

Evaluation of the antibacterial activity of *Solanum torvum* fruit extracts on hospital strains of enterobacteria

Abstract

Enterobacteriaceae are among the most pervasive bacteria globally and are the most commonly identified in clinical consultations. It is unfortunate that these disease-causing germs are now increasingly resistant to the standard antibiotics that are generally used. In order to propose probable therapeutic alternatives, this study focused on evaluating the antibacterial activity of *Solanum torvum* fruits on a few strains of enterobacteria. To this end, 70% hydroethanolic and 100% ethanolic extracts were prepared from *Solanum torvum* fruits and tested, respectively and separately, on a reference strain of *Escherichia coli* (ATCC), on sensitive strains of *Salmonella sp.* and *Shigella sp.*, and on resistant strains of *Escherichia coli*, *Citrobacter koseri*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Citrobacter freundii*. All susceptible and resistant strains of Enterobacteriaceae were isolated from various biological products of patients. The results demonstrated that these extracts exhibited antibacterial activity with a bactericidal effect on these strains. These extracts could therefore be employed in the manufacture of phytomedicines for the treatment of enterobacterial infections.

Keywords : Enterobacteriaceae, resistance, bactericidal effect, *Solanum torvum*.

Introduction

The Enterobacteriaceae are a vast family of Gram-negative bacilli. The family comprises over 130 species, which are grouped into 40 genera. They colonize humans, animals, and the environment (Zrardi, 2020; Prével, 2021). They are typically commensal organisms of the digestive tracts of humans and animals, which is reflected in their nomenclature, "Enterobacteriaceae" (Morice, 2003; Joly and Reynaud, 2007; Bentabet, 2021). They are frequently the causative agents of both community-acquired and nosocomial infections in human pathology. Additionally, they are implicated in a multitude of infections, including urinary, pulmonary, intra-abdominal, bacteremia, and meningitis. The enterobacteria most commonly implicated in clinical pathology are *Escherichia*, *Salmonella*, *Shigella*, *Yersinia*,

Klebsiella, *Enterobacter*, *Serratia*, *Morganella*, *Proteus*, *Providencia*, and *Citrobacter* (Verhaegen, 2002; Zrardi, 2020; Maddi and Menasra, 2022).

In recent years, there has been a notable increase in the resistance of enterobacteria to antibiotics. This phenomenon may be attributed to the overuse and/or inappropriate application of antibiotics. This constitutes a genuine public health concern. Indeed, these bacteria are capable of producing enzymes that can render beta-lactam antibiotics inactive through the production of extended-spectrum β -lactamases (ESBL). The production of extended-spectrum β -lactamases (ESBL) is frequently associated with resistance to specific antibiotic classes, including aminoglycosides and fluoroquinolones (Iabadene *et al.*, 2010; Gadou, 2019). As stated by Patterson (2001), Masterton *et al.*, (2003), and Bentabet (2021), infections caused by ESBL-producing Enterobacteriaceae (ESBL-PE) are typically associated with high morbidity and mortality rates. Indeed, antimicrobial resistance is estimated to be responsible for approximately 700,000 deaths globally (Maddi & Menasra, 2022). In light of the emergence of antibiotic resistance among Enterobacteriaceae, there is an urgent need to identify novel molecules with alternative mechanisms of action (Okou, 2012). Among the various avenues of investigation, traditional pharmacopoeia represents a promising source of potential candidates (Okou, 2012). The objective of this study is to assess the antibacterial efficacy of *Solanum torvum* fruits against a range of hospital-acquired strains of enterobacteria.

Materials and methods

Biological material

The biological material consisted of powdered dried fruits of *Solanum torvum* harvested in the town of Vavoua (Haut-Sassandra Region, Côte d'Ivoire) on May 2, 2023, and a number of bacterial strains of human origin that had been responsible for bacterial infections. The strains were isolated from various diagnostic samples and were obtained from the Biobank of the Institut Pasteur de Côte d'Ivoire. Table I below summarizes the phenotypic profile of the different bacterial strains used.

