

Chapter

Salmonellosis Infections and Treatment Proposals

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Abstract

Enterobacteriaceae are among the most widespread bacteria in the world, and the most frequently encountered in clinical consultations. Unfortunately, these disease-causing germs are now increasingly resistant to the standard antibiotics generally used. In order to propose probable therapeutic alternatives, this study focused on evaluating the antibacterial activity of *Solanum torvum* fruit (a food plant) on around thirty strains of enterobacteria such as *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Citrobacter koseri*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Citrobacter freundii*. Among these strains of enterobacteria were nine strains of *Salmonella sp.* To do this, 70% hydroethanolic and 100% ethanolic extracts were prepared from the fruits of *Solanum torvum* and then tested on them. All strains of *Salmonella sp.* were isolated from various biological samples (stool and/or urine) from patients. Qualitative and quantitative analyses revealed the presence of several phytochemicals such as polyphenols, tannins, coumarins, saponosides, and so on in the 70% hydroethanolic and 100% ethanolic extracts. Finally, the antioxidant activity of both extracts showed that they were capable of inhibiting free radicals. The results revealed that these various extracts have antibacterial activity with a bactericidal effect on these strains. These extracts could therefore be used to produce phytomedicines to combat *Salmonella* infections.

Keywords: Salmonellosis, medicinal plants, antibacterial, antioxidant activities, phytochemicals

1. Introduction

Enterobacteriaceae are Gram-negative bacilli comprising 40 genera and over 130 species. They are found in humans, animals, and the environment [1, 2]. They are present in the digestive systems of humans and animals [3, 4]. They are a constant cause of urinary tract, pulmonary, and intra-abdominal infections; bacteremia; and meningitis and are encountered both in the community and in hospitals. *Escherichia*, *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella*, *Enterobacter*, *Serratia*, *Morganella*, *Proteus*, *Providencia*, and *Citrobacter* are the most common pathogens [1, 5].

Over the last few decades, the overuse and/or inappropriate use of antibiotics has led to therapeutic failures with these bacteria. In fact, they can produce extended-spectrum β -lactamases (ESBLs) that can inhibit beta-lactam antibiotics. These enzymes can render antibiotics such as aminoglycosides and fluoroquinolones ineffective. This

situation represents a real public health problem [6, 7]. Indeed, ESBL infections can increase overall patient morbidity and mortality. Consequently, around 700,000 deaths worldwide are due to antimicrobial resistance [4, 5, 8]. Therapeutic failures are prompting research teams to turn their attention to traditional African pharmacopoeia. Indeed, the WHO confirms that in Africa, 80% of the population relies on traditional medicine and pharmacopoeia to deal with health problems [9]. According to reference [10], the constant reinforcement of antimicrobial compounds through the discovery of new molecules keeps us one step ahead of the risk of therapeutic impasses. However, modern medicine is characterized by a crying lack of new treatments. What's more, antibiotics, regarded as the almost universal solution to serious infections, are increasingly showing their limitations [11, 12]. In this context, traditional medicine can provide a solution to these problems, as it is of prime importance in the health care of populations. As reference studies [13–15] have shown, medicinal plants have always been used for their cosmetic, chemical, dietetic, pharmaceutical, agri-food, and industrial properties. In particular, the work of reference studies [16–20] has shown that medicinal plants contain various biologically active chemical principles capable of exerting antioxidant, anti-inflammatory, analgesic, antibacterial, and other activities. Similarly, according to reference [21], medicinal plants are part of the history of every continent. Specifically, they have been organized, documented, and passed down from generation to generation for centuries in China and India. In Africa, as reference [22] points out, there are almost 5000 medicinal species. This study was carried out with the aim of contributing to the scientific valorization of medicinal plants. It aims to evaluate the antibacterial activity of *Solanum torvum* fruits on a few strains of hospital-acquired salmonellosis.

2. General information on the *Salmonella* genus

It was first discovered by Theobald Smith (1859–1934) in 1885. It is a straight bacillus, about 3 μm long, aero-anaerobic and often motile, except for *S. gallinarum*. According to Le Minor and Kaufmann-White, it comprises two subspecies: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* comprises 6 subspecies (*indica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *enterica*) [23].

