

## Research Article

# Antibacterial activity of the aqueous extract of *Thonningia sanguinea* against Extended-Spectrum- $\beta$ -Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* strains

JD N'guessan<sup>1</sup>, MR Dinzedi<sup>1,2</sup>, N Guessennd<sup>2</sup>, A Coulibaly<sup>1</sup>, M Dosso<sup>2,3</sup>, AJ Djaman<sup>3</sup>, F Guede-Guina<sup>1</sup>

<sup>1</sup>Laboratoire de Pharmacodynamie Biochimique, 22 BP 582 Abidjan 22, Côte d'Ivoire

<sup>2</sup>Département de Bactériologie & Virologie- Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan 01, Côte d'Ivoire

<sup>3</sup>Laboratoire de Biochimie - Institut Pasteur de Côte d'Ivoire, 01 BP 490 Abidjan, Côte d'Ivoire

## Abstract

**Purpose:** The aim of this study was to evaluate the antimicrobial activity of *Thonningia sanguinea* against two sensitive and two multi-drug resistant (ESBL) Enterobacteria strains namely *Escherichia coli* and *Klebsiella pneumoniae*.

**Method:** The confirmation of the ESBL producing strains was done by the double-disc synergy tests and the broth dilution method was used for the determination of the antimicrobial parameters (MIC and MBC) on these sensitive and ESBL producing strains.

**Results:** The two sensitive strains had the same MIC and MBC values respectively 3.125 mg/ml and 12.50 mg/ml. The ESBL producing strains also had the same MIC of 6.25 mg/ml and MBC values of 25 mg/ml. The extract was bactericidal for all tested strains.

**Conclusion:** The results suggest that the flowers of *T. sanguinea* can be used in association with antibiotics for alternative therapy of diseases caused by ESBL producing *E. coli*, *K. pneumoniae*.

**Key words:** antimicrobial activity, *Thonningia sanguinea*, ESBL producing strains; *E. coli*.

---

\*Corresponding Author: E-mail: [nguess100jd@yahoo.fr](mailto:nguess100jd@yahoo.fr) Tel : (00225) 05-78-57-89

## INTRODUCTION

Medicinal plants have long been utilized as a source of therapeutic agents worldwide. Recently, herbal medicines have increasingly been used to treat many difficult diseases including several infections. In Ivory Coast, *Alternanthera repens* (Zakrékokréko) and *Kalankoe crenata* (Kpakolo), respectively, are used for the treatment of paludism and asthma<sup>1</sup>. Plants are known to produce certain chemicals which are naturally toxic to bacteria<sup>2</sup>. In fact, the extract of each plant contains different secondary metabolites<sup>3</sup>.

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day<sup>4</sup>. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world<sup>5</sup>. Among the wide array of antibiotics,  $\beta$ -lactams are the most varied and widely used agents accounting for over 50% of all systemic antibiotics in use<sup>6</sup>. The most common cause of bacterial resistance to  $\beta$ -lactam antibiotics is the production of  $\beta$ -lactamases. Bacterial resistance to  $\beta$ -lactam antibiotics has significantly increased in recent years. This increase has been attributed to the spread of plasmid-mediated extended spectrum  $\beta$ -lactamases (ESBLs)<sup>7</sup>. ESBLs occur predominantly in the family of *Enterobacteriaceae*. *Klebsiella pneumoniae* and *E.coli* are the main species in which ESBL enzymes have been most commonly reported worldwide, and it is responsible for 5% – 20% of outbreaks of nosocomial infections in intensive care units, as well as burn, oncology and neonatal units<sup>8</sup>. ESBL-producing strains are usually resistant to all aminoglycosides, third- and fourth-generation cephalosporins and monobactams<sup>9</sup>.

For the time being, these strains remain generally susceptible to cephamycins, carbapenems and  $\beta$ -lactamase inhibitor- $\beta$ -lactam combinations<sup>9</sup>.

The present scenario of emergence of multiple drug resistance of human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants<sup>10</sup>. In previous studies, the extract of the flowers of *Thonningia sanguinea* showed promising

antibacterial activity against a MDR strain of *Salmonella* Enteritidis Lysotype 6, a member of *Enterobacteriaceae* family<sup>11</sup>.

