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Evaluation of the therapeutic activity of *Thonningia sanguinea* Vahl. (Balanophoraceae) on enteritis in pigeons from traditional breeding in Côte d'Ivoire.

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ABSTRACT

The pigeon is an ornamental animal and a popular source of protein. It is exposed to infections causing enteric disorders and the responsible strains are likely to be transmitted to humans. The objective of this work is to treat enteritis in pigeons with a plant extract in order to improve their breeding and reduce the reservoir of zoonotic strains. From an experimental breeding, pigeons presenting enteric disorders naturally through diet were treated with the aqueous extract of *Thonningia sanguinea* (10 mg/mL) compared to a standard antibiotic (colistin at 37.5 mg /ton) orally. The pigeon droppings were analyzed for 30 days using standard microbiology methods to identify the strains responsible for these disorders and their evolution during treatment. The analysis showed co-infection with strains of enteropathogenic *Escherichia coli* (EPEC), *Salmonella sp* and a high level of *Aspergillus sp*. Treatment with the plant extract allowed elimination of the two bacterial strains (EPEC and *Salmonella*) in a manner similar to colistin and a drastic reduction in the level of *Aspergillus sp* in animals. Furthermore, these treatments improved the morbidity score compared to the control. In view of these results, *T. sanguinea* could be recommended as a veterinary product in the fight against diarrheal diseases.

Keywords: Enteritis, pigeon, *Thonningia sanguinea*, colistin

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INTRODUCTION

The list of domestic animals for consumption is diversified to meet the demand for protein which is becoming increasingly important. Thus, the pigeon has become a very popular additional source of protein [1]. In addition to being used for food needs, the pigeon is a source of joy for children, the elderly or lovers in cities [2]. However, if the methods and techniques of their breeding are easily mastered, maintaining and improving their state of health is less so. Indeed, breeders are faced with numerous pathologies which cause enormous economic losses as well as a health risk for populations [3]. Among these diseases, there is enteritis which is gastrointestinal inflammation with a fatal effect in poultry. These enteritis are caused by several strains including *Salmonella sp*, enteropathogenic *Escherichia coli* (EPEC) causing diarrhea, poor general condition and weakness of the animal [4, 5]. Transmission generally occurs through ingestion of contaminated food and contaminated pigeon droppings. The germs cross the wall of the intestine and pass into the blood to create septicemia and cause mortality, especially in young pigeons. Animals can also carry the bacteria without showing symptoms. They then become healthy carriers and excrete the bacteria to contaminate other pigeons and humans. Treatment of the disease is generally done with antibiotics (tetracyclines, ampicillin, colistin, amoxicillin and quinolones) over a long period. There is also vaccination, but this causes a lot of side effects in poultry [5].

Despite the advances in veterinary medicine, there is an increasingly worrying appearance of resistance to the proposed drugs causing their ineffectiveness on enteritis in pigeons. To this must be added the high cost of these medications for small breeders whose breeding is often traditional. Faced with these difficulties, the use of phytomedicines is offered to us as an alternative solution to explore. It is with this in mind that we evaluated the therapeutic activity of the aqueous extract of *Thonningia sanguinea* (Balanophoraceae), a tropical plant from the undergrowth in Côte d'Ivoire on strains responsible for enteritis in pigeons.

MATERIAL AND METHODS

Plant material

The plant material consists of inflorescences of *Thonningia sanguinea* collected during the morning in Sandegue (North-East of Côte d'Ivoire) from July to August 2022. They were authenticated by the National Floristic Center of Felix University HOUPHOUËT-BOIGNY of Cocody-Abidjan (Côte d'Ivoire) and were preserved there under number 8355.

Preparation of the aqueous extract of *Thonningia sanguinea*

After washing, plant organs were cut up and dried away from the sun then ground with a laboratory grinder (RETSCH AS 200 type) to obtain a fine powder. Then, 20 g of this powder were diluted in 2 liters of distilled water. Mixture was homogenized at room temperature in the laboratory (22 °C) using a magnetic stirrer (IKA). Homogenate was filtered seven times through cotton wool and once through Whatman paper (3 mm). Filtrate obtained was evaporated under vacuum at 30 °C using a Büchi type rotavapor. Remaining solution was freeze-dried using a rotary evaporator (Rotavapor Büchi) and dried in an oven to obtain the aqueous extract of *T. sanguinea* [6, 7].

Animal material

Study involved 24 young domestic pigeons (*Columba livia domestica*) aged 4 months and with an average weight of 180± 0.5 g, divided into 3 groups of 4 pairs of pigeons (males and females) each: a control group (batch 1) and two experimental batchs (batch 2 and batch 3). These pigeons were purchased on the poultry market in the commune of Attecoube (Abidjan, Côte d'Ivoire) in September 2022.

