**Antibacterial activity of two *Solanum torvum* fruit extracts on *Staphylococcus aureus* and *Pseudomonas aeruginosa***

**Abstract :**

Bacterial infections are a major public health concern, accounting for almost 17 million deaths worldwide. The bacteria responsible include *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are sometimes resistant to several families of antibiotics. To overcome this problem, research into new antibiotic molecules is becoming a necessity. The aim of this study was to assess the antibacterial activity of *Solanum torvum* fruits against strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. To achieve this, 70 % hydroethanol and 100 % ethanol extracts were prepared from *Solanum torvum* fruit and then tested in solid and liquid media respectively and separately on reference strains (ATCC), clinical, sensitive and resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All these susceptible and resistant strains were isolated from various biological products from patients. It was found that the tests on the various extracts had bactericidal activity on all the bacterial strains tested. It can therefore be said that these extracts can be used as potential therapeutic sources for the treatment of these bacteria.

**Key words:** *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Solanum torvum*

**Introduction**

The discovery and use of antibiotic therapy has considerably reduced the mortality and morbidity rates of infectious diseases. However, bacterial resistance to antibiotics is becoming increasingly important and is characterized by the emergence of new resistances (Mirabaud, 2003, Debabza, 2015). These therapeutic failures can be due to the inappropriate or abusive use of antibiotics, and this constitutes a serious health problem worldwide (Gassama, 2004, Cesur and Demiröz, 2013; Gadou 2019). According to Sadikalay (2018), these facts represent one of the major health challenges of the 21st century. According to the Organisation for Economic Co-operation and Development (OECD), 2.4 million people in Europe, North America and Australia will die from multi-drug-resistant (MDR) bacterial infection over the next 30 years (Hofer, 2019 ; Gravey, 2022). Similarly, WHO (2023) and Guillaumy (2024) predict that by 2050, ten million people, including 4.1 million living in Africa, will lose their lives to infections caused by antibiotic-resistant organisms, leading to the loss of up to 5 % of their gross domestic product. In fact, bacteria are becoming increasingly resistant to antibiotics, even those of last resort such as carbapenems and vancomycin. These include Pseudomonas aeruginosa and Staphylococcus aureus. These bacteria, which cause numerous infections, have established themselves as the predominant pathogens in hospital environments (nosocomial infections) (Tankovic *et al.*, 1997; Saulnier and Andremont, 1997 ; Köhler and van Delden, 2009 ; Cholley, 2010 ; Durand, 2013 ; Meradji, 2017). The multi-resistant bacteria that cause nosocomial infections have become a global threat, as they are regularly encountered in intensive care units (Floret *et al.*, 2009 ; Meradji, 2017). Faced with this resistance, there is an urgent need to search for new molecules with mechanisms of action different from those hitherto present (Okou, 2012). To overcome this problem, the search for new antibiotic molecules is becoming a necessity. Among the many avenues explored, traditional pharmacopoeia is one of the most promising. According to Mehani (2015), there are over 10,000 plants with medicinal properties, including 5,000 in Africa (Okou, 2012). The aim of this study was to evaluate the antibacterial activity of *Solanum torvum* fruit extracts on *Pseudomonas aeruginosa* and *Staphylococcus* *aureus* strains responsible for infections in hospitals.

**Materials and methods**

**Plant material**

The plant material consisted of *Solanum torvum* fruits. The fruits were harvested in the town of Vavoua (Haut-Sassandra region, Côte d'Ivoire) on May 2, 2023. They were sorted, washed and then dried at room temperature for three weeks in a ventilated area away from the sun. Once dry, they were ground to powder using a Retsch-SK100 mill to prepare the various extracts.

**Biological material**

The material consisted of a few bacterial strains of human origin from the Biobank which had been responsible for bacterial infections, and two reference strains. They were isolated from various diagnostic samples and profiled at the Antibiotics, Natural Substances and Monitoring of Microorganism Resistance to Anti-infectives Unit (ASSURMI), then stored at the Institut Pasteur de Côte d'Ivoire Biobank. Table I below summarizes the phenotypic profile of the different bacterial strains used.

