



**IN-VITRO EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF *THONNINGIA SANGUINEA*, VAHL (BALANOPHORACEAE) ON MULTI-RESISTANT STRAINS OF *ESCHERICHIA COLI* ISOLATED FROM CHICKENS WITH COLIBACILLOSIS.**

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**ABSTRACT**

The aim of this study was to evaluate the in vitro activity of the aqueous extract of *Thonningia sanguinea* on multiresistant strains of *E.coli* of avian origin. The method of the wells in agar and that of the dilution in liquid medium (Muller Hinton broth) were used for the determination of the antibacterial parameters (MIC and MBC). The tests were carried out on 4 strains of *E.coli* isolates from dead broiler chickens. The MIC obtained was 1.25 mg/mL for the *E.coli* No. 011 and No. 221 followed by 2,5 mg/mL for the *E.coli* No. 131 and No. 179. For each of the 4 strains, MBC was 2.5 mg/mL. The extract was bactericidal on all the strains studied. These results suggest that *T.sanguinea* could be used as an alternative in the treatment of colibacillosis in broiler chickens.

**KEYWORDS:** Broiler chicken, *Thonningia sanguinea*, aqueous extract, *Escherichia coli*, colibacillosis.

**1. INTRODUCTION**

The increasing demand for animal proteins due to the strong demographic situation in the world has forced man to master all aspects of animal husbandry. In this way, we have been interested in animals with a cost-saving cycle such as broiler chickens. In order to increase the productivity and profitability of these animals, there is an industrialization that takes into account veterinary care in relation to genetically improved varieties.<sup>[1]</sup>

According to the FAO, world poultry production is second only to pork production, with production of more than 112 million tonnes in 2015.<sup>[2]</sup> In Ivory Coast, poultry consumption accounts for 23,6% of consumption of meat. Chicken production reaching 44,451 tons in 2015 against 22,364 tons in 2011 almost doubled in just four years.<sup>[3]</sup>

Alongside this spectacular development of Ivorian poultry farming, many diseases have appeared on farms and have settled there. These diseases are decimating livestock and causing considerable economic losses for

livestock farmers. These pathologies are of all kinds: parasitic, viral, fungal and especially bacterial.<sup>[4, 5, 6, 7]</sup>

Among bacterial infections, colibacillosis caused by pathogenic strains of *Escherichia coli*, are diseases that do more damage in avian farms after salmonellosis.<sup>[8]</sup> The treatment of these diseases is essentially based on chemotherapy.<sup>[9]</sup>

However, the difficulties encountered during the various treatments related to the development of resistant colibacillosis have led to the exploration of other antibacterial agents, particularly herbal products.<sup>[10]</sup> It is in this perspective that the aqueous extract of *Thonningia sanguinea* was tested in vitro on 4 strains of *Escherichia coli* isolated from chickens with colibacillosis.

**2. MATERIAL AND METHODS**

**2.1 Materials**

**2.1.1 Plant material**

It consists of the inflorescences of *Thonningia sanguinea* harvested at Sandegue in the eastern region of Ivory Coast (West Africa) and authenticated by the National

Floristic Center (CNF) of the Felix Houphouët-Boigny University in Cocody-Abidjan.

### 2.1.2 Microbial material

The bacterial support used in this study consists of 4 strains of *E.coli* (No. 011, No. 131, No. 179 and No. 212) isolated from the blood of dead chickens of colibacillosis at the Central Veterinary Laboratory of Bingerville (Ivory Coast).

## 2.2 Methods

### 2.2.1 Preparation of the aqueous extract

The inflorescences of *T. sanguinea* were washed, cut and dried out of the sun for two weeks. Once dried, these plant elements are ground. Thus, 20 g of this powder are dissolved in 2 liters of distilled water. The mixture is homogenized at room temperature using a magnetic stirrer. The homogenate is filtered seven times on hydrophilic cotton and once on 3 mm Whatman paper. The filtrate obtained is evaporated under vacuum at 30° C in a Buchi-type rotavapor. The evaporate is lyophilized and rendered dry and then recovered in the form of a powder which constitutes the total aqueous extract.<sup>[11, 12]</sup> The vegetable extract thus obtained is stored in the refrigerator for the anti-bacterial tests on the different strains of colibacilli.

