

# Assessment of Anti-*Salmonella* Activity of Aqueous and Ethanolic Extract of *Senna siamea*, Used in Traditional Management of Salmonellosis in Benin

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## Abstract

Recent ethnopharmacological data cited *Senna siamea* as one of the most widely used medicinal plants in the management of salmonellosis in Benin. However, data related to its activity on non-typhoidal *Salmonella* spp are limited. This study aimed to assess the antibacterial activity of *Senna siamea* on multidrug-resistant *Salmonella*. Ethanolic and aqueous extracts of *S. siamea* were tested for their antibacterial activity on four multidrug-resistant *Salmonella*: *Salmonella* Typhimurium ATCC 14028 and three *Salmonella* spp. isolated from animals intended for human consumption in Benin. Well diffusion technique combined with the determination by microdilution of Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) were used for antibacterial testing. From antibacterial testing, inhibition diameters of the extracts ranged from 7 to 11 mm, for the susceptible strains. Colistin (reference antibiotic) was active on all *Salmonella* spp. with inhibition diameters between 18 and 19 mm. The MICs ranged from 3.125 to 25 mg/ml while MBCs of the extracts are greater than 100 mg/ml, so none of the extracts have antibacterial power (p.a). From these results, it appears that the use of *Senna siamea* in the traditional treatment of salmonellosis is justified. These results must be valued in the development of anti-*salmonella* phytomedicines.

## Keywords

*Salmonella* spp., *Senna siamea*, Salmonellosis, MIC

## 1. Introduction

Non-typhoid *Salmonella enterica* associated with various foods, particularly poultry products, are major causes of bacterial gastroenteritis worldwide [1]. Non-typhoid salmonellosis is endemic in sub-Saharan Africa [2]. Approximately 1.4 million people are affected each year in the United States, with nearly 15,000 hospitalizations and more than 400 deaths [3].

Salmonellosis is a public health problem in Benin (West Africa). A recent study led in southern Benin revealed *Salmonella* spp. in the faeces of animals intended for human consumption. These strains were resistant to aminoglycosides, cephalosporins of generations 1 and 2 and penicillins, with the presence of virulence genes such as *invA*, *fimA* and *stn* [4]. These data show how emergent it is to implement an effective strategy to control multidrug resistance and virulence of *Salmonella* circulating in Benin.

Fluoroquinolones are involved in the treatment of serious *Salmonella* infections [5]. Unfortunately, there is an increase in the resistance of *Salmonella* to quinolone [2] [6] [7]. Lack of sanitation, limited access to safe drinking water and inappropriate use of antimicrobials also complicate the management and treatment of salmonellosis. In recent years, several alternatives to conventional antibiotics have been proposed in the fight against antimicrobial resistance. The use of medicinal plants is one of the most explored alternatives in West Africa.

In Benin, an ethnopharmacological survey performed in Benin identified 57 medicinal plants used by herbalists in the traditional management of *Senna siamea* (Lam) was one of the most quoted plants [8]. A study performed by Legba *et al.* [9] provided interesting data on the antibacterial activity of *Senna siamea* on enteric pathogens, and its chemical and toxicological characteristics. In addition, several authors demonstrated *in vitro* anti-*Salmonella* activity of *S. siamea*. In 2013, Dahiru *et al.* [10] demonstrated inhibition of *Salmonella* Typhi by *S. siamea* leaf extracts. Furthermore, in Doughari and Okafor [11] study, the aqueous extract of *S. siamea* leaves inhibited *Salmonella* Typhi with a MIC of 1 mg/ml and a MBC of 1.3 mg/ml. These data are of interest and show the potential of *S. siamea* in the control of salmonellosis, but there are two major shortcomings: There is a wealth of data on the antibacterial activity of *S. siamea* on *Salmonella* Typhi, but little information on its activity on non-typhoid *Salmonella*. In addition, no data on the antibacterial activities of the plant on multi-drug-resistant *Salmonella* isolated in Benin have been identified.

This work aims to assess the antibacterial activity of *S. siamea* on multidrug resistant strains of *Salmonella* isolated in Benin.

## 2. Methodology

### 2.1. Material

#### 2.1.1. Plant Material

Leaves of *Senna siamea* (Leguminosae, Caesalpinioideae) were collected in Porto-Novo (Benin) in March 2018. Samples were identified by Professor Houn-

nankpon Yedomonhan from National Herbarium of Benin, University of Abomey-Calavi (Benin). Reference number is AA6691/HNB.

### 2.1.2. Bacterial Strains

The reference strain *Salmonella* Typhimurium ATCC 14028 was obtained from Research Unit in Applied Microbiology and Pharmacology of natural substances, University of Abomey-Calavi, Benin. The three multiresistant *Salmonella* spp were isolated from animals intended for human consumption by Deguenon *et al.* [4]. The three strains were multidrug-resistant to first generation cephalosporins, some aminoglycosides and penicillins, and some **Table 1** below presents bacterial strains's characteristics.

