

## ANTIBACTERIAL POTENCIES OF TEN IVORIAN MEDICINAL PLANTS AGAINST MULTI DRUG RESISTANT STRAINS OF SALMONELLA

### ABSTRACT

**Aim:** The purpose of this study was to evaluate the antisalmonella activity of the aqueous and 70% ethanol extract of ten (10) Ivorian plants used in traditional medicine to cure infectious diseases against two (2) clinical multi-drug resistant strains of Salmonella typhi and Salmonella typhimurium.

**Methods:** The antimicrobial parameters (Minimal inhibitory Concentration MIC and Minimal Bactericidal Concentration MBC) were determined by the broth dilution method and the diameters of inhibition zone were determined by agar disc diffusion method.

**Results:** The aqueous extract of Thonningia sanguinea and 70% ethanol extract of Abrus precatorius had the same and the best MIC and MBC values respectively 5mg/ml and 10mg/ml for the multi drug resistant strains of Salmonella tested. Moreover, the inhibition diameters indicate that the aqueous extract of Thonningia sanguinea showed the best significant activity against the multi-drug resistant strain of Salmonella typhimurium ( $11 \pm 0, 57$ ).

**Conclusion:** The aqueous extract of Thonningia sanguinea and 70% ethanol extract of Abrus precatorius can provide an alternative therapy for the treatment of salmonellosis generated by multi drug resistant strains.

**Key words:** Minimal Inhibitory Concentration, Minimal Bactericidal Concentration, antimicrobial activity, Thonningia sanguinea, Abrus precatorius

### INTRODUCTION

Salmonella Spp is a primary cause of food poisoning worldwide. The center for disease control and prevention estimated that approximately 1.4 millions cases of salmonellosis were annually reported in the United States<sup>1</sup>. It is also a public health problem in developing countries. Salmonella enterica serovar Typhi causes approximately 10 million cases of typhoid fever each year mostly in developing countries<sup>2, 3</sup>. In developing countries, enteric fever (typhoid) is more severe due to poor hygiene, indiscriminate use of antibiotics, and a rapid rise in multi drug resistance. In Côte d'Ivoire (West Africa), the typhoid fever and the over forms of salmonellosis became these last years a problem of public health taking into account the degradation of the conditions of healthiness in relation to the civil war occurred from 2002 to 2011. In recent years there has been a rapid rise in multi drug resistance by Salmonella typhi all over the world<sup>4, 5, 6</sup>. The world health organisation estimated an annual rate of 12, 6 million typhoid fever infections with nearly 600000 deaths every year<sup>7</sup>. Resistance to the first line drugs, chloramphenicol, ciprofloxacin and amoxicillin, in the course of salmonellosis management has been reported<sup>8</sup>. 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 which could provide alternative therapies against salmonellosis.

Previous results revealed the antibacterial activities, with MIC varying from 5 mg/mL to 40 mg/mL, of aqueous and ethanolic extracts of ten Ivorian medicinal plants against two sensitive strains of Salmonella typhi and Salmonella typhimurium<sup>9</sup>. At the best of our knowledge, none study had been done to assess the sensibility of MDR strains of salmonella with the same plants except the aqueous extract of Thonningia sanguinea on Salmonella typhi DT 104<sup>10</sup>.

With regard to foregoing considerations, the present study was undertaken to evaluate the antibacterial activity of the ten plants against two clinical MDR strains of salmonella. The minimal inhibitory concentrations (MIC) and the minimal bactericidal concentrations (MBC) were determined for the aqueous and 70% ethanol extracts of each plant.

## **MATERIAL AND METHODS**

### **Collection of plant material**

All plants were collected from the wild in different area of Côte d'Ivoire and were identified and authenticated by PrAkéAssi of the department of Botany, university of Cocody-Abidjan. Vouchers specimens were deposited in the herbarium of "National Floristic Center" of Abidjan. The plant species, parts used, local name, voucher specimen numbers and the traditional uses of the plants are listed in Table 1.

### **Bacterial strains**

Bacteria (clinical strains) for testing purposes were kindly provided by the National Laboratory of Public Health of Côte d'Ivoire.

Salmonella typhi resistant to chloramphenicol and cefotaxim

Salmonella typhimurium resistant to cefotaxim and ceftriaxon

They were sub cultured on nutrient agar for 15 days and maintained on nutrient agar slants at 4°C. Fresh inoculums were taken for test.