In the present study, we have chosen this ivoirien medicinal plant *T. sanguinea* to screen its antimicrobial activity (MIC and MBC) against others multi-drug resistant (ESBL) *Enterobacteria* namely *Escherichia coli* and *Klebsiella pneumonia* and two sensitive strains of these bacteria.

## MATERIAL AND METHODS

### Plant material

*T. sanguinea* flowers were collected in Adzopé, Côte d'Ivoire (West Africa) and identified by Pr Aké-Assi of the Department of Botany, University of Cocody-Abidjan. A voucher specimen (Voucher no. 14162) is deposited in the herbarium of Centre National de Floristique (CNF) of Abidjan.

### Bacterial strains

The biological assays were carried out on 4 hospital isolates provided by the Laboratoire de Bactériologie & Virologie of Institut Pasteur de Côte d'Ivoire. The strains were house referenced. According to the activity of known antibiotics, two kinds of strain were distinguished: some strains were sensitive (*E. coli* no. 513/06; *Klebsiella pneumoniae* no. 515/06) and others were resistant (*E. coli* ESBL no. 1963; *Klebsiella pneumoniae* ESBL no. 1911).

### Extraction procedure

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 Whatman (No. 1) filter paper. The filtrate was concentrated *in vacuo* using a rotary evaporator to obtain the aqueous extract.

### Antibacterial tests

#### Confirmatory test for ESBL-producing *K. pneumoniae* and *E.coli* isolates

The double-disc synergy tests were used as screening tools to detect ESBL-producing strains. In the double-disc synergy test, cefotaxime (30 $\mu$ g), ceftazidime (30 $\mu$ g), cefepime (30  $\mu$ g) and Aztreonam (30 $\mu$ g) discs were placed

**Table 1:** Antibacterial activity of *Thonningia sanguinea*

Sensitive strains	Antibacterial parameters (mg/ml)		
	MIC	MBC	MIC/MBC
<i>E. coli</i> N°513/06	3.125	12.50	4
<i>K. pneumoniae</i> N° 515/06	3.125	12.50	4
<b>ESBL strains</b>			
<i>E. coli</i> N° 1963	6.250	25	4
<i>K. pneumoniae</i> N° 1911	6.250	25	4

on Mueller-Hinton agar adjacent to a co-amoxiclav disc (20µg amoxicillin plus 10µg clavulanate). All discs were purchased from Biorad (France). The procedures and interpretation of the double-disc synergy test were described previously<sup>12</sup>.

#### Determination of Minimum Inhibitory Concentration (MIC)

The Minimal Inhibitory Concentration (MIC) was determined according to Wilkinson and Gentry<sup>13</sup>. Two-fold dilutions (six) of the extract were carried out starting from the concentration of 5 mg/ml. The tubes were inoculated with a microorganism suspension at a final density of 10<sup>5</sup> cells/ml. The tubes were incubated at 37 °C for 24 h. 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 99.9% 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 subcultured on Mueller Hinton agar in Petri dishes for 24 h. MBC was determined as the lowest concentration that showed no bacterial growth in the subcultures<sup>15</sup>.

## RESULTS

### Confirmatory test

Phenotypic confirmation of ESBLs was carried out by the double-disc synergy tests (Figure 1). Of the four clinical isolates of *K. pneumoniae* and *E. coli*, two were confirmed to be ESBL producing isolates by the double disc synergy test according to Wu et al.<sup>12</sup>. Double-disc tests showed synergy for co-amoxiclav with cefotaxime, aztreonam and cefepime against both *E. coli* (no. 1963) and *K. pneumoniae* (no. 1911) strains. The tests were performed in triplicate.

### Antimicrobial activity of the extract

The sensitive strains tested showed some degree of sensitivity to the aqueous extract. The MIC results of the extract on the different strains are shown in Table 1.

The two sensitive strains had the same MIC and MBC values (3.125 mg /ml and 12.50 mg/ml, respectively). The calculation of MBC/MIC ratio shows that this value is equal to 4 for *K.pneumoniae* and *E.coli* (Table1). The aqueous extract of the flowers of *Thonningia sanguinea* is bactericidal for these tested strains.

The ESBL producing strains tested also showed various degrees of sensitivity to the aqueous extract. The resistant strains had the same MIC value of 6.25 mg /ml) and MBC value of 25 mg/ml.

