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Solanum torvum Sw. (Solanaceae): Phytochemical screening, antisalmonellal and antioxidant properties of leaves extracts

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Abstract
Salmonellosis remains a major public health problem in developing countries, where it remains endemic due to the precariousness of lifestyle combined with the misuse and inappropriate use of antibiotics. In the contination to search for new anti-salmonellosis substances, *in vitro* antisalmonellal and antioxidant properties of *Solanum torvum* leaves extracts were evaluated.
The Minimal Inhibitary and Minmal Bactericidal Concentrations (MBCs) were determine using broth dilution method. The in vitro antioxidant activity of thes extracts were alsomine using DPPH and Nitric oxide tests, Hydroxyl radicals scavenging assays and iron reduction. Total phenol as well as flavonoid contents and phytochemical screening were performed by standard methods.
In vitro antimicrobial test results showed that the activity of the 95° ethanol extract were significant (MIC <100 µg/mL) on all bacterial isolates and strain. Follow by ethanol extract 70% whose activity was significant on 4/5 of the bacteria tested. The antioxidant test results showed that 95° and 70% ethanol extracts have a high antioxidant potential (IC_{50} < 20 µg/mL) on DPPH. This extract (95° ethanol) showed the best percentages of nitric oxide (62.43%) and hydroxyl radical (49.97%) trapping and has a good ability to chelate iron. These activities correlate significantly with the levels of phenolic compounds and flavonoids. The greater the amount of these compounds, the greater were the radical scavenging effect. The phytochemical test of extracts of *S. torvum* revealed the presence of anthocyanins, saponins, steroids, tannins, anthraquinone, alkaloids, flavonoids, triterpenes, phenols.
These results show that the 95° ethanol extract of *Solanum torvum* has interesting antisalmonellal and antioxidant activities *in vitro* and could be use for the formulation of antisalmonellal phytomedicines.

Keywords: *Solanum torvum*, antisalmonellal, antioxidant, phytochemical screening

1. Introduction
Typhoid fever is a global distribution infectious disease with (about 22 million cases per year with 216,500 deaths) (WHO, 2014) [8], which mostly occur in developing countries where antibiotic resistance is steadily increasing (Gordana *et al.*, 2012) [9]. Amount bacteria that cause diarrhea responsible for the high rate of mortality and morbidity in developing countries, *Salmonella* continues to occupy a prominent place. Typhoid fever is prevalent in developing countries, particularly in Asia, South America and Africa in general, where there is widespread endemicity (WHO, 2014) [8], because of the precarious hygiene conditions. The situation has worsened in recent years with the emergence of major antimicrobial resistant bacterial strains used in treatment such as ampicillin and phenicolates (Ashok *et al.*, 2010) [10]. The high cost, resistance, and toxicity of some of these antibiotics are commonly encountered, as with chloramphenicol, which the uses of was limited because of its bone marrow toxicity (Shukla *et al.*, 2011) [11].
In addition, *salmonella* infection causes the body to produce superoxide and nitric oxide ions, which react together to form peroxynitrite, a potent biological oxidant that can damage certain tissues (Rastaldo *et al.*, 2007) [12]. There is a need for new, low-cost, effective therapies that, in addition to antisalmonellal activity, could reduce the level of free radicals produced during *salmonellal* infection.
Medicinal plants and knowledge about medicinal plants are an important heritage of the African continent (Sanogo, 2006) [13]. In this regard, *Solanum torvum* has so far been used in traditional medicine to fight fevers, diarrhea, and pain. Some authors have shown that *Solanum torvum* has analgesic and anti-inflammatory activities (Ndebia *et al.*, 2007) [14], cardiovascular

And Antiplatelet aggregation (Nguelefack *et al.,* 2008) [15], antifungal (Bari *et al.,* 2010), antimicrobial (Lalitha *et al.,* 2010, Silvaprya *et al.,* 2011) [16], Antioxidant (Waghulde *et al.,* 2011; Abdulkadir *et al.,* 2016) [3, 2], antihyperglycaemic and antidiabetic (Gandhi *et al.,* 2011) [18], Immunomodulatory and erythropoietic (George *et al.,* 2011) [1], antimicrobial and antimycobacterial (Chandrasekhar *et al.,* 2012) [6]. The aim of this work was to evaluate the antisalmonellal and antioxidant activities of the hydroethanolic, ethanolic and aqueous extracts of *Solanum torvum*.

2. Méthodes

2.1 Plant material

The plant material consisted of leaves of *Solanum torvum*, harvested in May 2016 at Dschang in the Menoua Division, Western Region Cameroon. The identification of this plant was made at the National Herbarium of Cameroon (Yaoundé) in comparison with the reference sample kept under the number 1651SRFK.

2.2 Microorganisms and culture media

The microorganisms used consisted of a reference strain of *Salmonella* Typhi from the American Type Culture Collection (ATCC 6539) and *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Paratyphi B and *Salmonella* Typhimurium isolates from the Pasteur Center Bacteriology Laboratory of Yaoundé (Cameroon). Bacterial activation was performed with *Salmonella*/Shigella (Italy Liofilchem) agar streak technique and Mueller Hinton broth (MHB) (Accumix TM, Belgium) was used for the determination of Minimal Inhibitory and Bactericides Concentrations (MIC and MBC).

2.3 Preparation of extracts

After been harvested, the leaves of *Solanum torvum* were dried at room temperature (23 °C ± 2) and ground to obtain a powder. This powder was used to prepare the Hydroethanol, ethanol and aqueous extracts:

2.3.1 Hydroethanol and ethanol extracts: The Hydroethanol and ethanol extracts were prepared by maceration of 50 g of powder in 0.5 L of each solvent (95° ethanol, 70% ethanol, 50% ethanol, 30% ethanol) for 48 hours. The solutions were stirred, filtered through Whatman N°1 paper and the complete evaporation of the solvents was made posible with the aid of a vacuum rotary evaporator (Buchi R200).