3. Pathogenicity of the genus *Salmonella*

Salmonellosis is a disease caused by the *Salmonella* genus. They remain a major health problem, especially in underdeveloped countries. *Salmonella* are responsible for both major and minor salmonellosis (non-typhoidal salmonellosis). Major salmonellosis is the cause of typhoid and paratyphoid fevers. Typhoid and paratyphoid fevers are strictly human pathologies caused by the serovars *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi B*, and *S. Paratyphi C*, while minor salmonellosis is the cause of gastroenteritis due to food poisoning by serovars such as *S. typhimurium*, *S. enteritidis*, and *S. panama* [24, 25].

4. Antibiotics and definition

Antibiotics derive their name from two Greek words, anti: against, and bios: life. They are natural, semisynthetic, or synthetic substances capable of inhibiting or destroying the growth of bacteria without harming the body. They were discovered in

1929 by Alexander Flemming. He was the first to discover penicillin. This discovery made it possible to combat certain diseases and paved the way for the discovery of several other molecules between 1940 and 2005. Antibiotics are therefore a large group of drugs composed of several families depending on their mode of action on bacteria and their chemical structures [7, 10, 26, 27].

5. Some figures on bacterial resistance

Bacterial resistance to antibiotics is becoming increasingly significant and is characterized by the emergence of new forms of resistance [28, 29]. This, according to reference [30], represents one of the major health challenges of the twenty-first century. The Organization for Economic Cooperation and Development (OECD) estimates that 2.4 million people in Europe, North America, and Australia will die from multidrug-resistant bacterial infections (MRB) over the next 30 years [31, 32]. In Europe, antibiotic-resistant bacteria cause 400,000 infections per year, with at least 25,000 deaths annually [33, 34]. In Africa, this phenomenon of resistance is poorly assessed. Nonetheless, studies report that the African continent is not immune to this phenomenon [34, 35]. Nevertheless, reference studies [36, 37] predict that by 2050, approximately 4.1 million people living in Africa will lose their lives due to MRB infections.

6. General information on the therapeutic use of *Solanum torvum*

In Dominica, the young leaves of *Solanum torvum* are used in combination with the leafy stem of *Bidens pilosa* to treat dysuria, and its flowers are used to treat abdominal pain in infants in the form of an infusion. In Guadeloupe, the roots macerated in water are used to treat renal colic [38]. In Cameroon, a decoction of the fruit is used to treat female infertility [39], and the bark, fruit, and leaves are used to treat poisoning, gastric problems, female infertility, and pneumonia [40]. In Central America, India, and Gabon, the leaves are used to treat cuts, wounds, and diabetes [41]. In Ghana, the leaves are used to treat wounds and boils [42], and in southern Nigeria, they are used to treat sickle cell anemia [43]. In southern Ivory Coast, a decoction of the leaves is used to treat anemia [44]. In Madagascar, a paste made from heated fruit is used to treat ringworm [45].

7. Some chemical compounds isolated from *Solanum torvum*

Solanum torvum contains a variety of bioactive compounds that give it its multiple pharmacological properties. As reported in reference studies [48–50], the chemical compounds identified in this plant and studied for their biological effects are: isoflavonoid sulfate and antiviral steroidal glycosides, torvanol A (a new isoflavonoid sulfate), torvoside H (a new steroidal glycoside), torvoside A (a previously recognized glycoside) and a tocopherol, solanolactosides A and B, and two newspirostanol glycosides (torvosides M and N). Certain structures have been identified, namely, solanolide 6-O-[α -l-rhamnopyranosyl-(1 $\rightarrow$ 3)-O- β -d-quinovopyranoside], solanolide 6-O-[β -d-xylopyranosyl-(1 $\rightarrow$ 3)-O- β -d-quinovopyranoside], yamogenin 3-O-[β -d-glucopyranosyl-(1 $\rightarrow$ 6)-O- β -d-glucopyranoside], neochlorogenic acid 3-O-[β -d-glucopyranosyl(1 $\rightarrow$ 6)-O- β -d-glucopyranoside], and so forth (**Figure 1**).