**Table I:** Phenotypic profile of bacterial strains studied

|  |  |  |  |
| --- | --- | --- | --- |
| **Bacterial strains** | **Identification number** | **Organic products** | **Phenotypes** |
| *Pseudomonas aeruginosa* | ATCC | - | - |
| 771C/24 | Urine | Toto-R |
| 363C/24 | Sonde urinaire | Toto-R |
| *Staphylococcus**Aureus* | ATCC | - | - |
| 2541C/23 | Sonde urinaire | Wild |
| 1086C/23 | Urine | FQR, MLSbC, KTG, Méti R |

*FQR: fluoroquinolone-resistan, MLSbC : constitutive MLSb phenotype, KTG : Kanamycine Tobramycine Gentamicine resistant, Méti-R : méticilino resitance*

**Preparation of extracts**

The 100% ethanolic extract was prepared according to the method of Zirihi *et al.* (2005). To this end, 100 g of *Solanum torvum* fruit powder was macerated in 1000 mL of distilled water in a blender for 5 min. The resulting homogenate was filtered and then evaporated to dryness in a BLENDER oven for three days at 50 °C to obtain the aqueous extract. This extract was then diluted in 300 mL ethanol and macerated for 5 minutes in a blender. After settling, the supernatant was separated from the pellet and dried in a BLENDER oven for three days at 50 °C. The pellet evaporate obtained constituted the 100 % ethanolic extract (Eth 100 %).

For the 70 % hydroethanol extract, 100 g of powder was macerated in 1000 mL of 70 % ethanol (ethanol-distilled water: 70/30 (v/v)) in a blender for 5 minutes. The filtrate obtained was evaporated to dryness in a BLENDER oven for three days at 50°C to obtain the 70 % hydroethanol extract (HydroEth 70 %) (Zirihi *et al.*, 2003).

**Antibacterial tests**

**Preparation of concentration ranges**

A 100 mg/mL solution of each extract was prepared from 1 g of powder homogenized in 10 mL of distilled water. The other concentrations were then prepared using the double dilution method in liquid medium. Concentration ranges varied for each extract from 100 mg/mL to 3.125 mg/mL.

**Susceptibility testing on solid media**

Bacterial inoculum was prepared using one or two bacterial colonies of a given strain. They were homogenized in 85 % NaCl solution to obtain a 0.5 Mc Farland calibre. The inoculum was then swabbed onto a Muller-Hinton (MH) agar plate, and wells were dug into the agar using a Pasteur pipette. A pre-prepared range of extract was added to each well, and the plate was incubated for 18 to 24 hours at 37°C. After this incubation time, the assay was read for the presence or absence of a zone of inhibition.

**Susceptibility testing in liquid media**

**Preparation of inoculated broth**

Inoculated broth was prepared from two or three young colonies of a given bacterium, 18 to 24 hour old, diluted in 10 mL of MH broth and incubated for 3-5 hours at 37°C to obtain a pre-culture. A volume of 0.3 mL of the pre-culture broth was then diluted in a test tube containing 10 mL of MH broth to obtain a bacterial population of around 106 CFU/mL. This latter broth constituted the inoculated broth of the bacterial strain to be tested.

**Determination of antibacterial parameters**

**Determination of the Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) was determined using the following method: a volume of 1.8 mL of broth inoculated with the initially prepared bacterial strain to be tested was taken and transferred to 7 tubes to form the experimental series. In 6 tubes of the experimental series, 0.2 mL of a given extract at different concentrations was added individually. In the 7th tube, instead of plant extract, 0.2 mL of sterile distilled water was added to form the growth control tube. Thus, in each tube of the experimental series, the final volume was 2 mL. The entire preparation was then incubated at 37°C for 18 to 24 hours. After this incubation time, the preparation was checked for turbidity with the naked eye and in daylight. According to Marmonier (1990) and Okou (2012), the minimum inhibitory concentration is the lowest concentration of a compound or substance that inhibits bacterial growth. It is given by the concentration of the first tube in which the culture is not cloudy.

**Bacterial count**

The bacterial inoculum was diluted from 10 to 10 until the 10-4 dilution was reached. Four successive dilutions were obtained: 10-1, 10-2, 10-3 and 10-4. The initial inoculum and the four dilutions were inoculated with a 2 µl platinum loop in 5 cm strips onto an MH agar plate to form plate A. This plate was incubated for 18 to 24 hours at 37°C.

**Determination of the Minimum Bactericidal Concentration (MBC)**

To determine the MBC, each bacterial culture in the experimental series was seeded separately on a 5 cm streak using a platinum loop calibrated at 2µl on an MH agar plate to form plate B. This plate was then incubated at 37°C for 18 to 24 hours. It was then incubated at 37°C for 18 to 24 hours. After this incubation period, the number of colonies on the various streaks of plate B was compared with that of the 10-4 dilution of plate A, to determine the BMC. Indeed, according to Sirot (1990) and Okou (2012), determination of the minimum bactericidal concentration (MBC) is possible by looking for the concentration leaving at most 0.01% surviving bacteria. However, according to Marnonier (1990):

- if the MBC/MIC ratio ≤ 4, the substance tested is bactericidal.

- if the ratio CMB/CMI ˃ 4, the substance tested is bacteriostatic.

