

# Antibacterial activity of *Thonningia sanguinea* against some multi-drug resistant strains of *Salmonella enterica*

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## Abstract

**Background:** The emergence of strains of *S. enterica* with multiple drug resistance (MDR) is of great concern worldwide. The extracts of flowers of *Thonningia sanguinea* are used in traditional medicine in Ivory Coast to treat diarrhoeal diseases including salmonellosis. Previous studies had shown inhibition of the MDR strain *Salmonella* Enteritidis lysotype 6.

**Objectives:** The present study focused to investigate the effect of the extract of the flowers of *Thonningia sanguinea* on some clinical MDR strains of *Salmonella* namely *S. Typhi*, *S. Typhimurium*, *S. Hadar* and a sensitive strain (*S. Enteritidis*).

**Methods:** The antimicrobial parameters were determined by double dilution with agar slant method. This method led us to determine MIC, IC<sub>50</sub> and MBC.

**Results:** The MDR strain of *S. Typhimurium* presented the highest MIC (2.5 mg/ml) whereas the other two MDR strains (*S. Hadar*, *S. Typhi*) and the sensitive one (*S. Enteritidis*) had the same MIC (1.25 mg/ml). The four strains presented the same MBC (2.5 mg/ml). The MDR strain of *S. Typhi* is the most susceptible strain to the aqueous extract of the flowers of *Thonningia sanguinea* according to the IC<sub>50</sub> values.

**Conclusions:** The aqueous extract of *Thonningia sanguinea* can provide an alternative therapy for the treatment of salmonellosis, mainly for typhoid fever caused by MDR strains of *S. Typhi*. The extract also inhibits *S. Hadar* a MDR emerging strain in Ivory Coast.

Key-words: *Thonningia sanguinea*; *Salmonella*, MDR strains, Ivory Coast

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## Introduction

*Salmonella* spp. are considered one of the major foodborne pathogens<sup>1</sup>, resulting in considerable morbidity and occasionally death<sup>2</sup>. It is a public-health problem in developing countries<sup>3</sup>. *Salmonella enterica* serovar typhi causes approximately 10 million cases each year mostly in developing countries<sup>4,5</sup>. Most of these infections have been attributed to consumption of poultry meat and eggs<sup>6</sup>.

The emergence of strains of *S. enterica* with multiple drug resistance is of great concern worldwide. Increasing rates of antibiotic resistance have been reported in various regions throughout the world and certain antibiotic resistant clones, such as *S. Typhimurium* DT 104, seem to have spread in areas rather distant from each other<sup>7</sup>.

In Ivory Coast, the typhoid fever and the other forms of salmonellosis became these last years a prob-

lem of public health taking into account the degradation of the conditions of healthiness in relation to the war. Ouattara<sup>8</sup> have put in evidence the emergence of MDR strains of *Salmonella* belonging mainly to the serovar Hadar. The pathogenic role of *Salmonella* infection in the development of human diseases and the impact of resistance on the clinical outcome stimulated the search for newer treatments and natural products could provide alternative therapies against salmonellosis.

*Thonningia sanguinea* is used in traditional medicine for the treatment of haemorrhoids and anal lesions, bronchial asthma<sup>9,10,11</sup>, skin diseases, dysentery, sore throat and as vermifuge<sup>12</sup>. In Ivory Coast, the flowers of *Thonningia sanguinea* are used for the treatment of diarrhoea<sup>13,14</sup> which is known to be one of the symptoms of salmonellosis.

Previous studies have shown inhibition of the MDR strain *Salmonella* enteritidis lysotype 6 by the crude aqueous extract of *Thonningia sanguinea*<sup>15,16</sup>. The antibacterial activity of *T. sanguinea* have also been studied by Ohiri and Uzodinma<sup>17</sup>. Two ellagitannins (*Thonningianins* A and B) with strong antioxidant activities have been isolated from *Thonningia sanguinea*<sup>18</sup>.

In this study, as a part of our contribution in the fight against salmonellosis, we have evaluated the anti-

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microbial effects of the aqueous extract of *T. sanguinea* on three clinical MDR strains of *Salmonella* including *S. Hadar* and a sensitive one.

## Material and methods

**Plant material:** *Thonningia sanguinea* is a parasitic plant and the flowers are the main part of the plant. The flowers of *T. sanguinea* were collected in Adzopé, Ivory Coast and identified by Pr Aké-Assi of the Department of Botany, University of Cocody-Abidjan. A voucher specimen (Voucher n° 14162) is deposited in the herbarium of "Centre National de Floristique" of Abidjan.

**Extraction:** The freshly collected flowers of the plant were air dried at room temperature for 7 days and powdered. Briefly 20g of powder was soaked in 500ml distilled water for 24 h with constant stirring. The suspension was further filtered through Whatmann (N°1) filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator to obtain the aqueous extract.

**Bacterial strains:** Bacteria for testing purposes were kindly provided by the Laboratory of Bacteriology and Virology of the Pasteur Institute of Ivory Coast. It was in-house reference strains.

