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Antibacterial Properties of an Aqueous Extract of *Solanum torvum* (Solanaceae) on a Few Multidrug Resistant Bacterial Strains to Common Antibiotics

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Abstract

This study aimed to evaluate the antibacterial activity of the aqueous extract of *Solanum torvum* leaves (EASt) on multidrug-resistant bacterial strains to common antibiotics. This plant is used in traditional medicine against diarrhoea. Solid agar diffusion and liquid dilution methods were respectively used to assess the sensitivity of bacterial strains to EASt and to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Tests performed on ten (10) bacterial strains, isolated from various biological products, showed that *Staphylococcus aureus* 234 UB/17 and *Escherichia coli* 135 UB/17, were significantly sensitive to EASt, with a higher sensitivity for the latter strain. This sensitivity was shown by an inhibition of bacterial growth in solid agar and liquid medium, by a decrease in turbidity caused by EASt sensitive germs growth as the concentration of this plant extract increased (25 to 100 mg/ml). This demonstrate that EASt is an antibacterial substance. Thus, treated with EASt at a dose of 100 mg/ml, the MICs of *S. aureus* 234 UB/17 and *E. coli* 135 UB/17 were 6.25 and 12.5 mg/ml respectively, and the MBCs were 25 and 50 mg/ml respectively. The ratio of these bactericidal parameters (MBC/MIC) equaled to four (4) for these two bacterial strains, indicating that EASt is bactericidal for these bacterial strains. The antibacterial and bactericidal properties of EASt on multiple antibiotic resistant germs could justify the use of this plant in traditional medicine for the treatment of some bacterial infections causing diarrhoea.

Keywords: *Solanum torvum*; antibacterial; bactericidal; multidrug-resistant bacteria.

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1. Introduction

Since their discovery a century ago, antibiotics played a key role in medical therapeutic [2].Nowadays, the upsurge and widespread of infections caused by antibiotics resistant in human populations is due to therapeutic failures. Indeed, antibiotic resistance has become a public health issue and a real concern for scientific communities, the challenge is to find new natural antibacterial agents from medicinal plants used for the treatment of infectious diseases [6].

It is the case of *Solanum torvum* (Solanaceae), used in traditional medicine against diarrhoea [4]. Previous studies showed that the leaf extract of this medicinal plant exhibited an antibacterial and antiviral activities [9] and can be used to relieve anite and shingles [3].

The objective of this work was to evaluate the antibacterial properties of *Solanum torvum* (Solanaceae). For this purpose, the effects of the aqueous leaf extract of this plant were evaluated on the *in vitro* growth of bacterial strains resistant to usual antibiotics.

2. Material and methods

2.1. Material

2.1.1. Plant material

Fresh leaves of Solanum torvum (Solanaceae) were collected from the district of Cocody (Abidjan, Ivory Coast). Plant material was identified and authenticated by the National Floristic Center of the University of Felix Houphouet-Boigny (Abidjan, Ivory Coast). The voucher specimen number was 8377.

2.1.2. Bacterial strains

For this study, seven (7) strains of enterobacteriaceae and three (3) strains of multidrug-resistant Staphylococcus aureus, provided by ASSURMI (Institut Pasteur of Côte d'Ivoire), were used (**Table1**).

[1])

ESBL : *Extended-spectrum beta-lactamases* (*ESBL*)

KT : Kanamycin-Tobromycin

KTG : Kanamycin-Tobromycin-Gentamycin

Meti-R : Meticillin-resistant

KGNet : Kanamycin-Gentamycin-Netilmycin

Table 1: Profiles of tested bacteria

Code	Bacterial strain	Phenotypic profiles	Origin
076 CO/17	*Shigella sp*	-Fluoroquinolone-resistant strain -Betalactamine-resistant strain	Faeces
150 PI/17	*Salmonella para typhi B*	-Phenotype KTGN -Fluoroquinolone resistant strain -Betalactamine-resistant strain	Faeces
214 CA/17	*Shigella flexneri*	-Aminioside-resistantstrain -Bêtalactamine-resistantstrain	Faeces
118 UB/17	*Escherichia coli*	- *ESBL* - Fluoroquinolone-resistantstrain	Blood
135 UB/17	*Escherichia coli*	*ESBL*	Blood
146 PI/17	*Escherichia coli*	*ESBL*	Blood
238 UB/17	*Escherichia coli*	- *ESBL* - Fluoroquinolone-resistant strain	Blood
129 UB/17	*Staphylococcus aureus*	- Betalactamase - KT Phenotype	Blood
234 UB/17	*Staphylococcus aureus*	- Phenotype KTG - Meti-R Phenotype	Blood
241 UB/17	*Staphylococcus aureus*	- KTG Phenotype - Meti-R Phenotype	Blood

3. Methods

3.1. Preparation of plant extract

The preparation of the aqueous extract of *Solanum torvum* (EASt) was carried out according to the method described by [7]. Two hundred fifty (250) g of fresh leaves of this plant were sliced into small pieces and boiled for 15 minutes in 1.5 L of distilled water. The hot decoction was filtered twice on hydrophilic cotton, then once on Wattman filter paper No. 1. Filtrate was dried in an oven at 50 °C for 72 hours. After drying, the aqueous dry

extract of *S. torvum* (EASt) was obtained.