## 2.2. Methodology

### 2.2.1. Obtaining Extracts

The leaves were sorted (decomposed leaves exclusion), washed with distilled water (to avoid contamination), dried in the laboratory's temperature (16°C) for 10 days. Thus, leaves were powdered using a Retsch SM 2000/1430/Upm/Smf type mill. The extracts were prepared from powders using the Maceration technique [9]. After extraction, the crude extracts obtained were kept at 4°C.

### 2.2.2. Antibacterial Tests

- Preparation of Extracts

For the antibacterial tests, aqueous and ethanol extracts were dissolved in sterile distilled water at a concentration of 100 mg/ml.

- Preparation of bacterial suspension

From young colonies of 18 to 24 hours, a bacterial suspension was prepared at 0.5 Mc Farland, with sterile distilled water [12].

- Antibiogram by well diffusion technique

Each inoculum was swabbed onto Petri dishes containing Mueller Hinton agar [12]. Wells of 6 mm diameter were dug, using a sterile Pasteur pipette tip. 50 µl of each extract were deposited in the wells. One well containing sterile distilled water was used as a negative control while colistin (reference antibiotic) was used as a positive control. Petri dishes were left for 1 hour at room temperature for pre-diffusion of the extracts and then incubated at 37°C for 18 hours. The inhibition diameters were measured at the end of the incubation period. To

**Table 1.** Characteristics of bacterial strains (Deguenon *et al.*, 2019).

Strains	Virulence genes				
	invA	spvR	spvC	fimA	Stn
<b>P14</b>	+	-	-	+	+
<b>P16</b>	+	-	-	+	+
<b>P19</b>	+	+	+	+	+
<b><i>Salmonella</i> Typhimurium ATCC 14028</b>	+	+	+	+	+

P14; P16; P19 are identified *Salmonella* Spp.; + = Presence; - = Absence.

avoid bias and to determine the means and standard deviations, the test was carried out three times.

The standard used for interpreting the results of the antibiogram tests is presented in **Table 2**.

- Determination of MIC and MBC.

Microdilution method with 96-well plate was used for determination of MIC [13]. 100  $\mu$ l of the stock solution of each extract prepared at 200 mg/ml were added to 100  $\mu$ l of Mueller-Hinton Broth (MHB). Then, a series of two-fold dilution from well to well was made then 100  $\mu$ l of different bacterial suspensions were added. Positive and negative controls were prepared respectively by adding 100  $\mu$ l of MHB to 100  $\mu$ l of bacterial suspension and 100  $\mu$ l of MHB to 100  $\mu$ l of the extracts. The microplates were coated with parafilm paper and incubated at 37°C for 24 hours. Resazurin is used as an indicator of cell viability. After incubation, each well was cultured on MH Agar and incubated at 37°C for 24 hours for the determination of MBC. MBC is the lowest concentration of extract to which no colony of bacteria can be observed. The antibiotic power (p.a) of each extract was then calculated with the formula MBC/MIC.

### 2.2.3. Data Analysis

The experiments were done with three replicates ( $n = 3$ ) and the results were subjected to Two-way ANOVA according to Turkey's multiple comparison test,  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Results

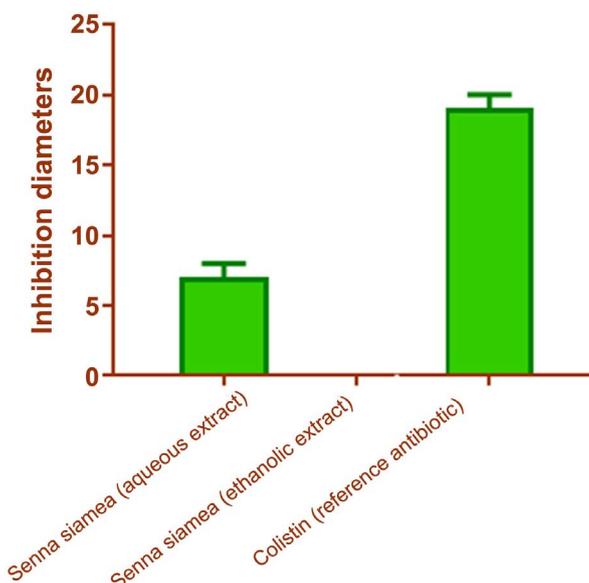
The anti-*Salmonella* activity of the aqueous and ethanolic extracts was evaluated *in vitro* by performing antibiogram and MIC and MBC determinations. Four strains were used: *Salmonella* Typhimurium ATCC 14028 (reference strain) and three strains of *Salmonella* spp isolated in Benin

### 3.2. Antibiogram results

**Figure 1** presents Antibacterial activity of leaves extracts of *Senna siamea* and Colistin (reference antibiotic) on *Salmonella* Typhimurium ATCC 14028. The reference strain was sensitive to aqueous extract of *Senna siamea* (7 mm) and Colistin (19 mm), but resistant to *Senna siamea* ethanolic extract.