The calculation of MBC/MIC shows that this value is equal to 4 for all the ESBL producing strains (Table 1). The aqueous extract of the flowers of *Thonningia sanguinea* is bactericidal for these tested strains. The extract was bactericidal at the MBC concentrations for all the tested strains.

## DISCUSSION

The pathogenic role of *E. coli*, *K. pneumoniae* infection in the development of human diseases and the impact of resistance on the clinical outcome stimulated the search for newer treatments and the use of natural agents as alternative therapies. The therapeutic challenges resulting from emerging antimicrobial resistances have compromised chemotherapy for hospitalized patients with severe infections. ESBL is one of the prevalent resistance problems. Most of the clinical isolates producing ESBL have originated from hospitalized patients and have frequently caused nosocomial outbreaks<sup>16</sup>.

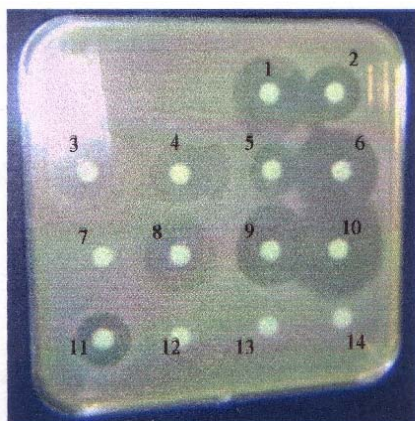
In the present investigation, we have evaluated the antimicrobial activity of the aqueous extract of *T. sanguinea* against sensitive and ESBL strains of *E. coli* and *K. pneumoniae*.

Our results clearly indicated the inhibitory effects of the extract of *T. sanguinea* on these bacteria. Ohiri and Uzodinma<sup>17</sup> had already shown the inhibitory activity of *T. sanguinea* on sensitive strains of *E. coli*, *K. pneumoniae*.

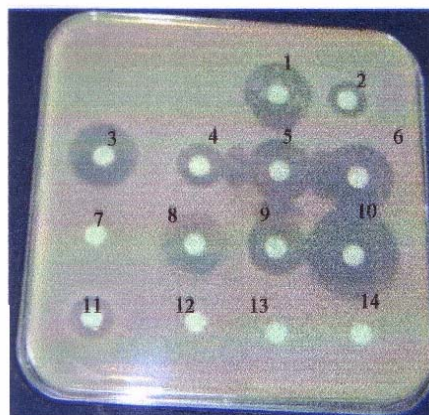
In our study, an interesting finding is the inhibition of the ESBL strains by the aqueous extract of *T. sanguinea*. The results showed significant inhibition with promising antibacterial

parameters (MIC values between 3.125 and 6.250 mg/ml). The minimum bactericidal concentration (MBC) was always found to be 4-fold higher than MIC values. The results revealed that the extract exhibited bactericidal activity against the tested strains. *T. sanguinea* is traditionally used in Côte d'Ivoire for the treatment of diarrhoeal diseases. Thus, these results support its use in traditional medicine. Previous phytochemical screening of the aqueous extract of the flowers of *T. sanguinea* has shown the presence of saponins, quinones, flavonoids<sup>18</sup>.

The flavonoids in the extract may account for the antimicrobial activity against *K. pneumoniae*. This group of secondary metabolites have also been detected in *T. sanguinea* by Ohiri and Uzodinma<sup>17</sup>. Flavonoids are common secondary metabolites in plants. They were described in previous papers as exhibiting antibacterial activities against gram-positive [e.g. methicillin-resistant *Staphylococcus aureus* (MRSA)] and *K. pneumoniae* bacteria<sup>10, 19</sup> as well as inhibiting the growth of lactic acid bacteria of human gastrointestinal tract origin<sup>20</sup>. Lin et al.<sup>10</sup> proposed the combination of antibiotics and flavonoids as a potential new strategy for the development of new therapies for infections



ESBL producing *E. coli*



ESBL producing *K. Pneumoniae*

**Figure 1:** Confirmatory tests of ESBL strains (1 = Ceftazidime; 2 = Ceftriaxone; 3 = Cefoxitin; 4 = Cefotaxime; 5 = Amoxicilin+Clavulanic acid; 6 = Aztreonam; 7 = Cefalotin; 8 = Meticilinam; 9 = Cefepime; 10 = Imipenem; 11. Piperaciline; 12 = Ticarcilin; 13 = Amoxicilin; 14 = Ampicilin)

caused by ESBL-producing bacteria in the future.