Table I: Phenotypic profile of bacterial strains

Bacterial strains	Identification number	Biological products	Profile
<i>Escherichia coli</i>	ATCC 25922	-	SAVAGE
<i>Escherichia coli</i>	1017KOR/23	Stool	ESBL/FQR
<i>Citrobacter koseri</i>	3350C/22	Urine	ESBL /FQR
<i>Citrobacter freundii</i>	1002C/22	Urine	ESBL /FQR

<i>Enterobacter cloacae</i>	383C/22	Urine	FQR/HPCASE
<i>Klebsiella pneumoniae</i>	908C/23	Urine	ESBL /FQR
<i>Salmonella sp</i>	710C/23	Stool	SAVAGE
<i>Shigella sp</i>	579C/23	Stool	SAVAGE
<i>Proteus mirabilis</i>	603C/23	Urine	HPCASE

HPCASE: probable plasmid cephalosporinase; **ESBL:** extended-spectrum β -lactamases; **FQR:** fluoroquinolone-resistant

Preparation of the various plant extracts

The *Solanum torvum* fruits were sorted, washed, and dried at room temperature for a period of three weeks in a ventilated area, situated at a distance from the sun. Once the fruits had been dried, they were pulverised to a powder using a Retsch-SK100 type grinder in order to prepare the various extracts. The 100% ethanolic extract was prepared in accordance with the methodology outlined by Zihiri *et al.* (2005). To this end, 100 grams of powder were macerated in 1 liter of distilled water for a period of five minutes in a blender. The resulting homogenate was filtered and subsequently evaporated to dryness in a blender oven for a period of three days at a temperature of 50°C, thereby obtaining the aqueous extract. Subsequently, the aqueous extract was macerated in a blender for five minutes with 300 mL of pure ethanol. The resulting supernatant and pellet were then separated and dried in a blender oven for three days at 50°C. The pellet evaporate obtained constituted the 100% ethanolic extract, designated as Eth 100%. The 70% hydroethanol extract was prepared in accordance with the methodology outlined by Zihiri *et al.* (2003) (Okou *et al.*, 2019). A total of 100 grams of powder was weighed and macerated in 1 liter of 70% ethanol (ethanol-distilled water: 70/30 (v/v)) for a period of five minutes in a blender. The resulting homogenate was filtered and subsequently evaporated to dryness in a blender oven for a period of three days at a temperature of 50°C, resulting in the production of the 70% hydroethanol extract (HydroEth 70%).

Calculation of extraction yield

The calculation of extraction yield is defined as the quantity of extract obtained from a plant material (Bssaibis *et al.*, 2009; Dinzedi, 2015; Okou *et al.*, 2019). The yield of the extract is expressed as a percentage of the dry matter, calculated according to the following formula: $R (\%) = M1 \times 100/M0$, where R is the yield of the extract expressed as a percentage, M1 is the mass of the extract in grams, and M0 is the mass of the plant powder in grams.

Evaluation of the in vitro antibacterial activity of different *Solanum torvum* extracts

Preparation of concentration series

One gram of each extract was weighed and dissolved in 10 mL of distilled water to obtain a concentration of 100 mg/mL (initial concentration or tube 1). The other concentrations were prepared from this by the double dilution method in liquid medium according to a geometric progression of reason 1/2. The concentration range varied from 100 mg/mL to 3.125 mg/mL from the starting tube to tube 6.

Agar Diffusion Sensitivity Tests

The bacterial inoculum was prepared using one or two bacterial colonies of a given strain. The samples were homogenized in 85% NaCl solution to obtain a 0.5 McFarland standard. Subsequently, the inoculum was swabbed onto a Muller-Hinton (MH) agar plate, and wells were dug into the agar using a Pasteur pipette. A pre-prepared extract range was placed in a given well, and the plate was incubated for 18 to 24 hours at 37°C. After this incubation period, the presence or absence of a zone of inhibition was observed, and readings were taken by measuring the diameter of the zone of inhibition.