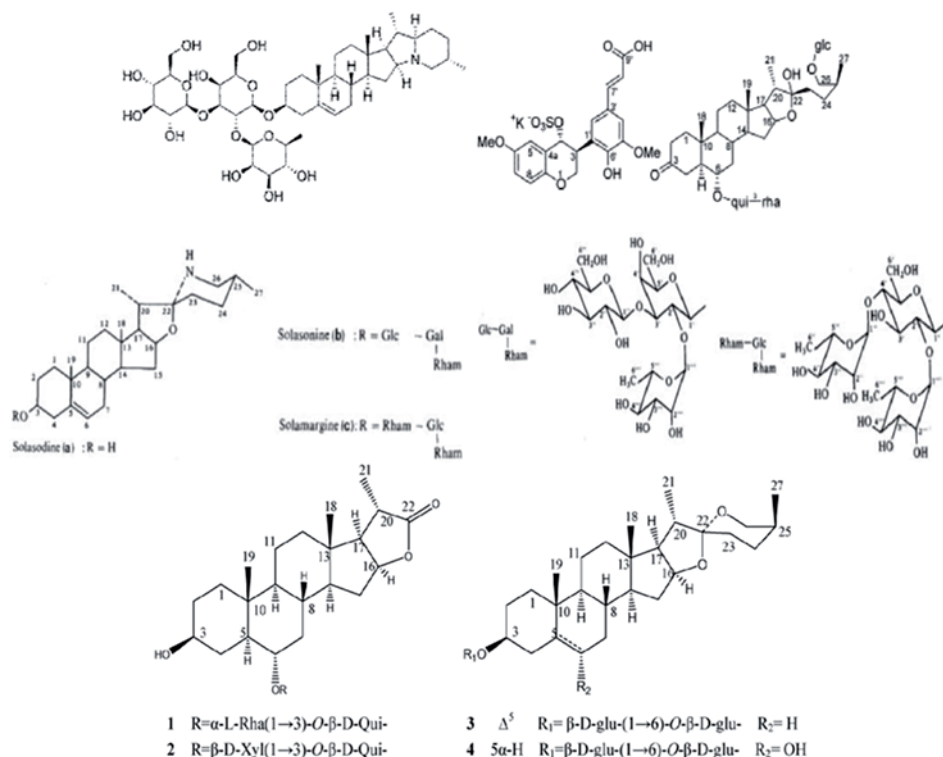


Figure 1.
Some isolated compounds from *Solanum torvum*.

8. Materials and methods

8.1 Biological materials

The biological material consisted of powder from dried *Solanum torvum* fruit and strains of *Salmonella sp* of human origin (stool and/or urine).

8.2 Method for preparing the various plant extracts

The fruits of the plant were harvested, sorted, washed, and dried at room temperature in the laboratory for three weeks. They were then pulverized in a Retsch-SK100 type mill to obtain a vegetable powder. This powder was then used to prepare the various extracts. The 100% ethanolic extract was prepared by dissolving 100 g of the previous plant powder in 1 liter of distilled water in a blender for 5 minutes to obtain a homogenate [49]. This was then filtered and evaporated in a blender-type oven for three days at 50 °C to obtain the aqueous extract. The latter was then macerated in 300 mL of pure ethanol in a blender for 5 minutes, then left to stand to obtain a supernatant and a pellet. These were subsequently further separated and evaporated in a Blender oven for three days at 50 °C. The 100% ethanolic extract (Eth 100%) obtained was taken from the pellet. In parallel with the preparation of the 100% ethanolic extract, another extract, the 70% hydroethanolic extract, was

prepared using the same procedure as above. The only difference is that the solvent used in this case is a mixture of ethanol and distilled water (70/30, v/v). Final preparation of this extract yielded the 70% hydroethanol extract (HydroEth 70%). The resulting 100% ethanolic (Eth 100%) and 70% hydroethanolic (HydroEth 70%) extracts were stored separately in hermetically sealed jars and stored at room temperature for future testing.

8.3 Extraction yield calculation method

It was based on the method described by reference studies [18, 50, 51]. Yield is the quantity of extract obtained from a plant material. It is expressed as a percentage of dry matter (plant powder) and was calculated using the following formula:

$$R(\%) = M_1 \times 100 / M_0$$

- R: Yield of extract expressed as a percentage (%),
- M_1 : Mass of extract (in g),
- M_0 : Mass of vegetable powder (in g).

8.4 Method for evaluating the *in vitro* antibacterial activity of various *Solanum torvum* extracts

8.4.1 Method for preparing concentration ranges

This was achieved by dissolving 1 g of a previously prepared extract in 10 mL of distilled water to give an initial concentration of 100 mg/mL (tube 1 = T1). Concentrations in the other tubes (T2 to T6) were prepared from tube 1 (T1) using the liquid double dilution technique. To do this, a volume of 5 mL of sterile distilled water was distributed from T2 to T6. Next, a 5 mL volume of T1 was withdrawn, diluted with T2 and homogenized. This operation was repeated until T6. From T6 onward, the excess was discarded. Concentration ranges oscillated between 100 mg/mL and 3.125 mg/mL from T1 to T6.

