extracts.

Conclusion: In view of the results obtained in the present work, this plant could be used as a phytomedicine to combat pathologies linked to *Salmonella typhymurium* in broiler chickens and as an antibacterial agent in food products that may harbor strains of *Salmonella typhymurium*.

Keywords : Anogeissus leiocarpus, Extracts, Salmonella typhymurium, Multiresistant

1. INTRODUCTION

Poultry products have rapidly become one of the most consumed sources of protein in the world, making them very important for the global food supply [1]. It should represent about half of meat production in the next ten years. This growth will be mainly due to developing countries, which will contribute about 73% of the additional production [2]. Indeed, the increase in poultry production is particularly visible in developing countries, due to the status of poultry as cheap meat [3]. To this, it should be added that poultry farming, which was of the traditional or semi-industrial type, has rapidly evolved with the installation of numerous industrial farms following the establishment of public supervisory structures. However, the spectacular development of poultry farming is not without difficulties. Indeed, most breeders are not professionals and do not necessarily master the application of the basic rules of hygiene in poultry farms. This has favored the emergence of many diseases on these farms. These diseases decimate farms and cause considerable economic losses for breeders. In addition to the economic losses generated among poultry farmers, the consumption of eggs and poultry meat causes several infectious diseases within populations, thus creating poisonings and therefore a public health problem. These pathologies are of all kinds: parasitic [4], viral [5], fungal [6] and especially bacterial [7]. Among bacterial diseases, salmonellosis is one of the most damaging diseases in poultry farming [7]. Therefore, antibiotic therapy based on an adequate diagnosis as well as prophylaxis, still remain the only means of combating this disease. However, these sanitary practices encounter many difficulties. In fact, in recent years, there has been an increasing appearance of antimicrobial resistance in salmonellosis [8]. Faced with these worrying therapeutic failures, the use of phytomedicines offers itself as a solution to be explored. *Anogeissus leiocarpus* is an important evergreen tree of the family Combretaceae, widely distributed in Africa [9,10] and is well known in African traditional medicine to treat skin sore, alopecia, headache, bronchitis, diarrhea, bruises, dysmenorrheal, constipation, dysentery, fever, cataract, anti-cold, inflammation, asthma, tumor, liver diseases and venereal diseases [11]. Extracts from this plant have shown antibacterial activities against several strains [12,13,14]. It is in this perspective that we evaluated the therapeutic effects of aqueous and hydroethanolic extracts of the leaves of *Anogeissus leiocarpus*, on two strains of multiresistant *Salmonella typhimurium* of avian origin.

2. MATERIAL AND METHODS

2.1 Plant material

It consists of leaves of *Anogeissus leiocarpus*. They were collected in the village of Lataha in the Korhogo region (North of Côte d'Ivoire) in March 2022 and authenticated by the National Floristic Center of the Felix HOUPHOUËT-BOIGNY University of Cocody-Abidjan.

2.2 Bacterial strains

Two bacterial strains of *Salmonella typhimurium* of avian origin were the subject of this study. They were provided by the Microbiology Unit of the Biotechnology Laboratory, of the UFR Biosciences of the Félix Houphouët-Boigny University of Côte d'Ivoire (Table 1).

Table 1. Origin and antibiotic profiles of the *Salmonella typhimurium* strains studied

Salmonella strains tested	Origin	Antibiotic profiles
Salmonella	broilers chickens	AMP-CHL-STR-SUL-TE-AUG-CTX-CIP-NA

typhimurium N°1	faeces	
Salmonella typhimurium N°2	broilers chickens faeces	AMP-CHL-STP-SUL-TE

AMP : ampicillin, CHL : chloramphénicol, STR : streptomycin, SUL : sulfamides, TE : tétracyclin, AUG : amoxicillin- clavulanic acid, CTX : cefotaxim, CIP : ciprofloxacin, NA : nalidixic acid

2.3 Preparation of aqueous and hydoethanolic extracts of *Anogeissus leiocarpus*

The leaves of *Anogeissus leiocarpus* were washed, cut and dried in the shade for two weeks. Once dried, these plant elements were ground. Thus, 100 g of this powder were diluted in 1 liter of distilled water. The mixture was homogenized at ambient laboratory temperature using a magnetic stirrer for 24 hours. The homogenate obtained was successively filtered twice on cotton wool and then once on Whatman paper (3 mm). The volume of the filtrate obtained is reduced using a Med Center Venticell type oven at 50°C to give a powder which constitutes the total aqueous extract [15]. The same operation was carried out using 70% ethanol instead of distilled water, to obtain the 70% hydroethanolic extract [16]. The two plant extracts obtained were stored in the refrigerator for the rest of the tests.