**Results**

**Solid-state sensitivity tests**

The results of the determination of the inhibition zones of the different extracts are shown in Table II. In this table, it is generally noted that for the HydroEth 70% and Eth 100% extracts, the inhibition diameters obtained are greater than 10 mm, irrespective of the concentration and strains used, with the exception of *Pseudomonas aeruginosa* strains. With regard to the reference molecule used (Ceftazidime) for *Pseudomonas aeruginosa* strains, the inhibition diameter for strain ATCC was greater than 17 mm, unlike strains 771C/23 and 363C/24. For *Staphylococcus aureus* strains, the inhibition diameters observed for the reference molecule (Cefotaxime) on strains ATCC and 2541C/23 are greater than 17 mm, unlike strain 1086C/23.

**Table II:** Determination of inhibition zones for different extracts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Bacterial strains | Eth 100% | HydroEth 70% | Antibiotiques |
| **Concentration (mg/mL)** | **CZD** | **FOX** |
| **100** | **50** | **Tm** | **100** | **50** | **Tm** |
| Zone d’inhibition (mm) | ***Pseudomonas aeruginosa*** | ATCC | 06 | 06 | 06 | 24 | 15 | 06 | 25 | - |
| 771C/23 | 06 | 06 | 06 | 20 | 17 | 06 | 6 | - |
| 363C/24 | 06 | 06 | 06 | 20 | 16 | 06 | 6 | - |
| ***Staphylococcus aureus*** | ATCC | 20 | 15 | 06 | 15 | 13 | 06 | - | 28 |
| 2541C/23 | 15 | 13 | 06 | 20 | 15 | 06 | - | 17 |
| 1086C/23 | 13 | 10 | 06 | 15 | 13 | 06 | - | 13 |

***CZD:*** *ceftazidime,* ***FOX:*** *cefoxitine*

**Susceptibility testing in liquid medium**

The antibacterial parameters of the various extracts tested are summarized in Table III. The results of this table gave MIC values ranging from 3.125 to 6.25 mg/mL for *Staphylococcus aureus* strains and from 6.25 to 50 mg/mL for all strains used for the Eth 100% and HydroEth 70% extracts respectively. CMB values ranged from 3.125 to 12.5 mg/mL for *Staphylococcus aureus* strains is from 12.5 to 50 mg/mL for all strains used for Eth 100% and HydroEth 70% extracts respectively. With regard to the MBC/MIC ratio for both extracts, the values obtained were less than or equal to 2 for all bacterial strains except *Pseudomonas aeruginosa*.

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

|  |  |  |
| --- | --- | --- |
|  | Eth 100 % | HydroEth 70 % |
| **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** | **POWER** | **CMI (mg/mL)** | **CMB (mg/mL)** | **CMB/CMI** | **POWER** |
| *Pseudomonas aeruginosa* | ATCC | - | - | - | - | 6,25 | 12,5 | 2 | Bactericide |
| 771C/24 | - | - | - | - | 12,5 | 12,5 | 1 | Bactericide |
| 363C/24 | - | - | - | - | 12,5 | 12,5 | 1 | Bactericide |
| *Staphylococcus areus* | ATCC | 3,125 | 3,125 | 1 | Bactericide | 50 | 50 | 1 | Bactericide |
| 2541C/23 | 3,125 | 6,25 | 2 | Bactericide | 50 | 50 | 1 | Bactericide |
| 1086C/23 | 6,25 | 12,5 | 2 | Bactericide | 50 | 50 | 1 | Bactericide |

***MIC:*** *minimum inhibitory concentration,* ***MBC:*** *minimum bactericidal concentration*

**Discussion**

**Susceptibility testing on solid media**

The aim of this study was to evaluate the antibacterial activity of *Solanum torvum* fruits on susceptible and resistant strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. For this purpose, two types of extraction were carried out to obtain the extract Ethanolic 100% (Eth 100%) and Hydroethanolic 70% (HydroEth 70%).

The results obtained with sensitivity tests on agar medium showed that for a concentration of 100 mg/mL of Eth 100% extract, inhibition diameters ranging from 13 to 20 mm were achieved for *Staphylococcus aureus* strains. For *Pseudomonas aeruginosa* strains, the diameters were all equal to 6 mm. For *Staphylococcus aureus* strains, the highest sensitivity was observed with the reference strain ATCC 29213 (20 mm), followed by the wild-type strain (15 mm) and the lowest with the resistant strain (13 mm).