**Table 2.** Interpretation of the susceptibility tests of the plant extracts [14].

Diameter of the inhibition zone ( $\Delta$ )	sensitivity of the germ	Symbol
$\Delta < 7$ mm	Resistant	–
$7$ mm $\leq \Delta < 8$ mm	Sensitive	+
$8$ mm $\leq \Delta < 9$ mm	Moderately sensitive	++
$\Delta \geq 9$ mm	Very sensitive	+++



**Figure 1.** Antibacterial activity of leaves extracts of *Senna siamea* and Colistin on *Salmonella* Typhimurium ATCC 14028.

Aqueous and ethanolic extracts of *Senna siamea* and Colistin had variable activities on *Salmonella* spp strains isolated from animals intended for human consumption. Colistin was active on all three strains with inhibition diameters between 18 and 19 mm. On *Salmonella* spp (P19), aqueous and ethanolic extracts of *Senna siamea* had inhibition diameters of  $8 \pm 1$  mm (moderately sensitive) and  $11 \pm 1$  m (very sensitive) respectively. On *Salmonella* spp (P14), only aqueous extract of *Senna siamea* was active with an inhibition diameter of  $7 \pm 0.57$  mm. Finally, *Salmonella* spp (P16) was resistant to all *Senna siamea* extracts with inhibition diameters less than 7 mm (Figure 2). Two-way Anova showed significant difference between inhibition diameter of extracts and Colistin ( $P = 0.0003$ ) and between sensitivity of strains ( $P < 0.0001$ ).

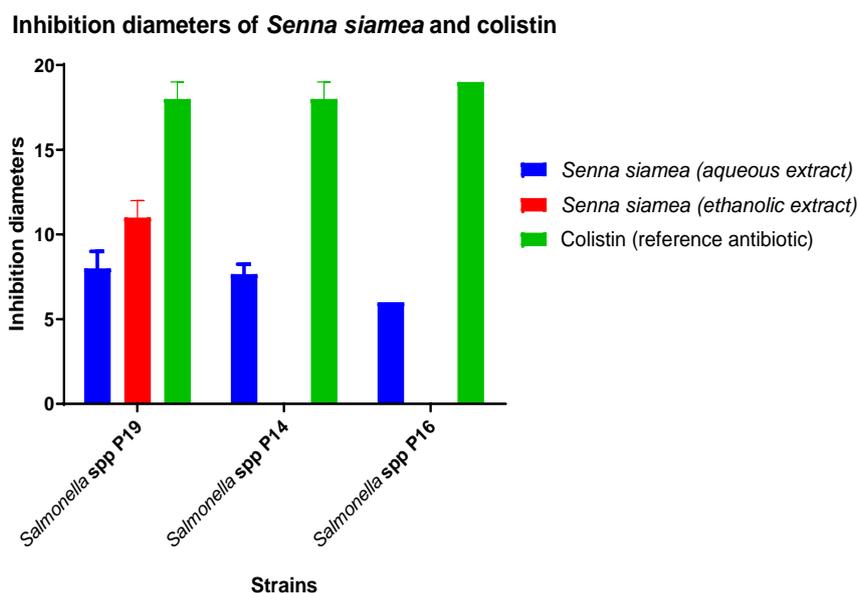
### 3.3. MIC and MBC

The well diffusion test was coupled with the determination of the MIC and MBC of the extracts in order to determine the antibacterial power of the extracts. Table 3 below shows MICMIC and MBC results obtained during this study.

The MICs of the extracts ranged from 3.125 to 25 mg/ml. The MBCs of the extracts are greater than 100 mg/ml, so none of the extracts have antibacterial properties.

## 4. Discussion

This study was aimed to assess the antibacterial activity of *Senna siamea* on multidrug resistant *Salmonella*. For Well diffusion test, the choice of 100 mg/ml concentration is explained by the fact that in previous work, aqueous and ethanolic extracts of *Senna siamea* have not been active on enteropathogens strains at concentrations lower than 100 mg/ml [9].



**Figure 2.** Antibacterial activity of leaves extracts of *Senna siamea* and Colistin on *Salmonella* spp. (P19, P14 and P16).

**Table 3.** MIC (mg/ml), MBC (mg/ml) and a. p. of the aqueous and Ethanolic extracts of *Senna siamea* on *Salmonella* spp.