2.4 Preparation of the bacterial suspension

The bacterial inoculum was prepared by homogenizing two (2) colonies of each *Salmonella Typhimurium* Multi-R in 10 mL of Mueller-Hinton broth. After an incubation of approximately 3 hours at 37°C, 0.1 mL of the broth is added to 10 mL of sterile Mueller-Hinton broth to constitute the inoculum at 10^6 bacteria/mL with a turbidity of 0.5 Mac Farland [15].

2.5 Antibacterial susceptibility screening of aqueous and hydroethanolic extracts

This test is based on the determination of growth inhibition zones. The method used was that of punch wells on Mueller-Hinton agar. Thus, as in the classic production of an antibiogram, the agar is inoculated by flooding (inoculum at 10^6 bacteria/mL). Then we make wells 6 mm in diameter. Then each well was filled with 80 µL of plant extract at a concentration of 200 mg/mL, taking care to separate two holes of at least 20 mm [17,18]. After a pre-diffusion of 45 minutes at room temperature under the hood, the whole was incubated in an oven at 37°C for 24 hours. After incubation, the action of each extract was assessed by measuring a growth inhibition zone (absence of colonies) around the well.

2.6 Determination of minimum inhibitory and bactericidal concentrations of aqueous and hydroethanolic extracts

First, a range of concentrations of each plant extract ranging from 25 to 0.39 mg/mL was prepared by the double dilution method in eight (8) test tubes. Then, 1 mL of each concentration of plant extract prepared is mixed with 9 mL of the bacterial inoculum whose bacterial load is estimated at 10^6 bacteria/mL. The Minimum Inhibitory Concentration (MIC) of the plant extract corresponds to the concentration of the first tube in which there is no growth visible to the naked eye of the bacteria tested after incubation for 24 hours at 37°C. To determine the Minimum Bactericidal Concentrations (MBC), the surface of a new Mueller-Hinton agar poured into a Petri dish was inoculated with 0.1 mL of the contents of the tubes having a concentration greater than or equal to the MIC using a calibrated loop. At the same time, double dilutions from the original suspension are carried out up to the 10^{-4} dilution. Then, these dilutions and the stock suspension are streaked onto Mueller-Hinton agar in another Petri dish. Seeding was done in parallel 5 cm streaks. All the plates containing the agars were incubated at 37°C for 24 hours. The MBC corresponds to the concentration of the extract presenting a number of colonies less than or equal to that of the 10^{-4} dilution. This MBC corresponds to the smallest concentration which allows at most 0.01% of the germs of the starting suspension to survive in 24 hours [15].

2.7 Phytochemical screening of to aqueous and hydroethanolic extracts

The phytochemical analysis of the different extracts of *Anogeissus leiocarpus* have been based on the coloration and precipitation tests [19]. To better estimate the quantity of chemical constituents in the extracts, scores ranging from 0-3 (absent or present) have been allocated. Thus, the absence was symbolized by a score of 0, the presence in small quantities by a score of 1, the presence in average quantity score 2 and finally, the abundance by a score of 3.

3. RESULTATS ET DISCUSSION

3.1 Antibacterial susceptibility screening of extracts

Table 2 presents the values of the diameters of the growth inhibition zones of *Salmonella typhimurium* germs tested with aqueous and 70% hydroethanolic extracts of *Anogeissus leiocarpus*. It appears that each of the extracts prepared has a well-defined activity on the growth of the bacterial strains studied. The inhibition diameters are between 13 and 22 mm. Thus, the aqueous extract gave an inhibition diameter of 13 mm on *Salmonella typhimurium* N^{o}1 and 19 mm on the strain of *Salmonella typhimurium* N^{o}2. As for the 70% hydroethanolic extract of *Anogeissus leiocarpus*, the inhibition diameters varied from 16 mm (*Salmonella typhimurium* N^{o}1) to 22 mm (*Salmonella typhimurium* N^{o}2). These first tests related to the determination of the zones of inhibition show the sensitivity of the bacteria studied to the plant extracts prepared because according to Biyiti *et al.* [20] an extract is judged to be active if it induces a zone of inhibition greater than or equal to 10 mm.
Similar studies were conducted by Prabhjot *et al.* [21] on aqueous and ethanolic extracts of *Tinospora cordifolia*, *Ziziphus mauritiana*, *Swertia chirata*, *Erythrina blakei* and *Asparagus densiflorus* to inhibit the growth of a strain of *Salmonella typhimurium*. The results of the work of these authors showed that the diameters of the zones of inhibition of the aqueous extracts of these plants on the *in vitro* growth of the strain studied varied between 8.7 and 10.3 mm while those of the ethanolic extracts were between 5.2 and 7.7 mm. Furthermore, Bahman *et al.* [22], in a study also showed that *Rhazya stricta* leaf extracts had varying effects on growth inhibition against eight (8) strains of *S. typhimurium*. Thus, among the plant extracts studied by these authors, the hydroalcoholic extract was the most effective in inhibiting the growth of strains of *S. typhimurium* with an average diameter of 12.25 mm. This extract was followed by the ethanolic extract with an inhibition diameter of 12.12 mm at different concentrations. As for Naphtali *et al.* [23], they showed that aqueous extracts at a concentration of 400 mg/mL from *Cymbopogan citratusmm*, *Psidium guajava* and *Anacadium occidental* recorded inhibition diameters of 11, 14 and 17 mm respectively. on a strain of *S. typhimurium*.