### **Preparation of aqueous extracts**

The plant extracts were prepared using the method of Guédé-Guinaet al.<sup>11</sup>. The freshly collected flowers, leaves and seeds of the plants were air dried at room temperature for 7 days and powdered. Briefly 20g of powder were soaked in 500mL distilled water for 24h with constant stirring. The suspension was further filtered through clean sterile muslin cloth and watmann N°1 filter paper. The filtrate was concentrated in vacuum using a rotary evaporator to obtain the aqueous extract.

### **Preparation of 70% ethanol extracts**

The plant extracts were prepared using the method of Zirihiet al.<sup>12</sup>. Briefly 20g of powder were soaked in 300ml 70% ethanol [ethanol/water (70/30, V/V)]. The mixture was stirred during 24 hours, and then filtered through a clean sterile muslin cloth. The filtrate was decanted for 24h. The hydroalcoholic solution was filtered through watmann N°1 filter paper and concentrated in vacuum at 40°C to obtain the 70 % ethanolic extract.

### **Preparation of different fractions**

Twenty (20)g of aqueous extract of Thonningiasanginea and 70% ethanolic extract of Abrus precatorius were soaked respectively in 100mL distilled water and 70% ethanol. This mixture was decanted in a bulb of 500mL with 100mL of cyclohexane. The solution was shaken for 20mn then was decanted. The cyclohexane fraction was collected and evaporated. The aqueous superior phase was extracted with 100mL of dichloromethane. The dichloromethane fractions were collected and evaporated. The aqueous superior phase was extracted at last with 100 mL of ethyl acetate. The ethyl acetate fractions were collected and evaporated. The different fractions were tested on the growth of Salmonella typhimurium.

### **Evaluation of antisalmonella activity**

#### **Determination of MIC**

The minimal inhibitory concentration (MIC) was determined by broth dilution method. The minimal inhibitory concentration (MIC) was determined according Wilkinson and Gentry<sup>13</sup>. Two fold dilutions of the extract were made in the concentration range of 1, 25 mg/ml to 80mg/ml. The tubes were inoculated with a microorganism suspension at a final density of 10<sup>6</sup> cells/mL. The tubes were incubated at 37°C for 24h. The lowest concentration of the tube which did not show any visible growth after macroscopic evaluation was considered as the MIC.

### Determination of MBC

The minimal bactericidal concentration (MBC) is defined as the concentration producing a 99.99% reduction in colony forming units (CFU) number in the initial inoculum. It was determined by subculture on nutrient agar as previously described<sup>14</sup>. The tubes without growth after 24 h of incubation were sub cultured on Mueller Hinton agar in Petri dishes for 24h. MBC was determined as the lowest concentration that showed no bacterial growth in the subcultures<sup>15</sup>.

### Determination of antimicrobial activity

The antibacterial activity of aqueous, cyclohexane, dichloromethane, ethyl acetate and residual fractions of *Thonningia sanguinea* and *Abrus precatorius* against *Salmonella typhimurium* were performed by the agar disc diffusion method<sup>16,17</sup>. The media (Mueller Hinton) along with the inoculum of *Salmonella typhimurium* ( $10^6$  cfu/mL) was poured into the Petri plate. The disc (0.7 cm) was saturated with 50  $\mu$ L of the test compound, allowed to dry and then placed on the upper layer of the seeded agar plate. The plates were incubated at 37°C for 24h. Antibacterial activity was determined by measuring the inhibition zone diameters (mm) surrounding bacterial growth. The results were compared with the standard antibiotic: ciprofloxacin (5  $\mu$ g/mL).

All the antibacterial parameters have been determined after triplicate assays.

### Statistical analysis

The data are presented as mean  $\pm$  SEM. All the data were analyzed by one-way ANOVA and differences between the means were assessed with Neuman-Keuls's multiple comparison tests. Differences were considered significant at  $p < 0.05$ . All analyses were carried out using Graph Pad software, version 5.01 (USA).

## RESULTS AND DISCUSSION

A total of 20 aqueous and 70% ethanol extracts from 10 different plant species were screened for antibacterial activity against two (2) clinical multi-drug resistant strains of *Salmonella* namely *Salmonella typhi* and *Salmonella typhimurium*. The results of the antisalmonella activity are shown in the table 2.