3.2. Determination of the extraction yield of Solanum torvum (Solanaceae)

The dry matter yield was determined by calculating the ratio between the weight of the dry matter and the weight of plant material used for extraction (fresh *Solanum torvum* leaves), according to the following formula :

$$\text{Yield \%} = \frac{m0}{m1} \text{ X } 100$$

m0 = Weight (g) of evaporated crude extract of S. torvum

m1 = Weight (g) of fresh leaves of S. torvum

3.3. Evaluation of the antibacterial activity of plant extract

3.3.1. Preparation of concentration range of the aqueous extract of Solanum torvum

The concentration range of the aqueous extract of *Solanum torvum*(EASt) was prepared in six test tubes numbered from 1 to 6 and one growth control tube (CT) by twofold dilution method, according to the method described by [8]. The prepared concentrations were 100; 50; 25; 25; 12.5; 6.25 and 3.12 mg/ml for EASt. However, for CT, the concentration was 0 mg/ml.

3.3.2. Inoculum preparation

For the inoculum preparation, two young colonies were collected from bacterial culture using a platinum loop calibrated at 2 µl and homogenized in 10 ml of Mueller Hinton broth, incubated for 3 hours at 37 °C, to obtain a pre-culture. Then, 0.1 ml of the pre-culture broth was collected for enterobacteriaceae and 0.3 ml for *Staphylococci* and introduced into a tube containing 10 ml of Mueller Hinton broth. This bacterial suspension was evaluated at about 10^{-6} cells/ml, representing the pure inoculum (inoculum 10^0) [15].

3.3.3. Inoculum count

The bacterial inoculum was diluted from 1/10 to 10^{-4} dilution. Four (4) successive dilutions, from 10^{-1} to 10^{-4}, were also obtained. The initial bacterial inoculum and the four successive dilutions are inoculated with a 2µl calibrated loop on Mueller Hinton agars with 5 cm long streaks, constituting box A [11]

3.3.4. Sensitivity test

Strains sensitivity to EASt was tested by the solid agar diffusion technique. Muëller Hinton agar was swabbed

as recommended by the Antibiogram Committee of the French Society of Microbiology [1]. Wells of approximately 6 mm in diameter were made in the agar using a sterile punchand each well received 80 µl of EASt at concentrations of 100; 50; 25; 12.5; 6.25 and 3.12 mg/ml. A cup, taken as a control, received 80 µl of sterile distilled water. After 30 minutes of pre-diffusion of the EASt at laboratory temperature (26 °C), the petri dishes were incubated at 37 °C for 24 hours. The presence or absence of an inhibition zone was observed after 24 hours of incubation [11]. The interpretation was made according to the indications of [13,5].

3.3.5. Inoculation

In a series of six (6) hemolysis tubes numbered T1 to T6, and in the growth control tube (CT), 1ml of pure inoculum was introduced. Then 1 ml of EASt was added to the 6 test tubes according to the prepared concentration range (100; 50; 25; 25; 12.5; 6.25 and 3.12 mg/ml). The control tube (CT) corresponding to the control received, instead of plant extract, 1 ml of sterile Mueller Hinton broth.

3.3.6. Determination of the minimum inhibitory concentration (MIC)

The MIC is the lowest concentration of the substance for which there is no growth visible to the naked eye after an incubation time of 24 h (Moroh and his colleagues 2008). Its determination is made by observation of the turbidity induced by the growth of organisms studied in each tube (T1 to T6 and CT).

3.3.7. Determination of the minimum bactericidal concentration (MBC)

MBC is the lowest concentration of substance that leaves at most 0.01 % of surviving germs [12]. Using a loop calibrated at 2 µl, the contents of the tubes in which no turbidity was observed, were collected and sown on a Mueller Hinton agar starting with the tube containing the MIC, as well as the growth control. Seeding were done by parallel striations 5 cm long on the surface of the agar (Box B).

After 24 hours of incubation in an oven at 37 °C, the number of colonies on the striae was compared to those in the inoculum count box (Box A).

4. Statistical analysis

Data analyses and graphical representations were carried out using Graph Pad Instat and Graph Pad Prism 4 softwares (Microsoft, San Diego, California, USA).

Values were expressed as mean ± standard of deviation. Data analysis was carried out using one way analysis of variance (ANOVA ONE WAY), followed by Tukey-kramer multi-comparison test and significance of difference was observed at $p < 0.05$.