### Conclusion

From the foregoing, our results suggest that the flowers of *T. sanguinea* can be used in association with antibiotics for alternative therapy of diseases caused by ESBL producing *E. coli*, *K. pneumoniae*.

### REFERENCES

- Zirih GN. Contribution au recensement à l'identification et à la connaissance de quelques espèces végétales utilisées en médecine traditionnelle chez les Bété du département d'Issia, Côte d'Ivoire. Université de Cocody, UFR Biosciences, Thèse de Doctorat de 3<sup>ème</sup> cycle 1991 ; 253 p
- Basile A, Giordano S, Lopez-Saez JA, Cobianchi RC. Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry* 1999; 52: 1479–1482.
- Singh B, Bhat TK. Potential therapeutic applications of some antinutritional plant secondary metabolites. *J Agric Food Chem* 2003; 51: 5579–5597.
- Iqbal A, Arina ZB. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol* 2001; 74 (2001) : 113–123
- Piddock LJV, Wise R. Mechanisms of resistance to quinolones and clinical perspectives. *J Antimicrob Chemother* 1989; 23(4): 475-480
- Bronson JJ, Barrett JF. Quinolone, Everminomycin, Glycylcycline, Carbapenem, Lipopeptide and Cephem Antibacterials in Clinical Development. *Curr Med Chem* 2001; 8 :1775-93.
- Sanders CC, Sanders WE Jr. Clinical importance of inducible beta-lactamases in gram-negative bacteria. *Eur J Clin Microbiol* 1987; 6: 435–438.
- Kohler J, Dorso KL, Young K, Hammond GG, Rosen H, Kropp H, Silver LL. In vitro activities of the potent, broad-spectrum carbapenem MK-0826 (L-749,345) against broad-spectrum beta-lactamase and extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates. *Antimicrob Agents Chemother* 1999; 43: 1170–1176.
- Livermore DM. Beta-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995. 8: 557–584.
- Lin RD, Chin YP, Lee MH. Antimicrobial activity of antibiotics in combination with natural flavonoids against clinical extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae*. *Phytother Res* 2005; 19(7): 612-7
- Ouattara K, Coulibaly A, N'guessan JD, Djaman AJ, Guédé-Guina F. Activité anti-diarrhéique de *Thonningia sanguinea* (THOS) sur les infections à *Salmonella Enterica* sérotype Enteritidis lysotype 6 chez la poule pondeuse. *Re Ivoir Sci Technol* 2005; 06: 151-160
- Wu TL, Siu L.K. , Su LH, Lauderdale TL, Lin FM, Leu HS, Lin TY, Ho M. Outer membrane protein change combined with co-existing TEM-1 and SHV-1 betalactamases lead to false identification of ESBL-producing *Klebsiella pneumoniae*. *J Antimicrob Chemother.* 2001; 47: 755– 761.
- Wilkinson ID, Gentry LO. In vitro comparison of ceftazidime and nine other antimicrobial agents against hospital strains of Gram-negative bacteria. *J Antimicrob Chemother* 1981; 8: 53–56.
- Riffel A, Medina LF, Stefani V, Santos RC, Bizani D, Brandelli A. In vitro antimicrobial activity of a new series of 1,4-naphthoquinones. *Braz J Med Biol Res* 2002; 35(7) : 811-818
- Suffredini IB, Bacchi EM, Sakuda TMK, Ohara MT, Younes RN, Varella AD. Antibacterial activity of Apocynaceae extracts and MIC of *Tabernaemontana angulata* stem organic extract. *Rev Bras Ciênc Farm*; 2002; 38: 89-94.
- Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998; 11: 589–603.
- Ohiri FC, Uzodinma VC. Antimicrobial properties of *Thonningia sanguinea* root extracts. *Fitoterapia* 2000; 71 (2): 176-178.
- Kouakou AV, N'guessan JD, Kra AKM and Guéde-Guina F. (2006) Etude de l'action antifongique et tri phytochimique de *Thonningia sanguinea* (THOS). *J Soc ouest-afr chim* 2006; 22: 21-25
- Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother Res*; 2001. 15: 39–43.
- Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from berries. *J Appl Microbiol* 2001; 90: 494–507.