- *Salmonella typhi* (330) resistant to ceftazidim, amoxicilin and clavulanic acid;
- *Salmonella typhimurium* (n° 4, 5, 12, i : 1, 2 12/02) resistant to Ceftazidim and Trimethoprim
- *Salmonella hadar* n°86.3 (337) resistant to Amoxicilin, Trimethoprim and Ceftazidim
- *Salmonella* Enteritidis (n°9,12 : g, m :-12/02) sensitive

## Antibacterial tests

### Determination of MIC and IC<sub>50</sub>

The Minimal Inhibitory Concentration (MIC) was determined by the agar slant method<sup>19</sup>. A 2-fold serial dilution (0–5000 µg/ml) of the crude extract in Mueller Hinton agar had been prepared in slant tubes. An aliquot of 10 µl of standardized suspension of bacteria (10<sup>3</sup> cells/ml) were added to each tube and incubated at 37 °C for

24 h. The lowest concentration of the tubes which did not show any visible growth after macroscopic evaluation was considered as the MIC.

IC<sub>50</sub> defined as the drug concentration that produces 50% of the maximal effect [19] was determined as following. After one day (24 h) of incubation at 37°C for 24 h, the colonies were then counted for each tube. The dose - response curve was established in term of percentage of survivors:

$$S (\%) = \frac{n}{N} \times 100$$

Where

n = number of UFC counted in experimental tube

N = number of UFC in control tube

The test had been conducted in triplicate.

### Determination of MBC

The Minimal Bactericidal Concentration (MBC) is defined as the concentration producing a 99.9 % reduction of colony number in the initial inoculum. It was determined by subculture on nutrient agar as previously described<sup>19</sup>. After the MIC determination, the tubes without growth (concentration = MIC) were sub-cultured on Mueller Hinton agar in Petri dishes at 37°C for 24 h. MBC was defined as the lowest concentration of the sub-cultured tubes which did not show any visible growth after macroscopic evaluation.

All the antibacterial parameters have been determined after triplicate assays.

## Results

The extract exhibited some degree of antimicrobial action on the three MDR strains.

The MDR strain of *S. typhimurium* presented the highest MIC value (2.5 mg/ml). The MDR strains of *S. Typhi*, *S. hadar* and the sensitive strain of *S. enteritidis* presented the same MIC (1.25 mg/ml) (Table 1). All the tested strains had the same value of MBC (2.5 mg/ml).

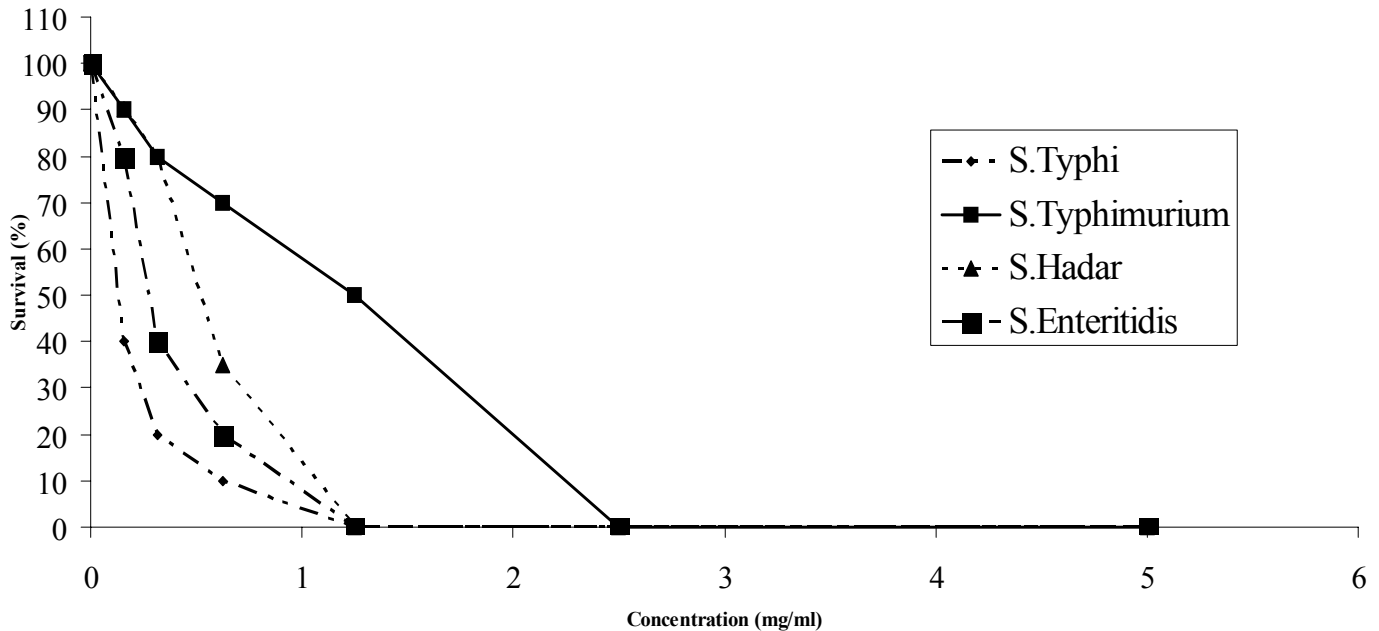
**Table 1 : Antibacterial parameters of the aqueous extract of *Thonningia sanguinea***