Extracts	Parameters	P19	P14
<i>S. siamea</i> aqueous extract	MIC	25	3125
	MBC	>100	>100
	a.p.	-	-
<i>S. siamea</i> ethanolic extract	MIC	25	-
	MBC	>100	-
	a.p.	-	-

**Legends:** MIC: Maximum inhibitory concentration, MBC: Minimum Bactericidal Concentration, PA: Antibacterial Power.

#### 4.1. An Interesting Anti-Salmonella Activity

The aqueous extract of *Senna siamea* was active on the reference strain (*Salmonella* Typhimurium ATCC 14028) and on two of the three *Salmonella* isolates, while the ethanolic extract was only active on one strain of *Salmonella*. However, the largest inhibition diameter was obtained with the ethanolic extract of *Senna siamea* which had an inhibition diameter of 11 mm on *Salmonella* spp. (P19). The MICs of the extracts ranged from 3.125 to 25 mg/ml. The interesting data reported reinforces some previous studies. For example, in 2013, Dahiru *et al.* [10] showed that *S. siamea* leaf extracts have antibacterial activity on *Salmonella* Typhi. In Doughari and Okafor [11] study, leaves aqueous extract of *S. siamea* has *in vitro* inhibitory activity on *Salmonella* Typhi with a MIC of 1 mg/ml and a MBC of 1.3 mg/ml. In another study, at 100 mg/ml, the aqueous extract of *Senna siamea* leaves had an inhibition diameter of  $15.45 \pm 0.26$  mm on *Salmonella* Ty-

phi while the ethanolic extract had an inhibition diameter of  $17.20 \pm 0.20$  mm on the same strain [15]. However, these studies differed from ours in that they focused on strains of *Salmonella* Typhi, the cause of typhoid fever, whereas we worked on Non Typhoid *Salmonella* isolated from animals intended for human consumption.

The interesting antibacterial activity of *Senna siamea* on these *Salmonella* strains encourages its traditional use in the management of salmonellosis, according to reports by Dougnon *et al.* [8]. However, the great inhibition diameters of Colistin could be due to the fact that the reference molecule is a pure molecule while the extract used is not yet one. In our study, extracts are raw, unpurified. The difference in activity between the aqueous extract and the ethanolic extract could be due to the likely variability in chemical composition between the two extracts. The differences in polarity between the two solvents (Water and ethanol) certainly led to the extraction of different chemical compounds not having the same pharmacological activities. Moreover, this result reinforces the traditional use of the plant. The decoction with water from the leaves is the essential method of preparation reported [8].

#### **4.2. Chemical Composition as a Source of Antibacterial Activity?**

Work on medicinal plants is unanimous on the fact that the activity of the plant extracts is mainly linked to the presence of molecules usually known under the term “Secondary metabolites”. The works of Legba *et al.* [9] revealed an interesting composition of aqueous and ethanolic extracts in polyphenols and flavonoids, molecules known for their antibacterial properties. Abdurasheded *et al.* [16] also identified alkaloids, flavonoids, tannin and steroids in *Senna siamea* leaves while Nas *et al.* [15] identified glycosides, tannin, anthraquinone, flavonoid saponin, phenol, terpenoid and steroid. We hypothesize that the presence of these molecules explains the activity of the extracts.

#### **4.3. Can *Senna siamea* Be Potential Source of Phytomedicines against Salmonellosis?**

*In vitro* antibacterial activity is not enough. *In vivo* anti-*Salmonella* activity tests are needed to support the plant activity data. The model developed by Legba *et al.* [13] seems interesting for this type of test. The evidence of non-toxicity of *Senna siamea* exists and attests to the harmlessness of this plant. Using the model *Artemia salina*, Legba *et al.* [9] showed that the extracts were non-cytotoxic. In addition, *in vitro*, leaves aqueous and ethanol extracts were devoid of toxicity against vero cells [17]. A few cases of toxicity were reported *in vivo*, but these were at very high doses up to 8000 and 9600 mg/kg body weight [18] [19]. *Senna siamea* could be valued in the development of anti-salmonellosis phytomedicine.

### **5. Conclusion**

The results of this study showed that aqueous and ethanolic extract of *Senna*

*siamea* have an antibacterial activity on multiresistant *Salmonella* spp., with inhibition diameters ranged from 7 to 11 mm and MICs ranged from 3.125 to 25 mg/ml. The traditional use of *Senna siamea* leaves in the treatment of salmonellosis is justified. *Senna siamea* could be a good candidate for the development of anti-Salmonellosis phytomedicine, and *in vivo* efficacy testing, quality control of the powder, plus testing of formulations will be required.

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## Limitations

Our study did not exhibit the way the extract gives the beneficial effect on *Salmonella* spp. Further investigations will assess it.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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