8.4.2 Agar diffusion sensitivity test method

In this step, the bacterial inoculum was prepared from 1–2 colonies of *Salmonella sp.* These were then diluted in 0.85 NaCl solution and homogenized to obtain a 0.5 Mc Farland caliber. Next, an accurate Muller-Hinton (MH) agar plate was inoculated with the inoculum previously prepared by swabbing. Wells were subsequently drilled into the inoculated agar using a Pasteur pipette. Each well was filled with a given concentration of the initially prepared extract. It was also incubated for 18 to 24 hours at 37 °C. After this incubation time, diffusion of each concentration was used to delimit a zone of bacterial growth inhibition in the seeded agar plate. Finally, the inhibition diameter was measured to determine whether the bacterial strain tested was sensitive or resistant to the extract used. This agar diffusion method is useful for assessing the bacterial activity of different extracts [52].

8.4.3 Dilution sensitivity test method

8.4.3.1 Method for preparing inoculated broth

It was performed using 2 young *Salmonella sp* colonies aged 18 to 24 hours. Colonies were picked from the agar plate, mixed with a platinum loop in 10 mL of MH broth and incubated for 3 to 5 hours at 37 °C to obtain a pre-culture. Again, 0.1 mL of the pre-culture broth was removed and homogenized in a further 10 mL of MH broth to achieve an opalescence of 10^6 CFU/mL.

8.4.3.2 Method for determining Minimum Inhibitory Concentration (MIC)

This was achieved by taking 1.8 mL of the previously prepared *Salmonella sp* inoculated broth and transferring it into 7 tubes to form the experimental series. Within the experimental series, a volume of 0.2 mL of each concentration of initial extract prepared was placed separately in 6 tubes. The 7th tube was the growth control tube, to which 0.2 mL of sterile distilled water was added. Thus, the final volume in the 7 tubes of the experimental series was 2 mL. All were then incubated at 37 °C for 18 to 24 hours. After this incubation time, the MIC was determined by identifying the absence of turbidity with the naked eye and in daylight. The MIC corresponds to the lowest concentration of a compound or substance capable of inhibiting bacterial growth. It is given by the concentration of the first tube in which the culture is not turbid [22, 53].

8.4.3.3 Bacterial count method

This was carried out by diluting the bacterial culture in the growth control tube, preincubated at 37 °C for 18 to 24 hours (7th tube), from 10 to 10 down to 10^{-4} . Each dilution was then streaked 5 cm apart with a 2 μ L calibrated platinum loop onto an MH agar plate (Box A). The plate was also incubated for 18 to 24 hours at 37 °C. After this time, Box A was used to determine the minimum bactericidal concentration.

8.4.3.4 Method for determining Minimum Bactericidal Concentration (MBC)

This involved inoculating each *Salmonella sp* culture from the experimental series separately on a 5 cm streak using a 2 μ L calibrated platinum loop on a MH agar plate (Box B). It was followed by incubation at 37 °C for 18 to 24 hours. After this incubation period, the MBC was determined by comparing the number of *Salmonella sp* colonies on the various streaks on the B plate with those on the 10^{-4} dilution of the A plate. Indeed, according to reference studies [22, 54], the determination of the minimum bactericidal concentration (MBC) is possible by looking for the concentration leaving at most 0.01% surviving bacteria. However, according to reference [53]:

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

8.5 Chemical test method for *Solanum torvum* fruit extracts

8.5.1 Qualitative analysis

They consisted in identifying compounds such as:

- polyphenols by reaction with ferric chloride (FeCl_3),
- flavonoids using the chloride vanillin and ammonia tests,
- coumarins with 96% ethanol, 10% NaOH and concentrated HCl,
- tannins with 80% methanol and 10% FeCl_3 ,
- saponosides with the foam test.

8.5.2 Quantitative analysis

8.5.2.1 Determination of total phenols

It was carried out using the Folin–Ciocalteu method, which is based on the redox reaction.

8.5.2.2 Determination of condensed tannins

It was tested using the vanillin method with HCl.

8.5.2.3 Total flavonoid content

It was produced using the colorimetric method with aluminum trichloride (AlCl_3).