Table 2. Diameters of inhibition of extracts of *Anogeissus leiocarpus* on the strains of *Salmonella typhimurium* studied

Salmonella strains tested	Origin	Antibiotic profiles	Aqueous extract (200 mg/mL)	70% hydroethanolic extract (200 mg/mL)
Salmonella typhimurium N^{o}1	broilers chickens faeces	AMP-CHL-STR-SUL-TE-AUG-CTX-CIP-NA	13±1 mm	16±0.5 mm
Salmonella typhimurium N^{o}2	broilers chickens faeces	AMP-CHL-STP-SUL-TE	19±1 mm	22±0.5 mm

AMP: ampicillin, CHL : chloramphénicol, STR : streptomycin, SUL : sulfamides, TE : tétracyclin, AUG : amoxicillin- clavulanic acid, CTX : cefotaxim, CIP : ciprofloxacin, NA : nalidixic acid

3.2 Antibacterial parameters of extracts

The values of the antibacterial parameters (MIC and MBC) as well as those of the MBC/MIC ratios determined are reported in Table 3. On the strain of *Salmonella typhimurium* N^{o}1, the aqueous extract of *Anogeissus leiocarpus* recorded a minimum inhibitory concentration (MIC) which is equal to the minimum bactericidal concentration (MIC = MBC = 25 mg/mL). On this same strain of *Salmonella typhimurium* N^{o}1, the 70% hydroethanolic extract of *A. leiocarpus* gave a minimum bactericidal concentration of 25 mg/mL which was twice the minimum inhibitory concentration (12.5 mg/mL).
In the presence of the strain of *Salmonella typhimurium* N^{o}2, the MIC of the aqueous extract of *A. leiocarpus* was 12.5 mg/mL with also a MBC of 12.5 mg/mL. On the other hand, the 70% hydroethanolic extract of *A. leiocarpus* gave a minimum inhibitory concentration of 6.25 mg/mL and a MBC of 12.5 mg/mL on this germ of *Salmonella typhimurium* N^{o}2. However, the MBC/MIC ratio of each extract prepared with respect to the two strains of *Salmonella typhimurium* studied was equal to 1 or 2. These ratios thus concluded that the plant extracts prepared were bactericidal for the two *Salmonella typhimurium* germs which is the subject of study. Indeed, according to Fauchere and Avril [24], an extract is said to be bactericidal if the MBC/MIC ratio ≤ 2.

Table 3. Antibacterial parameters of the extracts of *Anogeissus leiocarpus* on the strains of *Salmonella typhimurium* studied

Salmonella strains tested	Antibiotic profiles	Extracts of *Anogeissus leiocarpus*	Antibacterial Parameters		Ratios MBC/MIC	Effect of the extract
			MIC (mg/mL)	MBC (mg/mL)		
Salmonella typhimurium N^{o}1	AMP-CHL-STR-SUL-TE-AUG-CTX-CIP-NA	Aqueux extract	25	25	1	bactericidal
		70% hydroéthanolic extract	12,5	25	2	bactericidal
Salmonella typhimurium N^{o}2	AMP-CHL-STP-SUL-TE	Aqueux	12,5	12,5	1	bactericidal
		70% hydroéthanolic extract	6,25	12,5	2	bactericidal