	Antibacterial parameters		
	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
<i>S. Typhi</i> (MDR)	1.25	2.5	2
<i>S. Hadar</i> (MDR)	1.25	2.5	2
<i>S. Typhimurium</i> (MDR)	2.5	2.5	1
<i>S. Enteritidis</i> (sensitive)	1.25	2.5	2

MIC and MBC led to the calculation of MBC/MIC (table 1) and this value was equal to 1 for the MDR strain of *S.typhimurium* and 2 for the other MDR strains. These values (MBC/MIC) indicated that the aqueous extract of the flowers of *Thonningia sanguinea* showed a bactericidal action on all the four tested strains.

The dose-response curve (Figure 1) led to the determination of the  $IC_{50}$  (table 2). The  $IC_{50}$  values for *S.typhi*, *S.Hadar*, *S.typhimurium* were respectively 0.10 mg/ml, 0.45 ml and 1.25 mg/ml. According to the values of  $IC_{50}$ , between the MDR strains, *S.Typhi* was the most susceptible to the extract and *S.Typhimurium* is the less susceptible one.

**Figure 1: Dose response curves for the  $IC_{50}$  determination**



**Table 2:  $IC_{50}$  values of the aqueous extract on the *Salmonella* strains tested**

	$IC_{50}$ (mg/ml)
<i>S. Typhi</i> (MDR)	0.10
<i>S. Enteritidis</i> (Sensitive)	0.25
<i>S. Hadar</i> (MDR)	0.45
<i>S. Typhimurium</i> (MDR)	1.25

The comparison of the antimicrobials parameters of the MDR strains with those of the sensitive strain showed that they had the same MBC (2.5 mg/ml). *S. Typhi* and *S. Hadar* possessed the same MIC (1.25 mg/ml) with the sensitive strain. The  $IC_{50}$  of the sensitive strain is higher than the  $IC_{50}$  of *S. Typhi* but it is smaller than the  $IC_{50}$  of the others MDR strains. *S. typhi* is most susceptible to the extract than all the others tested strains including the sensitive one.

### Discussion and conclusion

In this study, the aqueous extract of *T.sanguinea* was tested against one sensitive strain namely *Salmonella* Enteritidis and three MDR strains (*S. Typhi*, *S. Hadar* and *S. Typhimurium*). The red colour of the aqueous extract of *Thonningia sanguinea* in solution created interferences with the turbidity so it was difficult to determine with certainty the MIC by the recommended broth dilution method. This technical difficulty led us to use an alternative method (agar slant method) to evaluate the antimicrobial parameters of this kind of extract. Our results showed bactericidal effect of the aqueous extract of *Thonningia sanguinea* for all the MDR and sensitive tested strains. Moreover, *S. Typhi* (MDR), *S. Hadar* (MDR) and *S. Enteritidis* (sensitive) presented the same susceptibility to the aqueous extract of *Thonningia sanguinea*. These results indicate that the aqueous extract of *Thonningia sanguinea* contains some substances having target sites other than those used by antibiotics at

which these strains are resistant. The inhibition of The MDR strains of *S. Typhi* responsible of Typhoid fever and *S. Hadar* which is an emerging MDR strains in Ivory Coast<sup>8</sup> is a promising result for the treatment of salmonellosis induced by these MDR strains.

The antimicrobial activities of the flowers of *T. sanguinea* have already been reported by M'baïasbé<sup>15</sup>. Using the disk diffusion method to evaluate the antimicrobial activity of the aqueous extract of *T. sanguinea* on the growth of *S. Enteritidis*, this author found IC<sub>50</sub> value of 4.68 mg/ml. Ouattara *et al.*<sup>16</sup> using the broth dilution method (recommended method for the antibacterial assays) have also tested the antimicrobial effect of this extract on *Salmonella* Enteritidis. They reported MIC and IC<sub>50</sub> values respectively 1.25 mg/ml and 0.189 mg/ml. Using in this study the agar slant method, our results gave the same MIC on *S. Enteritidis* with those of Ouattara *et al.*<sup>16</sup> and the IC<sub>50</sub> values are similar. It is possible to say that the agar slant method can be also used as an alternative method to evaluate the antibacterial activity of coloured extracts.

The phytochemical screening of the extract of the flowers of *Thonningia sanguinea* have shown the presence of saponins, quinons, polyphenols<sup>20</sup>. These three classes of secondary metabolites are known to possess antibacterial activities<sup>21</sup> and they may be responsible of the antimicrobial activity of the flowers of *T. sanguinea*.

The results of this study suggest that *Thonningia sanguinea* can provide alternative solution for the treatment of salmonellosis particularly in Ivory Coast where this pathology is always a public health problem<sup>8</sup>.

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