*8.5.2.4 Method for assessing the antioxidant activity of *Solanum torvum* fruit extracts using the DPPH test*

It is based on a compound's ability to reduce the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical.

9. Results

9.1 Yield of different extracts

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

9.2 Agar diffusion sensitivity tests

The results of agar diffusion sensitivity tests gave inhibition diameters of 9 mm for Eth 100% extract and 10 mm for HydroEth 70% extract. According to reference studies [55, 56], a bacterium is said to be:

- resistant to a plant extract when the inhibition diameter described around this extract is less than or equal to 8 mm,
- sensitive, if it is between 9 and 14 mm,
- very sensitive when it is between 15 and 19 mm,
- extremely sensitive when the diameter is greater than or equal to 20 mm.

9.3 Dilution sensitivity tests for MIC and MBC determination

The results of sensitivity tests using the dilution method yielded minimum inhibitory concentrations (MIC) of:

- 12.5 mg/mL of HydroEth 70% extract,
- 50 mg/mL of Eth 100% extract.

Similarly, these results enabled us to determine minimum bactericidal concentrations (MBC) of:

- 25 mg/mL of HydroEth 70% extract,
- 50 mg/mL of Eth 100% extract.

The MBC/MIC ratio gave a value of:

- 2 for HydroEth 70% extract,
- 1 for Eth 100% extract.

9.4 Chemical testing of *Solanum torvum* fruit extracts

The results of qualitative analyses of both extracts showed the presence of phyto-compounds such as polyphenols, tannins, flavonoids, coumarins, and saponosides. Quantitative analyses yielded:

- 151.15 ± 0.95 mgEAG/gMS total phenols,
- 18.24 ± 1.31 mgEC/gMS condensed tannins,
- 88.37 ± 1.13 mgEQ/gMS total flavonoids in Eth 100% extract.
- 92.99 ± 2.9 mgEAG/gMS total phenols,
- 9.42 ± 1.63 mgEC/gMS condensed tannins,
- 49.11 ± 0.3 mgEQ/gMS total flavonoids in HydroEth 70% extract.

9.5 Evaluation of the antioxidant activity of *Solanum torvum* fruit extracts using the DPPH test

Results for the anti-free radical activity of vitamin C, Eth 100% extract, and HydroEth 70% extract yielded IC₅₀ values of 0.007, 0.39, and 0.36 mg/mL, respectively.

10. Discussion

10.1 Yield of different extracts

The results of the yield calculation show that the Eth 100% extract is just over 2 times higher (13.94/5.64) than the HydroEth 70% extract. This may be justified by the fact that the extraction of bioactive substances may depend on the solvent and method used. So, water can isolate certain compounds such as polypeptides, lectins, starches, saponins, terpenes, and so forth, while ethanol can help reveal propolis, alkaloids, flavonoids, sterols, and so on [22, 57]. In terms of method, it should be noted that the aqueous extract served as the basis for the preparation of the Eth 100% extract. Subsequently, the Eth 100% extract could contain both substances soluble in both solvents (water and pure ethanol) and those specifically extractable with one of the solvents. These explanations could account for the high presence of the various compounds mentioned above and influence yield. In contrast, the HydroEth 70% extract can only isolate substances with solubility intermediate between ethanol and water.

10.2 Agar diffusion sensitivity tests

Inhibition diameters were 10 mm for the HydroEth 70% extract and 9 mm for the Eth 100% extract on *Salmonella sp* at a concentration of 100 mg/mL. These results demonstrate that *Salmonella sp* is sensitive to these two extracts, as their inhibition diameters ranged from 9 to 14 mm.

10.3 Dilution sensitivity tests for MIC and MBC determination

Antibacterial parameters (MIC and MBC) for the HydroEth 70% extract were 12.5 and 25 mg/mL, respectively, and the MBC/MIC ratio was 2. Similarly, the MBC/MIC ratio was 2. In the same vein, the Eth 100% extract produced an identical MIC and MBC value of 50 mg/mL. In addition, the MBC/MIC ratio was 1. These results thus confirm that both extracts are bactericidal on *Salmonella sp*, but with a higher bactericidal effect for the HydroEth 70% extract. Previous work by reference [58] on Eth extract from 100% of *Solanum torvum* leaves revealed bactericidal activity against *Salmonella sp*. These two results confirm that whatever the *Solanum torvum* organ used, antibacterial activity against *Salmonella sp* is observed.