With HydroEth 70% extract, the different diameters observed ranged from 15 to 17 mm for *Pseudomonas aeruginosa* strains and from 20 to 15 mm for *Staphylococcus aureus* strains. *Pseudomonas aeruginosa* strains are 17 mm (771C/24), 16 mm (363C/24) and 15 mm (ATCC27853). As for *Staphylococcus aureus* strains, they are characterized by diameters of 20 mm for the wild strain 2541C/23 and 15 mm for the reference strains ATCC 29213 and resistant 1086C/23.

The results show that both extracts inhibited the growth of all *Staphylococcus aureus* strains. However, the best inhibition was obtained with the Eth 100% extract on the reference strain (ATCC) and the HydroEth 70% extract on the wild-type strain (2541C/23). Both strains are sensitive, so it can be said that the extracts (Eth 100% and HydroEth 70%) of *Solanum torvum* fruits are more active on sensitive strains of *Staphylococcus aureus* than on resistant strains. The inhibition diameter values obtained on resistant strains of *Staphylococcus aureus* are roughly equal to those of N'guessan *et al.* (2019) in their work on the aqueous extract of the same plant.

In the case of *Pseudomonas aeruginosa* strains, only the HydroEth 70% extract inhibited bacterial growth. However, the reference strain (ATCC) was more sensitive to this extract (24 mm) than the Toto-Resistant strains (20 mm). It can be said that this extract is more active than the reference molecule used (Ceftazidime). Nevertheless, the use of this antibiotic indicates resistance to a family of antibiotics. This would suggest that the HydroEth 70% extract is more effective than currently available antibiotics, despite the fact that this extract is still an aggregate of molecules. These results concur with those of Konan (2015), who tested the efficacy of T. glaucescens stem bark on ESBL-producing Enterobacteriaceae. In this study, this author revealed that plant extracts in general can be more effective than commercially available antibiotics.

A comparison of the activity of the different extracts at 100 mg/mL concentration on the various strains tested showed that the HydroEth 70% extract had good inhibition on all the strains tested. However, this inhibition was more marked on *Pseudomonas aeruginosa* strains (Gram-negative) than on *Staphylococcus aureus* strains (Gram-positive). It would be fair to say that this extract is more active on Gram-negative than on Gram-positive bacteria.

**Sensitivity tests using the dilution method**

The absence of turbidity indicating a lack of bacterial growth for MIC determination was observed for the Eth 100% extract from :

- 3.125 mg/mL for *Staphylococcus aureus* ATCC and 2541C/23 ;

- 6.25 mg/mL for *Staphylococcus aureus* 1086C/23.

With the same extract, turbidity was not observed for *Pseudomonas aeruginosa* strains.

With the HydroEth 70% extract, however, turbidity was observed from :

- 6.25 mg/mL for *Pseudomonas aeruginosa* ATCC ;

- 12.5 mg/mL for *Pseudomonas aeruginosa* 771C/24 and 363C/24 ;

- 50 mg/mL for all strains of *Staphylococcus aureus*.

According to Marmonier (1990) and Okou (2012), these various observed values constitute the minimum inhibitory concentrations (MICs) of the various strains studied.

Comparison of the number of colonies on the streak at 10-4 dilution in box A with the different streaks in box B gave the following values for the Eth 100% extract:

- 3.125 mg/mL for *Staphylococcus aureus* ATCC ;

- 6.25 mg/mL for wild *Staphylococcus aureus* ;

- 12.5 mg/mL for resistant *Staphylococcus aureus*.

Whereas with HydroEth 70% extract the values obtained are :

- 12.5 mg/mL for all strains of *Pseudomonas aeruginosa* ;

- 50 mg/mL for all strains of *Staphylococcus aureus*.

According to Sirot (1990) and Okou (2012), these different values obtained constitute the minimum bactericidal concentrations (MBC) of the various strains studied.

The results of the MBC/MIC ratio of the two extracts on all the strains studied were less than or equal to 2. Indeed, according to Marnonier (1990), when the MBC/MIC ratio is less than or equal to four (MBC/MIC ≤ 4), the substance solicited has a bactericidal effect on the strain tested. Consequently, the extracts tested are bactericidal.

**Conclusion**

This study demonstrated the antibacterial activity of two ethanolic extracts (Eth 100% and HydroEth 70%) from *Solanum torvum* fruits on a number of strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Susceptibility tests on agar media showed that both extracts were active on the strains tested. However, *Solanum torvum* fruit extracts were more active on sensitive strains of *Staphylococcus aureus* than on resistant strains. However, the HydroEth 70% extract is more effective than currently available antibiotics, despite still being an aggregate of molecules. Similarly, this extract is more active on Gram-negative bacteria than on Gram-positive bacteria. The BMC/MIC ratio of the two different extracts showed a dose-dependent bactericidal effect on all the strains tested. *Solanum torvum* fruit could therefore be used to develop a phytomedicine for the treatment of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria.

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