### 2.2.2 Study of the antibacterial activity of the various extracts

The inoculum was prepared by homogenizing 0.1 ml of an opalescent suspension of *E. coli* of 3 hours in 10 ml of Mueller-Hinton Broth concentrated twice so as to obtain an estimated bacterial load of  $5.10^6$  CFU / ml. In addition, a range of concentrations ranging from 25 to 0.39 mg / mL was prepared by the double dilution method.<sup>[13]</sup>

### 2.2.3 Determination of growth inhibition zones

The method of the punch holes in the MH agar was retained at the expense of the loaded discs method due to the limits in the latter method relating to the non-diffusion of the plant extracts. Thus, as in the standard embodiment of an antibiogram, each well of 6 mm diameter was filled with 80 µl of extract of concentration 200 mg / mL, taking care to separate two holes of at least 20 mm. A control well was prepared with 80 µl of DMSO/Sterile distilled water (V/V) solution mixture.<sup>[14, 15]</sup> After 45 minutes prediffusion at room temperature under the hood, the whole was incubated in an oven at 37° C for 18 to 24 hours. At the same time, cefotaxin (30µg), gentamicin (15µg), imipenem (10µg),

amoxicillin (20µg), ciprofloxacin (5µg) and nalidixic acid (30µg) served as positive controls.

After incubation, the action of the extract is evaluated by measuring an area of growth inhibition (absence of colonies) around the wells.

### 2.2.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The macromethod of dilution in liquid medium was used.<sup>[16]</sup> By this method the MIC of the extract corresponds to the concentration of the first tube in which there is no visible growth to the naked eye of the bacteria tested after an incubation of 18 to 24 hours at 37° C. In order to determine the MBC, the surface of a new Mueller-Hinton Agar cast in Petri dish is then inoculated with 0.1 ml of the contents of the tubes having a concentration greater than or equal to the MIC. This MBC corresponds to the lowest concentration which allows to survive at most 0.01% of the germs of the starting suspension in 24 hours. In addition, the MBC/MIC ratio of the extract was calculated in order to assess its antibacterial potency.<sup>[17]</sup>

## 3. RESULTS AND DISCUSSION

### 3.1 Determination of diameters of growth inhibition zones

The table 1 shows the diameters of the growth inhibition zones of the tested microorganisms. It appears that commercial antibiotics have different activities with respect to the bacteria studied. Indeed, on the two strains of *E. coli* (No. 011 and No. 221), except for nalidixic acid, where there is a fundamental difference, the inhibition diameters of the other antibiotics are between 23 and 25 mm. By recording the smallest inhibition diameters (0-11 mm), the other two *E. coli* (No. 131 and No. 179) were the most resistant to these antibiotics. However, *E. coli* strain No. 179 remains the most resistant with inhibition diameters varying from 7 to 8 mm. Moreover, this strain (*E. coli* No. 179) recorded no inhibition diameter (0 mm) on 4 antibiotics of the six studied.

As for our plant extract, it showed a well-defined activity on the growth of *E.coli* strains by inducing inhibition diameters of between 11 and 12 mm. Referring to Biyiti et al. (2004), our extract is found to be active on all the strains tested. According to these authors, an extract is considered active if it induces an inhibition zone greater than or equal to 10 mm.<sup>[17]</sup>

**TABLE 1: Diameters of the zones of inhibition of the aqueous extract of *T. sanguinea* on the strains of *E. coli***

Strains	Extract (200 mg)	FOX (30µg)	GEN (15µg)	IMP (10µg)	AMX (20µg)	CIP (5µg)	NAC (30µg)
	Diameters of the zones of inhibition (mm)						
<i>E.coli</i> No 011	11 ± 0,4	24 ± 0,2	23 ± 0,4	25 ± 0,5	24 ± 0,2	23 ± 0,3	25 ± 0,
<i>E.coli</i> No 131	12 ± 0,2	07 ± 0,2	11 ± 0,1	07 ± 0,1	08 ± 0,2	09 ± 0,1	0
<i>E.coli</i> No 179	12 ± 0,1	08 ± 0,2	07 ± 0,2	0	0	0	0
<i>E.coli</i> No 221	11 ± 0,3	23 ± 0,3	24 ± 0,3	25 ± 0,4	25 ± 0,2	23 ± 0,2	0