Pigeon breeding conditions

Experiment took place in three dovecotes at the animal house of the Félix HOUPHOUET-BOIGNY University of Cocody (Ivory Coast). Dovecotes with dimensions 2 m x 2 m x 2 m were made of wood and covered with plywood with a mesh part allowing ventilation and natural lighting. Each dovecote was equipped with a feeder and a water trough. Pigeons were kept in captivity for 10 days to tame them and

allow them to acclimatize. At the end of this period, they had the opportunity to leave and return to the dovecotes. They were fed every day between 5 p.m. and 6 p.m., a mixture of millet and corn. These cereals were also purchased at the Attecoube market at the same time as the animals and stored without treatment in order to promote natural microbial contamination of the pigeons.

Treatment of pigeons

Experiment lasted 30 days during which the pigeons received different treatments:

- Batch 1: simple water (tap water) as a drink during the experiment.
- Batch 2: aqueous extract of *T. sanguinea* (100 mL) daily in drinking water at 10 mg/mL. The plant solution was served as soon as the diarrhea appeared every two (2) days and was replaced by simple water in the event of total consumption by the pigeons [7].
- Batch 3: colistin in sulfate form (reference standard antibiotic against poultry diarrhea) at recommended dose of 37.5 mL/tonne live weight. The treatment for these animals lasted 5 days from appearance of diarrhea in the dovecote [8].

Microbiological analysis of droppings

Collection of pigeon droppings

At the start of experiment (D_0), a reference sample of liquid droppings was taken in the 3 batches, then on the following days: D_2, D_4, D_6, D_{10}, D_{16}, D_{23}, D_{30}. Samples were taken using sterile spatulas and placed in sterile 40 mL bottles before being transported to the laboratory in a cooler containing a cold preservative (0 - 4 °C) for microbiological analysis.

Search for germs responsible for enteritis in pigeons

For the preparation of the stock solution (SS), 1 g of droppings was taken and dissolved in 10 mL of buffered peptone water to obtain a broth thus allowing better development of the microflora. Isolation and identification of the strains were carried out according to the method of [9] and concerned the pathogenic bacterial strains (enteropathogenic *E.coli, Salmonella sp*) and a fungal strain (*Aspergillus sp*).

Testing for pathogenic *Escherichia coli*

Biochemical isolation and identification

From the stock solution (SS), dilutions with sterile distilled water were made from 10^{-1} to 10^{-5} using sterile disposable pipettes [10]. Then, 3 Petri dishes containing Eosin Methylene Blue (EMB) agar were streaked with 0.1 mL of each dilution. Then, the plates were incubated in an oven at 37°C for 24 hours. After incubation, a purple colony with a metallic appearance was taken per dish to make a suspension with sterile distilled water. Then, using a sterile Pasteur pipette, API 20E galleries (Biomérieux) were inoculated and incubated at 37°C for 24 hours for the identification of *E. coli* by the following specific biochemical characters: indole production, fermentation of glucose, lactose and mannitol in an aerobic environment, ß-galactosidase presence, absence of production of hydrogen sulfide and urease, tryptophan deaminase and the non-use of citrate as a carbon source [11].

Morphological identification

Morphological identification consisted of observation in the fresh state under an optical microscope (objective 40) allowing the morphology, mode of grouping, abundance and mobility of the bacteria to be assessed. This test was complementary to the biochemical identification of the strains. To do this, a colony of pure strain obtained after subculture for 24 hours from the EMB culture on Müeller-Hinton agar is inoculated in 2 mL of Heart-Brain broth (Bio-Rad, France) then incubated at 37 °C for 3 hours. After incubation, a drop of the inoculated broth is taken then placed on a slide and covered with a coverslip. Everything is taken under an optical microscope for visualization.

Serological identification of pathogenic *E.coli*

Serological identification of enteropathogenic strains was carried out according to the method described by [12]. This involves the slide agglutination technique using specific antisera (Bio-Rad, France) for *E.coli* responsible for enteritis. This technique made it possible to demonstrate serotypes by identification of O antigens. It consisted of directly suspending in a drop of antisera specific to the different strains, on a microscope slide, a pure culture of 18 hours carried out on Mueller-Hinton agar at 37°C. After having thoroughly mixed the colonies in the antisera, the mixture is observed with the naked eye above a concave mirror. Any *E.coli* belonging to one of the serotypes sought gives massive and immediate agglutination. But first, an auto-agglutination test was carried out on a clean glass slide, with the same strain in a drop of saline solution (NaCl) to eliminate auto-agglutinable strains. This study targeted enteropathogenic *E. coli* (EPEC) strains, namely serotypes O26, O55, O86, O111, O128, O127 due to their strong involvement in animal and human diarrhea, particularly in children [13]. The agglutination test was carried out using first nonavalent, then trivalent and finally monovalent antisera.