Susceptibility testing by the dilution method

Preparation of inoculated broth

Inoculated broth was prepared using two colonies of the bacterial strain to be tested that were 18 to 24 hours old. These colonies were picked and mixed with a platinum loop in 10 mL of MH broth, then incubated for 3 to 5 hours at 37°C to obtain a preculture. Next, 0.1 mL of the preculture broth was removed and homogenized in another 10 mL of MH broth. This last broth constituted the inoculated broth of the bacterial strain to be tested, with an opalescence of 10^6 CFU/mL.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the following methodology. A volume of 1.8 mL of inoculated broth of the bacterial strain to be tested, initially prepared, was transferred to seven tubes to form the experimental series. Each of the six tubes in the experimental series contained 0.2 mL of a given extract at varying concentrations. The seventh tube served as a growth control, receiving 0.2 mL of sterile distilled water in lieu of the plant extract. Subsequently, the entire preparation was incubated at 37°C for 18 to 24 hours. Following this incubation period, the preparation was visually inspected for turbidity in daylight. According to Marmonier (1990) and Okou (2012), the minimum inhibitory concentration is defined as the lowest concentration of a compound or substance capable of

inhibiting bacterial growth. It is determined by the concentration of the first tube in which the culture is not cloudy.

Results

Yield of different extracts

The yield of the Eth 100% extract was 13.94%, while the yield of the HydroEth 70% extract was 5.64%. Thus, the yield of the Eth 100% extract was just over 2 times higher (13.94/5.64) than that of the HydroEth 70% extract.

Agar Diffusion Sensitivity Tests

The results are presented in Table II below. At a concentration of 100 mg/mL, the results demonstrate the activity of both the HydroEth 70% and the Eth 100% extracts on the bacterial strains under investigation. The inhibition zones of the HydroEth 70% extract exhibited diameters ranging from 10 to 14 mm, while those of the Eth 100% extract ranged from 6 to 15 mm. In accordance with the findings of Ponce *et al.* (2003) and Atsain (2017), a bacterium is classified as resistant to a plant extract when the inhibition zone diameter is less than or equal to 8 mm, sensitive between 9 and 14 mm, very sensitive between 15 and 19 mm, and extremely sensitive when the diameter is equal to or greater than 20 mm.

Table II: Determination of Inhibition Zones for Extracts

Bacterial strains	Eth 100%		HydroEth 70%			Antibiotic		
	Concentration (mg/mL)		Tm	Concentration (mg/mL)		AMC	COX	
	100	50		100	50			
<i>Escherichia coli</i> ATCC	15	12	06	14	13	06	18	26
<i>Escherichia coli</i> 1017KOR/23	12	10	06	10	08	06	14	22
<i>Enterobacter cloacae</i> 383C/22	10	10	06	11	10	06	20	19
<i>Proteus mirabilis</i> 603C/23	15	12	06	13	10	06	21	27
<i>Salmonella sp</i> 710C/23	09	06	06	10	07	06	28	29
<i>Shigella sp</i> 579C/23	06	06	06	10	06	06	11	06
<i>Klebsiella pneumoniae</i> 908C/23	10	09	06	11	09	06	10	28
<i>Citrobacter freundii</i> 1002C/22	11	09	06	12	08	06	21	30
<i>Citrobacter koseri</i> 3350C/22	10	09	06	10	06	06	20	23