AMP : ampicillin, CHL : chloramphénicol, STR : streptomycin, SUL : sulfamides, TE : tétracyclin, AUG : amoxicillin- clavulanic acid, CTX : cefotaxim, CIP : ciprofloxacin, NA : nalidixic acid, MIC : Minimum inhibitory concentration, MBC : Minimum bactericidal concentration

Furthermore, we note from this study that the two aqueous and 70% hydroethanolic extracts gave MBCs of 25 mg/mL on *Salmonella typhimurium* N^{o}1 against MBCs of 12.5 mg/mL on *Salmonella typhimurium* N^{o}2 (Figure 1). These results could be explained by the intrinsic resistance presented by each strain of *Salmonella typhimurium*. These results confirm those of Bssaibis *et al.* [18] who proved that the antimicrobial activities are related to the origin of the extract and the strain tested as well as the nature of the solvent used.

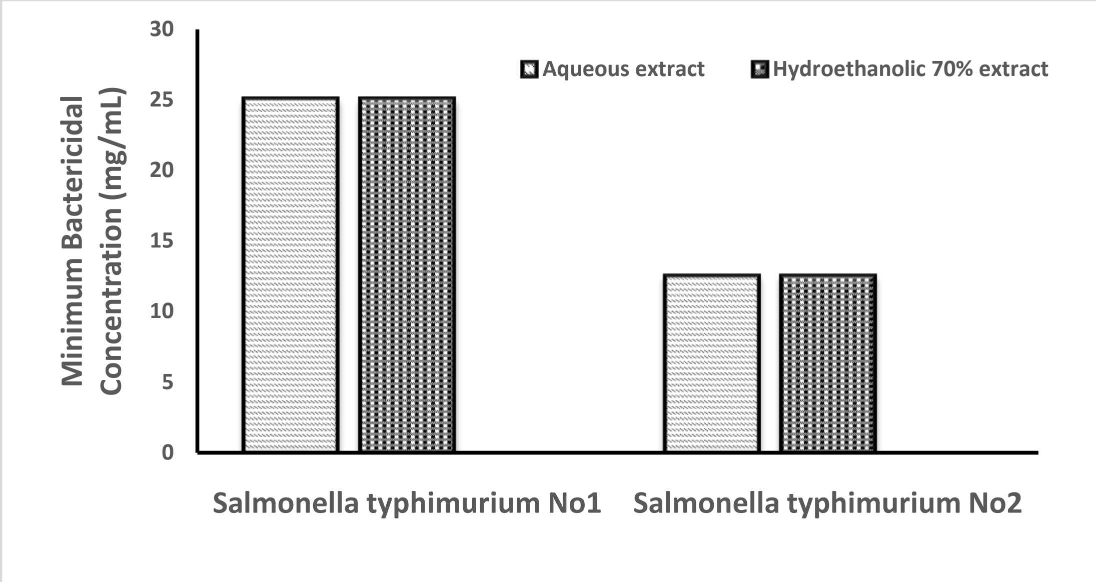

Fig.1. Comparated antibacterial effects of *Anogeissus leiocarpus* extracts.

Razieh *et al.* [25], in the evaluation of the antimicrobial activities of the hydroethanolic extract of *Satureja sahendica* against 20 isolates of *S. typhimurium* showed that the values of MIC and MBC ranged from 0.29 to 4.68 mg/mL and 75-150 mg/mL respectively.
A study was carried out by Dahiru *et al.* [26] on an ethanolic extract of *Anogeissus leiocarpus* obtained by Soxhlet extraction on three β-lacttamase-producing bacteria, namely *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. typhi*. Inhibition diameters ranging from 15.67 mm to 17.00 mm were obtained on bacteria tested during their study by the method of impregnated discs. These authors also showed that the minimum inhibitory concentration was between 66.67 mg/L and 16.67 mg/L while the minimum bactericidal and bacteriostatic concentrations were between 266.67 mg/L and 106.67 mg/L and 266.67 mg/L to 80.00 mg/L respectively. In their study, the MBC/MIC ratio showed a bacteriostatic effect for *E. coli* and *S. typhi* and bactericidal for *K. pneumoniae* and *P. aeruginosa*. These results further show that the antibacterial activity of a plant extract depends on several factors. These factors may take into account the nature of the plant extract, the nature of the strains studied and the methodology used.