Testing for Salmonella sp

Biochemical isolation and identification

The search for *Salmonella* began with selective enrichment of the samples after the pre-enrichment step with pepton water according to standard NF EN ISO 6579:2002. Indeed, 0.1 mL of the stock suspension (SS) was transferred into 10 mL of selenite broth to obtain an enriched broth which was then incubated at 37°C for 24 hours. Then, search for *Salmonella* was carried out using a selective medium, Hektoen agar in a Petri dish inoculated in streaks with 0.1 mL of the enriched broth and incubated at 37°C for 24 hours. After incubation, 3 gray-blue or black-centered colonies (suspect colonies) were taken to be subcultured each on new Mueller-Hinton agar which was then incubated at 37°C for 24 hours. Following incubation, a young colony was taken from each agar and homogenized in 10 mL of sterile distilled water to inoculate an API 20E gallery. This allowed the identification of strains, based on their biochemical characters. Furthermore, microscopic observation in the fresh state was also carried out in addition to the biochemical characterization.

Serological identification

Serological identification of *Salmonella* strains was carried out by serotyping from an 18 hours pure culture test carried out on Mueller-Hinton agar at 37°C. The method used is the agglutination test with specific multivalent and then monovalent antisera (Bio-Rad, France) [14]. Only antisera for the O antigen were used in this study.

Search for *Aspergillus sp*

Dilutions with sterile distilled water of the stock suspension (SS) were used for the search for *Aspergillus sp*. With 0.1 mL of each dilution, Sabouraud-chloramphenicol agar in a Petri dish was inoculated. For each dilution, 3 Petri dishes were inoculated and incubated at 30°C for 48 hours. After incubation, a count of the colonies was carried out and an observation in the fresh state under an optical microscope of the colonies obtained was carried out. The microscopic characteristics (*Aspergillus* head and spore production) and the macroscopic aspects (powdery, velvety and downy) of the colonies made it possible to identify the genus and species of *Aspergillus*.

Determination of morbidity and mortality

The cumulative morbidity of the pigeons in the 3 groups was observed daily during the experiment, according to the following notation [15]:

- Note 0: normal pigeon;
- Note 1: pigeon down and standing (feathers ruffled) with diarrhea;
- Note 2: pigeon slaughtered and lying down with diarrhea, which moves under moderate stress;
- Note 3: pigeon slaughtered and lying down with diarrhea, which does not move under moderate stress.

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At the end of the experiment, the total number of dead pigeons was recorded and the mortality rate was deducted.

RESULTS

In vivo effect of treatments on *E. coli*

Cultures on EMB medium showed throughout the experiment numerous purple colonies with a metallic appearance which are lactose positive enterobacteria. Microscopic and biochemical analyzes with the API 20E gallery carried out on these colonies made it possible to identify *E.coli* strains (glucose+, lactose+, gas+, mannitol+, mobility+, indole+, urease-, tryptophan deaminase-, citrate- H2S-). The results of antigenic tests (agglutination) are recorded in Table 1. It appears that in the batch treated with the aqueous extract (batch 2) and in that treated with colistin (batch 3), agglutination indicating the presence of EPEC was observed on D_{16}. Then these strains disappeared following treatment with the aqueous extract and colistin. On the other hand, in the untreated batch (batch 1), EPEC were identified from D_{10} and persisted in the droppings until the end of the experiment at D_{30}. Furthermore, different serotypes identified in the 3 batches were *E. coli* O86 and *E. coli* O55.

Table 1 : Presence of EPEC in pigeon droppings samples

Days / Batches	D_0	D_2	D_4	D_6	D_{10}	D_{16}	D_{23}	D_{30}
Batch1	-	-	-	-	+	+	+	+
Batch 2	-	-	-	-	-	+	-	-
Batch 3	-	-	-	-	-	+	-	-

Batch1 : untreated pigeons; Batch 2 : pigeons treated with *T. sanguinea* aqueous extract; Batch 3 : pigeons treated with colistin.