10.4 Chemical testing of *Solanum torvum* fruit extracts

Qualitative analysis of both extracts revealed the presence of polyphenols, tannins, flavonoids, coumarins, and saponosides. These results are in line with those of reference [59] on *Solanum torvum* fruits. In the course of their work, these authors

showed that *Solanum torvum* fruits contained alkaloids, and so on, in addition to the aforementioned compounds. Furthermore, the work of reference studies [56, 60, 61] stipulates that these bioactive substances possess antibacterial activities. Quantitative analysis revealed that both extracts contained varying levels of total polyphenols, flavonoids, and tannins. For the Eth 100% extract, the respective contents of total phenols, tannins, and flavonoids were 151.15 ± 0.95 mgEAG/gMS, 18.24 ± 1.31 mgEC/gMS, and 88.37 ± 1.13 mgEQ/gMS. For the HydroEth 70% extract, the respective contents of these same compounds were 92.99 ± 2.9 mgEAG/gMS, 9.42 ± 1.63 mgEC/gMS, and 49.11 ± 0.3 mgEQ/gMS. These results generally show a higher abundance of these compounds in the Eth 100% extract than in the HydroEth 70% extract. Nevertheless, the work of reference [57] on several medicinal plants has revealed that water is capable of isolating anthocyanins, tannins, saponins, terpenes, flavonols, and so forth. In the same way, this author showed that ethanol can highlight compounds such as tannins, polyphenols, polyacetylenes, flavonoids, sterols, alkaloids, propolis, and so on. Thus, during his work, the author revealed that ethanol can extract more bioactive substances than water. Consequently, the combination of these two solvents (water and ethanol) can extract a variety of bioactive molecules that are soluble in both water and ethanol, such as anthocyanins, tannins, saponins, terpenes, flavonols, polyphenols, polyacetylenes, flavonoids, sterols, alkaloids, propolis, and so forth. Therefore, the synergy of the bioactive substances that can be isolated by the water-ethanol mixture could justify the greater activity of the HydroEth 70% extract compared to Eth 100%, even though the compounds identified in this study are not present in high concentrations in this extract. In the same way, reference studies [62, 63] have established that these compounds possess anti-inflammatory, antibacterial, antiviral, antiallergic, antithrombotic, and vasodilatory activities. These results would justify the use of *Solanum torvum* in the treatment of antibacterial infections. Reference studies [51, 64] also highlighted the antibacterial activity of *Solanum torvum* fruit extracts on susceptible and resistant bacteria. Also, studies by reference [65] showed that the aqueous extract of *Solanum torvum* would possess antidiarrheal activities.

10.5 DPPH test

The anti-free radical activity results of the DPPH test for vitamin C, HydroEth 70%, and Eth 100% extracts gave IC_{50} values of 0.007, 0.36, and 0.39 mg/mL, respectively. These values correspond to the concentration of the substance required to inhibit 50% of free radicals in an antioxidant test. Therefore, the lower the value, the greater the anti-free radical activity. Consequently, vitamin C (positive control or reference) inhibited more than 51 ($0.36/0.007$) and 57 ($0.39/0.007$) times more free radicals in the medium than HydroEth 70% extract and Eth 100% extract, respectively. However, the HydroEth 70% and Eth 100% extracts show good anti-free radical activity, as they are still aggregates of molecules (polyphenols, tannins, flavonoids, coumarins, and saponosides), unlike the reference control (vitamin C, pure molecule). This work is in line with that of reference [48] when testing the ethanolic extract of *Solanum torvum* fruits.

11. Conclusion

This study revealed that the 100% ethanolic and 70% hydroethanolic extracts of *Solanum torvum* fruits had antibacterial activity against *Salmonella sp.* It showed that

extraction with pure ethanol concentrated more bioactive substances than hydroethanolic extraction. These compounds are essentially polyphenols, tannins, flavonoids, coumarins, and saponosides. Of these, secondary metabolites, total phenols and flavonoids, and the condensed tannins measured had anti-free radical properties. Nevertheless, sensitivity tests in solid and liquid media showed that the hydroethanol extract was more bactericidal on *Salmonella sp* than the ethanol extract. The bactericidal effect observed was generally dose-dependent, since it depended on the concentration used. The fruits of *Solanum torvum* (a food plant) could therefore be used to develop a phytomedicine for the treatment of salmonellosis.

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