The MIC values varied from 5 to 80 mg/mL and MBC values from 10 to 80 mg/mL. The aqueous extract of *Thonningia sanguinea* and 70% ethanol extract of *Abrus precatorius* had the same and the best MIC and MBC values respectively 5 mg/mL and 10 mg/mL against the two multi-drug resistant strains of salmonella. The aqueous extracts of *Abrus precatorius*, *Tectonagrandis*,

*Vernonia amygdalina*, *Nauclea latifolia* and *Olax subscorpioides* presented MIC values of 40 mg/ml against *Salmonella typhimurium*. The 70% ethanol extracts of *Nauclea latifolia* and *Ageratum conyzoides* exhibited the same MIC and MBC values respectively 40 mg/ml and 80 mg/ml against *Salmonella typhi*, and *Salmonella typhimurium*.

The 70% ethanol extracts of *Acanthospermum hispidum* and *Manihot esculenta* presented MIC values of 80 mg/ml against *Salmonella typhi*. The 70% ethanol extracts of *Tectonagrandis* exhibited MIC values of 80 mg/ml and MBC values of 80 mg/ml against *Salmonella typhimurium*. The aqueous extract of *Acanthospermum hispidum* presented MIC values of 40 mg/ml against *Salmonella typhimurium*. The extracts of *Cycas revoluta*, the aqueous extract of *Ageratum conyzoides*, *Manihot esculenta*, the 70% ethanol extract of *Vernonia amygdalina* showed no antibacterial activity against all the strains. The above results showed that eight (8) of the tested plants possessed weak antisalmonella activity against the multi-drug resistant tested strains. Two plants, namely *Thonningia sanguinea* and *Abrus precatorius* are the more potent antibacterial plants among the tested one against the two multi drug resistant MDR strains of salmonella according to the MIC and MBC values.

The aqueous extract of *Thonningia sanguinea* has previously showed good antibacterial activities against extended spectrum- $\beta$ -lactamases (ESBL) strains of *Klebsiella pneumoniae* and *Escherichia coli* with MIC of 6.25 mg/mL<sup>18</sup>. Moreover, N'guessan et al.<sup>10</sup> have also pointed out the antimicrobial activity of *Thonningia sanguinea* against *Salmonella enterica* (DT 104). Our results are in accordance

with these results and provide a supplementary proof of the presence of compounds with promising antimicrobial activities against MDR strains of bacteria.

Furthermore, the work of Bolou et al.<sup>9</sup> showed that these plants possessed interesting antibacterial activities on sensitive strains of *Salmonella typhi* and *Salmonella typhimurium*. Our results showed that these plants are also inhibitors of the growth of MDR strains of *Salmonella*.

The results on antibacterial activity of different fractions of *Thonningiasanguinea* and *Abrusprecatorius* were shown in table 3. The aqueous extract of *Thonningiasanguinea* showed the highest significant activity ( $11 \pm 0,57$ ) followed by the ethyl acetate fraction of *Thonningiasanguinea* ( $10,3 \pm 0,4$ ), the ethyl acetate fraction of *Abrusprecatorius* ( $10 \pm 0,0$ ), the residual fraction of *Thonningiasanguinea* ( $9 \pm 1,15$ ), 70% ethanol extract of *Abrusprecatorius* ( $9 \pm 1,0$ ), the residual fraction of *Abrusprecatorius* ( $8 \pm 0,57$ ), the dichloromethane fraction of *Thonningiasanguinea* ( $8 \pm 0$ ), the dichloromethane fraction of *Abrusprecatorius* ( $7,6 \pm 0,33$ ) and the cyclohexane fraction of *Thonningiasanguinea* ( $6,6 \pm 0,33$ ) against the MDR strain of *Salmonella Typhimurium*. The cyclohexane fraction of *Abrusprecatorius* showed no pronounced antibacterial activity against *Salmonella typhimurium*.

The results showed no significant improving of the antisalmonella activities of the aqueous extract of *Thonningiasanguinea* and 70% ethanol extract of *Abrusprecatorius*.

This study may not be adequate to suggest potential antibiotic agent considering the zone of inhibition which could be affected by the solubility and rate of diffusion in agar medium or its volatilization which could affect the results. However, this approach could be considered as preliminary step to find out promising candidates<sup>19</sup>. It is essential to apply other fractionation methods.