Tm: Control; AMC: Amoxicillin + clavulanic acid; COX: cefotaxime

Susceptibility testing using the dilution method

Determination of MIC and BMC

The results of the MIC and BMC determinations are presented in Table III. Regarding MICs, it should be noted that they generally range from 3.125 to 50 mg/mL and from 6.25 to 50 mg/mL for Eth 100% and HydrEth 70% extracts, respectively, regardless of the bacterial strain used. As for the CMBs, they range from 6.25 to 50 mg/mL and from 12.5 to 50 mg/mL for Eth 100% and HydroEth 70% extracts, respectively, regardless of the bacterial strain used. However, for Eth 100% extract, the MIC was lower (3.125 mg/mL) for the reference strain (*Escherichia coli* ATCC 25922) and higher (50 mg/mL) for *Enterobacter cloacae*, *Salmonella sp*, *Shigella sp*, and *Citrobacter koseri* strains in succession. It was 25 mg/mL for resistant strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii*. *Proteus mirabilis* with its HPCASE phenotype had an MIC of 6.25 mg/mL. The MBC was low (6.25 mg/mL) for *Escherichia coli* ATCC 25922 and higher (50 mg/mL) for strains of *Escherichia coli*, *Enterobacter cloacae*, *Salmonella sp*, *Shigella sp*, *Klebsiella pneumoniae*, and *Citrobacter koseri*. For *Proteus mirabilis* and *Citrobacter freundii* strains, it was 12.5 and 25 mg/mL, respectively. The lowest inhibitory value was observed with the resistant *Proteus mirabilis* strain (6.25 mg/mL) for the HydrEth 70% extract, followed by *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, and *Salmonella sp*. The lowest inhibitory value was observed with the *Shigella sp* strain (12.5 mg/mL), followed by the resistant *Escherichia coli*, *Citrobacter koseri*, and *Citrobacter freundii* strains (25 mg/mL). The highest value was found with the *Enterobacter cloacae* strain (50 mg/mL). At the BMC level, the lowest inhibitory value was observed with the *Proteus mirabilis* strain (12.5 mg/mL), while the highest was noted with the *Enterobacter cloacae* strain (50 mg/mL). For strains of *Citrobacter freundii*, *Salmonella sp*, *Shigella sp*, *Citrobacter koseri*, *Klebsiella pneumoniae*, and resistant *Escherichia coli*, the lowest inhibitory value was 25 mg/mL.

Table III: Summary of antibacterial parameters for the various extracts tested

	Eth 100%				HydroEth 70%			
	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	POWER	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	POWER
<i>Escherichia coli</i> ATCC 25922	3,125	6,25	2	Bactericide	12,5	25	2	Bactericide
<i>Escherichia coli</i> 1017C/23	25	50	2	Bactericide	25	25	1	Bactericide
<i>Enterobacter cloacae</i> 383C/23	50	50	1	Bactericide	50	50	1	Bactericide
<i>Proteus mirabilis</i> 603C/23	6,25	12,5	2	Bactericide	6,25	12,5	2	Bactericide
<i>Salmonella sp</i> 710C/23	50	50	1	Bactericide	12,5	25	2	Bactericide

<i>Shigella sp</i> 579C/23	-	-	-	Bactericide	12,5	25	2	Bactericide
<i>Klebsiella pneumoniae</i> 908C/23	25	50	2	Bactericide	12,5	25	2	Bactericide
<i>Citrobacter freundii</i> 1002C/23	25	25	1	Bactericide	25	25	1	Bactericide
<i>Citrobacter koseri</i> 3350C/23	50	50	1	Bactericide	25	25	1	Bactericide

Discussion

Yields of the different extracts

The results of the yield calculation revealed that the Eth 100% extract exhibited a slightly higher yield (13.94/5.64) than the HydroEth 70% extract. This observation can be explained by the fact that, according to the literature (Cowan, 1999; Okou, 2012), the extraction of bioactive molecules is a function of the solvent and the method used. In consideration of the extraction of bioactive molecules, water can be employed to yield anthocyanins, tannins, saponins, terpenes, and other compounds. Conversely, ethanol can be utilized to isolate tannins, polyphenols, polyacetylenes, flavonoids, sterols, and other molecules. With regard to the method, it should be noted that the ethanolic extract (100%) was obtained from the aqueous extract. Consequently, this extract will contain compounds that are soluble in both water and pure ethanol, as well as substances that can only be extracted with one of the solvents. This may account for the higher concentration of bioactive molecules in this extract, which in turn may promote higher yields. In contrast to the 70% hydroethanol extraction, which isolated compounds with a solubility intermediate between ethanol and water.