5. Results

5.1. Yield of dry extract of Solanum torvum (Solanaceae)

19.66 g of dry aqueous extract (EASt) were obtained from 250 g of fresh leaves of *S. torvum*, with an extraction yield of 7.86%.

5.2. Sensitivity of bacterial strains to aqueous extract of Solanum torvum (EASt)

The sensitivity tests carried out showed that EASt, at concentrations ranging from 3.12 to 100 mg/ml, has a good growth inhibitory activity on *Escherichia coli* 135 UB/17 and *Staphylococcus aureus* 234 UB/17. Indeed, compared to control, the inhibition diameters induced by EASt on E. coli 135 UB/17 at concentrations of 50 and 100 mg/ml were 14.00 ± 0.00 mm ($P < 0.01$) and 18.00 ± 1.16 mm ($P < 0.001$) respectively and, on *S. aureus* 234 UB/17, the inhibition diameter was 13.33 ± 0.33 mm ($P < 0.01$) at a concentration of 100 mg/ml. However, a low sensitivity to EASt was observed at some other concentrations for these two bacterial strains; the growth inhibition diameters were 6 ± 0.00 mm ($P > 0.05$). Similarly, for the eight (8) other bacterial strains tested (*Shigella sp* 076 CO/17, *S. para typhi* B 150 PI/17, *S. flexneri* 214 CA/17, *E. coli* 118 UB/17, *E. coli* 146 PI/17, *E. coli* 238 UB/17, *S. aureus* 129 UB/17 and *S. aureus* 241 UB/17), the growth inhibition diameters were all 6 ± 0.00 mm ($P > 0.05$)at concentrations ranging from 3.12 to 100 mg/ml (Table 2). Figure 1 shows the growth inhibition diameters of different bacterial strains at a concentration of 100 mg/ml.

Table 2: Growth inhibition of bacterial strains by EASt

			Extract of *Solanum torvum*		
	Strains and codes	Origin	From 3,12 to 25 mg/ml	50 mg/ml	100 mg/ml
Diameter of bacterial growth inhibition zones (mm)	*Shigella*. sp 076 CO/17	Faeces	6 ± 0,00	6 ± 0,00	6 ± 0,00
	S. para typhi B 150 PI/17	Faeces	6 ± 0,00	6 ± 0,00	6 ± 0,00
	S. flexneri 214 CA/17	Faeces	6 ± 0,00	6 ± 0,00	6 ± 0,00
	E. coli 118 UB/17	Blood	6 ± 0,00	6 ± 0,00	6 ± 0,00
	E. coli 135 UB/17	Blood	6 ± 0,00	14,00 ± 0,00**	18,00 ± 1,16***
	E. coli 146 PI/17	Blood	6 ± 0,00	6 ± 0,00	6 ± 0,00
	E. coli 238 UB/17	Blood	6 ± 0,00	6 ± 0,00	6 ± 0,00
	S. aureus 129 UB/17	Blood	6 ± 0,00	6 ± 0,00	6 ± 0,00
	S. aureus 234 UB/17	Blood	6 ± 0,00	6 ± 0,00	13,33 ± 0,33**
	S. aureus 241 UB/17	Blood	6 ± 0,00	6 ± 0,00	6 ± 0,00