+ : Agglutination and presence of EPEC sought; - : No agglutination and absence of EPEC sought

In vivo effect of treatments on *Salmonella*

The culture on Hektoen agar of samples of pigeon droppings in the 3 batches during the experiment showed the presence of numerous gray-blue colonies with black centers which are suspicious colonies that could be *Salmonella*. The results of microscopic and biochemical tests with the API 20E gallery showed the presence of *Salmonella* (glucose+, lactose-, gas+, mannitol+, mobility-, citrate+, H2S+, indole-, urease-, tryptophan deaminase-) from D_{16} until at the end of the experiment for the control batch (batch 1) compared to only a presence on D_4 in the batch treated with the aqueous extract (batch 2) and on D_2, D_4 and D_6 in the batch treated with colistin (batch 3) (Table 2). These strains disappeared from the droppings following treatment respectively after D_4 with the aqueous extract and D_6 with colistin. Furthermore, the antigenic tests showed that all the strains of *Salmonella* isolated from the droppings were agglutinated with the versatile OMB antiserum and monovalent O:4;5 antiserum. These are therefore serogroup B *Salmonella*.

Table 2 : Presence of *Salmonella* in pigeon droppings samples

Days / Batches	D_0	D_2	D_4	D_6	D_{10}	D_{16}	D_{23}	D_{30}
Batch1	-	-	-	-	-	+	+	+
Batch 2	-	-	+	-	-	-	-	-
Batch 3	-	+	+	+	-	-	-	-

Batch1 : untreated pigeons; Batch 2 : pigeons treated with *T. sanguinea* aqueous extract; Batch 3 : pigeons treated with colistin.

+ : Agglutination and presence of *Salmonella* ; - : No agglutination et absence of *Salmonella*

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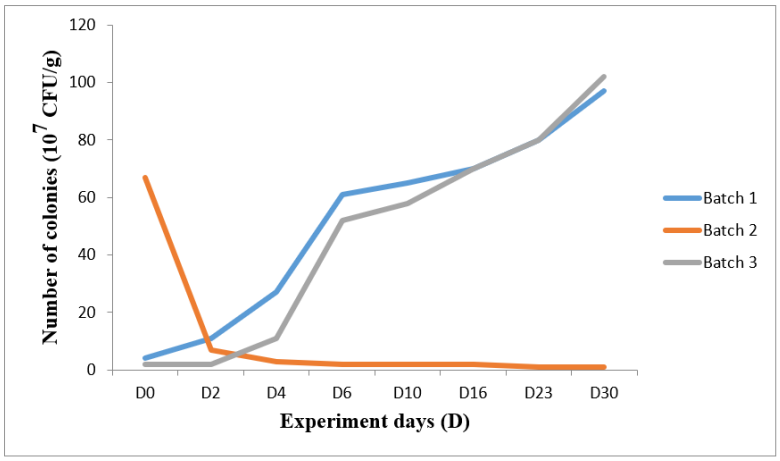
In vivo effect of treatments on *Aspergillus*

Macroscopic (bluish-green or dark green powdery colonies) and microscopic analyzes (septate mycelium, erect conidiophores ending in vesicles, round, smooth and brownish spores) of the cultures obtained on Sabouraud-chloramphenicol agar made it possible to identify numerous colonies of *Aspergillus fumigatus*. The evolution of the load of this fungus in the 3 batches of pigeons during the experiment is presented in Figure 1. It appears from the analysis of these results that the number of colonies decreased considerably in batch 2 continued to treatment with the aqueous extract of *T. sanguinea* from 67.107 ± 0.5 CFU/g to 1.107 ± 0.9 CFU/g compared to the two other batches (1 and 2) where the load increased respectively by 4.107 ± 1, 2 CFU/g to 97.107 ± 0.2 CFU/g (batch 1) and from 2.107 ± 0.77 CFU/g to 102.107 ± 0.55 CFU/g (batch 3).

Figure 1 : Evolution of *Aspergillus* load in the differents pigeons batches droppings



Batch1 : untreated pigeons; Batch 2 : pigeons treated with *T. sanguinea* aqueous extract; Batch 3 : pigeons treated with colistin.

Morbidity and Mortality

Concerning the cumulative morbidity, observations made it possible to note a normal condition of the pigeons from the 3 batches, that is to say a zero score from the start of the experiment until D_{16}. From this date, in batch 1 (control), this score was 1±0.7 corresponding to animals with ruffled feathers with fetid diarrhea until the end of the experiment. As for the batches treated with the aqueous extract and colistin, the scores were respectively 1±0.3 and 2±0.8 on D_{16}. However, the animals returned to their normal state in these two batches following treatment from D_{23} until the end of experiment. Furthermore, no mortality was observed in the 3 batches.