Agar Diffusion Sensitivity Tests

The results of sensitivity tests on agar media at a concentration of 100 mg/mL of Eth 100% extract yielded respective inhibition zones of 15 mm (*Escherichia coli* ATCC 25922 and *Proteus mirabilis*), 1 The inhibition zones were 2 mm (resistant *Escherichia coli*), 11 mm (*Citrobacter freundii*), 10 mm (*Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Citrobacter koseri*), 9 mm (*Salmonella sp.*), and 6 mm (*Shigella sp.*). These results corroborate the hypothesis that the sensitivity of the aforementioned strains (*Escherichia coli* ATCC 25922 and *Proteus mirabilis*, as well as resistant *Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Citrobacter koseri*, and *Salmonella sp.*) to the Eth 100% extract is variable. The extract demonstrates greater activity against *Escherichia coli* ATCC 25922 and *Proteus mirabilis* than against *Citrobacter freundii*, *Enterobacter cloacae*,

Klebsiella pneumoniae, *Citrobacter koseri*, and *Salmonella sp.* The *Shigella sp.* strain exhibits resistance to this extract.

The inhibition diameters obtained for the 100 mg/mL concentration of HydroEth in agar medium are 14 mm for *Escherichia coli* ATCC 25922, 13 mm for *Proteus mirabilis*, and so forth. The inhibition diameters obtained for the 100 mg/mL concentration in agar medium were 14 mm (*Escherichia coli* ATCC 25922), 13 mm (*Proteus mirabilis*), 12 mm (*Citrobacter freundii*), 11 mm (*Enterobacter cloacae* and *Klebsiella pneumoniae*), and 10 mm (resistant *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, and *Citrobacter koseri*). The results demonstrate that the bacterial strains utilized exhibit sensitivity to this extract. Based on these observations, it can be posited that the extracts under examination are active on all the strains tested, with the exception of *Shigella sp.* Indeed, according to Biyiti (2004), an extract is considered active when it induces a zone of inhibition greater than or equal to 10 mm.

Sensitivity tests using the dilution method

Determination of MIC and BMC

For MIC determination, the absence of turbidity was observed for the Eth 100% extract from:

- 3.125 mg/mL for *Escherichia coli* ATCC 25922,
- 6.25 mg/mL for *Proteus mirabilis*,
- 25 mg/mL for resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freundii*,
- 50 mg/mL for *Enterobacter cloacae*, *Citrobacter koseri* and *Samonella sp.* With the same extract, turbidity was observed for *Shigella sp.*

In the case of HydroEth 70% extract, however, it was found that from:

- 6.25 mg/mL for *Proteus mirabilis*,
- 12.5 mg/mL for *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Shigella sp.* and *Salmonella sp.*,
- 25 mg/mL for resistant *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii*,
- 50 mg/mL for *Enterobacter cloacae*.

It can be said that the different concentrations observed represent the minimum inhibitory concentrations (MICs) of the different strains studied (Marmonier (1990) and Okou (2012)).

The dilution of the inoculated broth resulted in colonies isolated at a dilution of 10⁻⁴, which means that the work was carried out under standard culture conditions. A comparison of the number of colonies on the 10⁻⁴ dilution strip in Dish A and the strip in Dish B yielded the following values for the Eth 100% extract:

- 6.25 mg/mL for *Escherichia coli* ATCC 25922,

- 12.5 mg/mL for *Proteus mirabilis*,
- 25 mg/mL for *Citrobacter freundii*,
- 50 mg/mL for *Enterobacter cloacae*, *Citrobacter koseri*, *Samonella sp*, resistant *Escherichia coli* and *Klebsiella pneumoniae*.

Similar for HydroEth 70% extract:

- 12.5 mg/mL for *Proteus mirabilis*,
- 25 mg/mL for *Escherichia coli* ATCC 25922, resistant *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii*, *Citrobacter koseri*, *Samonella sp.* and *Shigella sp*,
- 50 mg/mL for *Enterobacter cloacae*.

According to Sirot (1990) and Okou (2012), these values represent the different BMCs of the studied extracts for each strain used.