FOX: Céfotaxin, GEN : Gentamicin, IMP: Imipenem, AMX: Amoxicillin, CIP: Ciprofloxacin, ACN: Nalidixic acid

### 3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (CMB)

For both strains of *E. Coli* (n° 131 and n° 179), the value of the registered MIC was 2.5 mg/mL while that obtained for the other two strains (n° 011 and n° 221) was 1.25 mg / mL. In addition, all strains had the same MBC value of 2.5 mg/mL.

The MBC/MIC ratio determined the bactericidal power. Indeed, according to Marmonier (1990), when this ratio is less than 4, the extract has a bactericidal effect and when it is greater than or equal to 4, it has bacteriostatic power. However, on all the strains tested, this ratio varied between 1 and 2, concluding that the plant extract studied is bactericidal on the different bacterial strains studied (Table 2).<sup>[18]</sup>

**TABLE 2: Antibacterial parameters of the aqueous extract of *T. sanguinea***

Strains tested	Antibacterial parameters (mg / mL)			
	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Antibacterial potency
<i>E.coli</i> No. 011	1,25	2,5	2	Bactéricide
<i>E.coli</i> No. 131	2,5	2,5	1	Bactéricide
<i>E.coli</i> No. 179	2,5	2,5	1	Bactéricide
<i>E.coli</i> No. 221	1,25	2,5	2	Bactéricide

Our results confirm the anti *E. coli* activity of *T. sanguinea* already shown by Vangah-Manda (1994) and N'guessan and *al.* (2007).<sup>[19, 20]</sup> Indeed, these authors showed the antibacterial effect of the total aqueous extract of *T. sanguinea* on hospital strains of *E. Coli* obtaining a CMB of 3.25 mg / mL. This extract was also bactericidal on a hospital germ of *E. Coli* multi-resistant beta-lactamase producer with a MBC of 6.25 mg / mL. However, the MBCs of our study are smaller than those obtained by these authors. This difference in MBC levels may be related to several factors, including the mode of extraction, the concentration of active ingredients and their mode of action, the nature of the germs and their origins.<sup>[21, 22]</sup> Studies have shown that the aqueous extract of *T. sanguinea* inhibits the growth of various strains of *Salmonella*.<sup>[23, 24, 25, 26]</sup> This extract also inhibits *Staphylococcus aureus*.<sup>[27]</sup> This plant was active on fungal strains including *Candida albicans*.<sup>[26]</sup>

The bactericidal activity of this plant could be linked to the active ingredients it contains. Moreover, several studies have been carried out in this direction by other researchers. The phytochemical sorting of the aqueous total extract of this plant by Kouakou and *al.*(2006) showed that it contained polyphenols, quinones, saponins and flavonoids in abundance.<sup>[28]</sup> The presence of polyphenols was also demonstrated in this plant by Nyarko and Addy(1994).<sup>[29]</sup> Two other molecules called *Thonningianin* A and B were found in the inflorescences of this plant by Ohtani and *al.* (2000).<sup>[30]</sup> All these classes of molecules are known for their antibacterial activities.<sup>[27, 31]</sup> These molecules could therefore be responsible for the inhibitory activity on these avian pathogenic strains of *E. coli*. Therefore, this plant could be advocated in the control of colibacillosis in the poultry environment.

### 4. CONCLUSION

From this study, which was intended to evaluate in vitro the antibacterial activity of the total aqueous extract of

*T.sanguinea* on avian pathogenic strains of *E.coli*, it is apparent that:

- all 4 avian strains of *E.coli* were sensitive to the total aqueous extract of *T. sanguinea*.
- the susceptibility of the strains to the extract is dose-dependent.

The aqueous extract exerted a bactericidal power on the different strains.

The sensitivity of these strains to *T.sanguinea* extract is of great importance in the treatment of avian colibacillosis which they cause as these strains exhibit increasing resistance to commonly used antibiotics.

In the remainder of our work, we intend to carry out a molecular characterization of the four (4) colibacilli studied by PCR in order to clearly mark the difference between these strains.

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