3.3 Phytochemical analysis

The results of the phytochemical analysis of the extracts studied reveal the presence of several families of secondary metabolites including phenolic compounds, cardiac glycosides, saponins, alkaloids and then sterols and terpenes. However, these secondary metabolites are distributed differently within the aqueous and 70% hydroethanolic extracts. Thus, in the present study, the aqueous extract of *Anogeissus leiocarpus* does not contain catechin tannins and sterols and terpenes. The 70% hydroethanolic extract also does not contain catechin tannins in this study. Also, the 70% hydroethanolic extract of *Anogeissus leiocarpus* contains more phenolic compounds compared to the aqueous extract (Figure 2).
The study by Dahiru *et al.* (2021) from an ethanolic extract of *Anogeissus leiocarpus* obtained by Soxhlet extraction made it possible to identify twenty-five (25) chemical compounds. Among these compounds, myristoleic acid; Z-7-tetradecenal; 4,5-dimethyl-4-

Hexene-3-un; 1,15-hexadecadiene and linoleoyl chloride were the most abundant. In this study, the observed antibacterial activities are explained by the results of the phytochemical analysis of the studied extracts. Indeed, the antimicrobial activity of most of these compounds, in particular flavonoids, tannins, alkaloids as well as terpenes, has already been demonstrated by several researchers [27,28,29]. The action mechanisms of such active substances are in forms of inhibiting the cell wall synthesis, the cell membrane function and the protein synthesis [30].

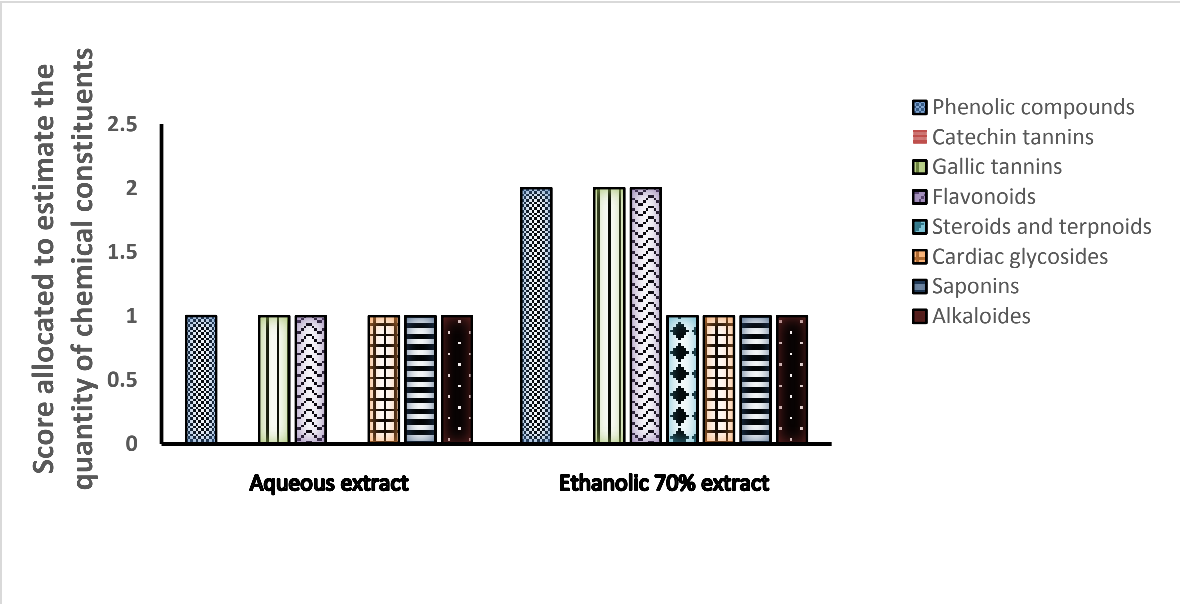

Fig.2. Phytochemical analysis of *Anogeissus leiocarpus* extracts.

4. CONCLUSION

This work allowed us to highlight the antibacterial properties of *Anogeissus leiocarpus* on two strains of multiresistant *Salmonella typhymurium* isolated from broilers chickens faeces. The aqueous and 70% hydroethanolic extracts prepared showed bactericidal powers on the two strains of multiresistant *Salmonella typhymurium* which were the subject of study. Thus, the aqueous and 70% hydroethanolic extracts of *Anogeissus leiocarpus* recorded minimal bactericidal concentrations of 12.5 mg/mL and 25 mg/mL on the two bacterial germs. In view of the results obtained in the present work, this plant could be used as a phytomedicine to combat certain pathologies linked to *Salmonella typhymurium* in broilers breeding. Also, it would be interesting to undertake studies with a view to using these extracts of *Anogeissus leiocarpus* as antibacterial agents in food products that may harbor strains of *Salmonella typhymurium*.

ETHICAL APPROVAL

It is not applicable.

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