## CONCLUSION

This study indicates that *Thonningiasanguinea* and *Abrusprecatorius* have the potential to generate novel antimicrobials metabolites against MDR strains of salmonella. *Thonningiasanguinea* and *Abrusprecatorius* can provide alternative solution for the treatment of salmonellosis particularly in Côte d'Ivoire.

species(family)	voucher number	Plant part tested	local name	popular use
Abrus precatorius Linn. (Fabaceae)	CNF/875	Seed	Alobogna	Dermatose, wound, cough
Acanthospermum hispidum Schrank. (Asteraceae)	CNF/16762	Leaf	Lukoubassa moni	Paludism, typhoid fever
Ageratum conyzoides Linn. (Asteraceae)	CNF/635	Leaf	Koitndré	Typhoid fever
Cycas revoluta Thunb. (Cycadaceae)	CNF/1235	Leaf	efou	Typhoid fever
Manihot esculenta Crantz (Euphorbiaceae)	CNF/9024	Leaf	Agbagna	Paludism, Typhoid fever
Nauclea latifolia SM. (Rubiaceae)	CNF/15927	Leaf	Tôle	Typhoid fever
Olax subscorpioides Oliv. (Olacaceae)	CNF/10756	Leaf	ifon	Paludism
Tectona grandis Linn. (verbenaceae)	CNF/842	Leaf	Tec	Typhoid fever
Thonningia sanguinea Vahl. (Balanophoraceae)	CNF/17954	Flower	Glouglan	Typhoid fever
Vernonia amygdalina Del. (Asteraceae)	CNF/11694	Leaf	Abohoui	Paludism, Typhoid fever

References for uses: 20 and 21

**Table 1: List of medicinal plants used in the antisalmonella assay**

**Table 2: Antibacterial activities of aqueous and 70% ethanol extracts of different medicinal plants against Salmonella strains.**

Family	Name of Plant	Plant part	Extract	MIC mg/ml		MBC mg/ml	
				S.Typhi	S.Typhim	S.Typhi	S.Typhim
Balanophoraceae	Thonningia sanguinea	F	AE	5	5	10	10
			ETH	80	80	80	80
Fabaceae	Abrus precatorius	S	AE	80	40	> 80	> 80
			ETH	10	5	10	10
Verbenaceae	Tectona grandis	L	AE	> 80	40	> 80	> 80
			ETH	> 80	80	> 80	80
Asteraceae	Vernonia amygdalina	L	AE	> 80	40	> 80	> 80
			ETH	> 80	> 80	> 80	> 80
Olacaceae	Olax subscorpioides	L	AE	> 80	40	> 80	> 80
			ETH	> 80	> 80	> 80	> 80
Rubiaceae	Nauclea latifolia	L	AE	80	40	80	> 80
			ETH	40	40	40	40
Asteraceae	Acanthospermum hispidum	L	AE	> 80	40	> 80	> 80
			ETH	80	> 80	80	> 80
Cycadaceae	Cycas revoluta	L	AE	> 80	> 80	> 80	> 80
			ETH	> 80	> 80	> 80	> 80
Euphorbiaceae	Manihot esculenta	L	AE	> 80	> 80	> 80	> 80
			ETH	80	> 80	80	> 80
Asteraceae	Ageratum conizoides	L	AE	> 80	> 80	> 80	> 80
			ETH	80	80	80	80

AE= Aqueous Extract

ETH= 70% Ethanol Extract

S.= Salmonella

Typ= Typhi

L= Leaves

S= Seed

Typhim= Typhimurium F=

Flower

MIC= Minimal Inhibitory Concentration

MBC= Minimal Bactericidal Concentration

**Table 3: Antibacterial activities of different fractions of *Thonningia sanguinea* and *Abrus precatorius* against *S. Typhimurium*.**

Name of the plant	Fractions	Diameter of inhibition zone (mm) <i>S. Typhimurium</i>
<i>Thonningia sanguinea</i> (Fl)	Aqueous	11± 0,57
	Cyclohexane	6,6± 0,33
	Dichloromethane	8
	Ethyl acetate	10,3± 0,4
	Residue	9± 1,15
<i>Abrus precatorius</i> (S)	70% ethanol	9± 1,0
	Cyclohexane	-
	Dichloromethane	7,6± 0,33
	Ethyl acetate	10
	Residue	8± 0,57
Ciprofloxacin 5µg		35

Fl: Flower, S: Seed, -: No Activity, S: Salmonella

Note: Values are highly significant at 5% level

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