n = 3 *** P < 0,01* **** P < 0,001compared to control*

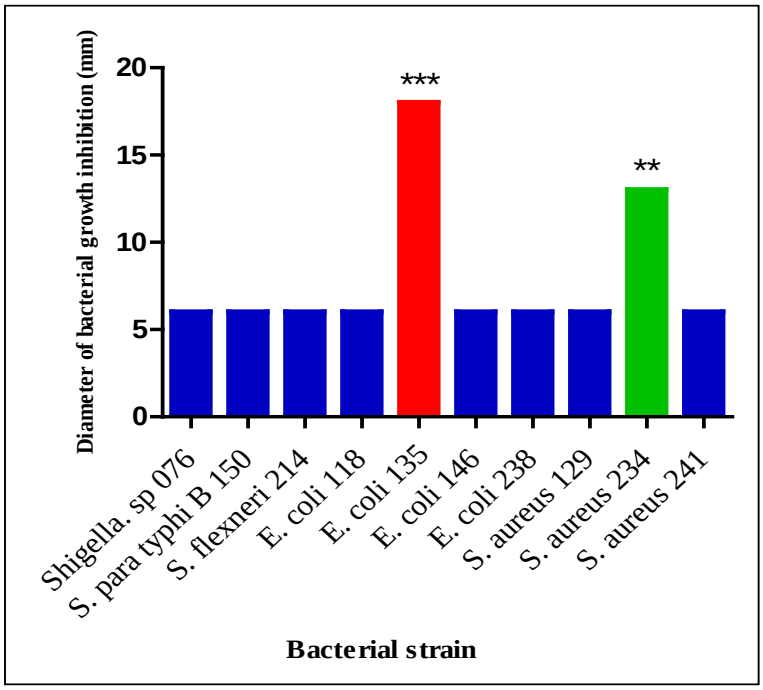

Figure 1: Sensitivity of bacterial strains to EASt at concentration of 100 mg/ml

n = 3 *** P < 0,01* *** P < 0,001compared to control*

5.3. Determination of antibacterial parameters (MBC and MIC) of the aqueous extract of Solanum torvum (EASt)

For susceptible strains (*E. coli* 135 UB/17 and *S. aureus* 234 UB/17), as the concentration of the plant extract increased, a gradual decrease in the intensity of turbidity induced by bacterial growth was observed. *S. aureus* 234 UB/17 gave the lowest values of MIC and MBC. Indeed, at a concentration of 100 mg/ml *S. aureus* 234 UB/17 and *E. coli* 135 UB/17 respectively exhibited MICs of 6.25 and 12.5 mg/ml and CMBs of 25 and 50 mg/ml. Thus, the MBC/MIC ratios for these 2 bacterial strains sensitive to EASt were 4 (Table 3).

Table 3: Antibacterial parameters and bactericidal activity of EASt

Bacterial strain	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC	Activity of EASt
*Escherichia coli*135 UB/17	12,5	50	4	Bactericidal
*Staphylococcus aureus*234 UB/17	6,25	25	4	Bactericidal

6. Discussion

The choice of *Solanum torvum* (fresh leaves) and the extraction method (decoction) was based on testimonies collected from the population and traditional therapists in Abidjan (Côte d'Ivoire). The extraction yield of *S. torvum* is similar to that of *Justicia secunda* (Acanthaceae) [7]. According to the results, this extraction method is satisfactory for the detection of the active ingredients of *Solanum torvum* and justifies the traditional use of the plant.

This study focuses on the antibacterial activity of the aqueous extract of *Solanum torvum* (EASt) on the *in vitro* growth of multi-resistant bacterial strains to common antibiotics. The sensitivity test of bacterial strains showed that *Escherichia coli* 135 UB/17 and *Staphylococcus aureus* 234 UB/17 were significantly sensitive to EASt. These two bacterial strains growth were significantly inhibited by EASt, their inhibition diameter zones were greater than 10 mm. On the one hand, the growth of eight (8) other bacterial strains studied has an insignificant inhibition diameter zone of 6 mm when tested by EASt, so these 8 bacteria were resistants to EASt. Indeed, according to [13, 5], an extract has an antibacterial activity if its inhibition diameter is greater than 10 mm. On the other hand, when the inhibition diameter is less than 10 mm, the bacterium is said to be resistant. In addition, for the two susceptible bacterial strains (E. coli 135 UB/17 and S. aureus 234 UB/17), compared to the growth control, as the concentration of EASt increased, a decrease in turbidity caused by the growth of EASt susceptible organisms was observed. Inhibition of bacterial growth justifies theantibacterial activity of the aqueous extract of *S. torvum*. This helped to determine the different antibacterial parameters for susceptible strains, such as MIC and MBC. At a concentration of 100 mg/ml, the lowest values of antibacterial parameters were obtained with the methicillin-resistant (Meti-R) strain of *S. aureus* 234UB/17, with a MIC of 6.25 mg/ml and a MBC of 25 mg/ml. The highest values were obtained with the *extended-spectrum beta-lactamases* (*ESBL*) producing *E. coli* 135 UB/17 strain, with a MIC of 12.5 mg/ml and a MBC of 50 mg/ml. Thus, for EASt, *S. aureus* strain was the most sensitive, while *E. coli* strain was the least sensitive. Comparison of antibacterial properties of this extract with other anti-infective plants showed that the aqueous extract of *S. torvum* was twice as active on *E. coli* as the aqueous extract of *Harungana madagascariensis* (Hypericaceae) [8]. and the aqueous extract of *Chromolaena odorata* (Asteraceae) [10]. According to these authors, both extracts inhibited the growth of *E. coliin vitro* with CMBs at concentrations of 100 mg/ml and 117.50 mg/ml respectively, while the CMB for EASt was at a concentration of 50 mg/ml. The CMB/CMI ratios for both bacterial strains susceptible to EASt were four (4). According to [15], when the CMB/CMI ratio value is less than or equal to 4, the activity of the extract studied is qualified as bactericidal. Thus, EASt is a bactericidal substance for *S. aureus* 234 UB/17 and *E. coli* 135 UB/17.

The antibacterial and bactericidal properties of the aqueous extract of *Solanum torvum* on multidrug-resistant bacterial strains to common antibiotics justify the use of this plant in traditional medicine for the treatment of some bacterial infections and specifically against diarrhea of bacterial origin.

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