Based on the BMCs, when comparing the two extracts requested and each strain used, it is observed that:

- The 100% ethanol extract (6.25 mg/mL) has a lower BMC than the 70% hydroethanol extract (25 mg/mL) for *Escherichia coli* strain ATCC 25922,
- The 100% and 70% ethanol extracts have identical BMC values for the strains *Enterobacter cloacae* (50 mg/mL), *Proteus mirabilis* (12.5 mg/mL) and *Citrobacter freundii* (25 mg/mL),
- Eth 100% extract had identical, higher BMC values (50 mg/mL) for resistant *Escherichia coli*, *Salmonella sp*, *Klebsiella pneumoniae* and *Citrobacter koseri* strains compared to 25 mg/mL for the same strains with HydroEth 70% extract. According to Okou *et al.*, (2019), the lower the CMB value, the higher the activity of the extract. Therefore, it can be concluded that:
 - Eth 100% extract has better activity on the reference strain *Escherichia coli* ATCC 25922 than HydroEth 70% extract,
 - both Eth 100% and HydroEth 70% extracts have the same activity on *Enterobacter cloacae*, *Proteus mirabilis* and *Citrobacter freundii* strains.
 - Eth 100% extract has weaker activity than HydroEth 70% extract on resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter koseri* and *Salmonella sp*,
 - Eth 100% extract has no activity on *Shigella sp*, unlike HydroEth 70% extract.

The results of the bacterial microdilution checkerboard method (BMC/MIC) ratios of the two *Solanum torvum* extracts tested on these bacterial strains generally yielded values that were consistently below 4 (≤ 4). This finding suggests that all extracts tested on the bacterial strains used have bactericidal activity. Indeed, as Maronnier (1990) asserts, when the CMB/CMI ratio is less than or equal to four ($\text{CMB/CMI} \leq 4$), the solicited substance has a bactericidal effect on

the strain tested. Conversely, if the ratio is precisely greater than four ($CMB/CMI > 4$), the substance exerts a bacteriostatic effect on the strain. However, this bactericidal effect is concentration-dependent, as it is influenced by the substance's dosage. Notably, this phenomenon does not apply to the *Shigella sp* strain in the presence of an Eth 100% extract. These findings are not consistent with those reported by Okou *et al.*, (2019). Indeed, the aforementioned study demonstrated that Eth 100% extract and HydroEth 70% exhibited bacteriostatic activity against the resistant *Escherichia coli* strain, as well as against *Klebsiella pneumoniae* and *Citrobacter koseri* strains. Additionally, the Eth 100% extract demonstrated bactericidal activity against the *Klebsiella pneumoniae* and *Citrobacter koseri* strains, whereas the HydroEth 70% extract exhibited bactericidal activity against the resistant *Escherichia coli* strain. The discrepancy in findings may be attributed to various factors, including the extraction methodology, the geographical origin of the plant material, soil quality, the time of plant harvesting, the duration of the extraction process, and other variables. Additionally, Okou *et al.*, (2018) demonstrated that an Eth 100% extract of *Solanum torvum* leaves exhibits bactericidal activity against *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella sp.* strains. This finding corroborates the notion that despite the organ utilized being disparate, the results align with those observed in this study. This would substantiate *Solanum torvum's* comprehensive antibacterial efficacy.

Conclusion

The present study demonstrated the antibacterial activity of two ethanolic extracts (Eth 100% and HydroEth 70%) derived from the fruits of *Solanum torvum* against a range of enterobacterial strains. The results demonstrated that the Eth 100% extract exhibited superior efficacy in the extraction of bioactive compounds. Furthermore, sensitivity tests on agar media demonstrated that both extracts exhibited antibacterial activity against the strains under investigation, with the exception of the *Shigella sp* strain, which demonstrated resistance to the Eth 100% extract. With regard to the GPC/MIC ratio, the various extracts demonstrated bactericidal activity against all strains tested. This bactericidal action is dose-dependent, as it is linked to increasing concentrations of the extract under study, with the exception of the *Shigella sp* strain and the Eth 100% extract. It may therefore be concluded that *Solanum torvum* fruit could be employed in the development of a phytomedicine for the treatment of enterobacterial infections.

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