DISCUSSION

Gastroenteritis are conditions linked to pathogenic microbial strains considered emerging in public health. The acquisition of virulence factors allows them to colonize several organs of the host. Many domestic animals are reservoirs of contamination to humans [3]. Indeed, the different serogroups involved in humans are also present in different animal species where they are often carried asymptomatically in the digestive tract. In this study, the contamination of the pigeons occurred naturally through their diet. This confirms that this, when it is not well preserved and lack of hygiene in pigeon breeding, constitutes main route of animals contamination [16].

The results show the presence of *E. coli* serogroups known in the etiology of gastroenteritis in both humans and pigeons. Indeed, the strains found in the droppings of animals showing signs of enteritis were *E.coli* O86 and *E.coli* O55. According to several studies, these strains are enteropthogenic *E.coli* (EPEC) which generally cause epidemics of severe diarrhea in children in developing countries [17]. These foodborne outbreaks cause attachment/effacement (A/E) damage to intestinal epithelial cells [18, 19].

Treatment with the aqueous extract of *T.sanguinea* made it possible to eliminate pathogenic *E.coli* strains compared to the control and colistin with an improvement in morbidity. Effectiveness of the aqueous extract of *T.sanguinea* confirms the results of [7]. Indeed, these authors effectively treated experimental colibacillosis in broiler chickens using this extract orally, consequently improving lesions in the organs and stopping diarrhea. Results obtained also confirm the antibacterial activity of *T. sanguinea* on *E. coli* as reported by several researchers. Indeed, [20] showed the in vitro antibacterial effect of the total aqueous extract of *T. sanguinea* inflorescences on clinical *E. coli* strains with a MBC of 3.25 mg/mL.

Concerning *Salmonella*, the strains observed were all from group B. Strains of this group such as *Salmonella typhimurium* present in birds (asymptomatic carriers) cause gastroenteritis and enteric fever in children under 5 years of age, in adults and elderly [21]. *T.sanguinea* aqueous extract at 10 mg/mL was effective on diarrhea resulting in the elimination of *Salmonella* in pigeon droppings. These results are in the same direction as those obtained by [22]. Indeed, these authors showed that *T.sanguinea* aqueous extract inhibited the growth of various *Salmonella* strains in broiler and laying hen farms as well as fungal strains in particular *Candida albicans*. The results of this study also confirm the effect of this plant against infectious diarrhea which are signs of enteritis, already demonstrated by other researchers. Indeed, [6, 23] treated experimental diarrhea due to *Salmonella enteritidis* phage type 6 and parasites of the *Emeria* genus with aqueous extracts of *T. sanguinea* in laying hens respectively.

Signs of enteritis observed as well as the morbidity of the pigeons would therefore be linked to a co-infection due to *E.coli* and *Salmonella*. However, in addition to enteric bacterial strains, *Aspergillus fumigatus* were also found at high levels in pigeon droppings. This high rate could be explained by a drop in immunity caused by the bacterial infection given that a priori, this fungal strain is not a primary cause of gastroenteritis in birds.

Moreover, it is clear that search for new plant-based antibacterial agents against avian digestive conditions is increasingly intensifying. Indeed, several previous studies have shown that plant extracts were effective in vitro on pathogenic *E. coli* strains of avian origin. This is the case in Algeria with the work of [24] which demonstrated the effectiveness of 6 medicinal plants essential oils on APEC strains isolated from broiler chickens. The in vivo antibacterial and antifungal activity of this plant is believed to be linked to the molecules it contains. Indeed, a phytochemical sorting of the aqueous extract of this plant already carried out showed that it contained total polyphenols, quinones, saponins and flavonoids in abundance [25]. All these molecules are known for their antibacterial and antioxidant properties [26].

In the batch treated with the reference standard antibiotic (colistin), there was also an elimination of pathogenic bacterial strains but an increase in the rate of *Aspergillus*. This could be explained by its ineffectiveness on fungus. Indeed, colistin is a polycationic polypeptide antibiotic, both hydrophilic and lipophilic, which increases membrane permeability leading to leakage of intracellular contents and death of the bacteria [27].

This plant therefore has anti-infectious effects and could be better used in the fight against pathologies in pigeons.

CONCLUSION

The objective of this study was to treat pigeon enteritis with the aqueous extract of *Thonningia sanguinea* compared to a standard reference antibiotic (colistin). At the end of the work, it appears that the strains involved in this condition were made up of enteropthogenic *E.coli* (EPEC), *Salmonella* and to a lesser extent *Aspergillus fumigatus*. Treatment with the aqueous extract allowed elimination of pathogenic bacterial strains in a manner similar to colistin and a reduction in the load of *Aspergillus fumigatus* in pigeon droppings with a cessation of diarrhea. These results could serve as a basis for the production of traditionally improved medicines (TIMs) to help combat enteritis in poultry.

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