2.3.2 Aqueous extracts: the aqueous extracts (infusion and decoction) were prepared following the methods proposed by Duke (2000).

- The aqueous macerate was obtained by maceration of 50 g of powder in 500 mL of distilled water for 48 hours. The resulting mixture was filtered on Whatman N°1 paper and dried in an oven (Memmert) set at 45 °C.
- The aqueous infusion was obtained by mixing 50 g of powder in 500 mL of distilled water boiled beforehand. After 15 minutes, the resulting mixture was filtered through Whatman N°1 paper and dried in an oven (Memmert) set at 45 °C.
- The aqueous decoction was obtained by introducing 50 g of powder into 500 mL of distilled water and boiling for 15 minutes. After cooling, the mixture obtained was filtered on Whatman N°1 paper and dried in an oven (Memmert) set at 45 °C.

2.4 Phytochemical screening of leaves extracts of *S. torvum*

Phytochemical tests were performed according to the standard methods as described by Harbone (1973) [28]. In order to determine the different classes of potential bioactive compounds such as anthocyanins, anthraquinones, steroids, tannins, phenols, saponins, flavonoids, triterpenes and alkaloids present in extracts of *S. torvum*.

2.5 Antimicrobial assay

Minimal Inhibitory Concentrations (MICs) and Bactericides (MBCs) of extracts from *Solanum torvum* and the reference antibiotic (Oxytetracycline) were determined by the micro dilution method using the rapid p-Iodonitrotetrazolium chloride (INT) (Sigma-Aldrich) colorimetric assay (Mativandlela *et al.*, 2006) [20].

Briefly, 100 mL of extracts of *Solanum torvum* were introduced into the first wells of a 96-well micro plate containing previously 100 mL of Mueller Hinton broth (MHB). After serial dilutions following a geometric progression of reason 2. 100 µl of bacterial in ocular prepared in the Mueller Hinton broth (MHB) at the concentration (1.5 × 10^6 CFU/mL) were introduced into all the wells. The plates were covered and incubated at 37 °C for 18 hours. Wells containing the inoculum as well as those containing only the culture media constituted the negative and neutral controls respectively. After incubation, 40 µl of an aqueous solution of INT (0.2%) were added to the wells for revelation. The pink stains appearing in the wells indicate the bacterial growth and the absence of staining is noted as inhibitory concentrations. The smallest concentration is the MIC. For each extract, three columns were made and the revelation was made on two columns. The third was used to determine minimum bactericidal concentrations. This test was performed three times.

After reading the different MICs, 50 mL of the contents of each well where there was inhibition of bacterial growth (absence of pink coloration) were removed and introduced into the corresponding wells of the new plate previously containing 150 mL of MHB. The coated plates were incubated at 37 °C for 48 hours. The wells containing the inoculum as well as those containing only the culture media were also made (negative and neutral controls respectively). The revelation was made with the INT as in the determination of the MIC. All extract concentrations for which no bacterial growth was noted were considered bactericidal and the smallest was noted as MBC. This test was repeated 3 times.

2.6 Antioxidant Assay

2.6.1 DPPH• radical scavenging assay

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH•) is a stable absorbent radical at 517 nm. It fixes a hydrogen atom released by the antioxidant (AH) to form the DPPH-H which no longer absorbs at 517 nm. The stable radical DPPH• is used as an oxidizing radical that can be reduced by the antioxidant (AH). Trapping of the DPPH• radical by leaves extracts of *Solanum torvum* was evaluated according to the method described by Mensor *et al.* (2001) [21]. To 100 µl of extracts dissolved in methanol at different concentrations (200; 100; 50; 25 and 12.5 µg/mL), 900 µl of a methanolic solution of DPPH (20 mg/l) initially quantified were added. The mixture was incubated in the dark and at room temperature for 30 minutes. The absorbance of the mixture was read at 517 nm against white. The blank consisted of 100 µl of dissolved extracts in methanol and 900 µl of methanol. Butylated Hydroxytoluene (BHT) was used as a counter. Optical densities were

converted to DPPH• ($SP_{DPPH•}$) scavenging percentages according to the formula below:

$SP_{DPPH•}$ = [(Absorbance of DPPH - Absorbance of sample)/Absorbance of DPPH] x 100

The regression lines were plotted using the values read from the different percentages of DPPH radical trapping to determine the inhibitory concentration 50 (IC_{50}).

2.6.2 Nitric oxide radical scavenging (NO) assay

The antiradical activity of nitric oxide was evaluated according to the method described by Chanda and Dave (2009) [23] with some modifications. In aqueous medium and at physiological pH, sodium nitroprusside generates nitric oxide which reacts with oxygen to produce nitrite ions (NO_2^-) (stable compound), which can be measured using the Griess reaction (Green *et al.*, 1982) [22]. The amount of nitrite ions (NO_2) produced is proportional to the concentration of nitric oxide.

To 0.5 mL of extract or standard (buthylhydroxytoluene (BHT)) at different concentrations (62.5-1000 µg/mL), 0.75 mL of sodium nitroprusside (10 mM) prepared in phosphate buffer was added. After incubation at room temperature for 60 minutes, 1.25 mL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% N- (1-naphthyl) ethylenedium dihydrochloride in distilled water) was added in ways to have the concentrations final values ranging from 12.5-200 µg/mL (12.5-25. 50 to 100 and 200 µg/mL) in the tubes. The mixture was incubated in the dark and at room temperature for 5 minutes and the optical densities (A_1) was read at 540 nm. White (A_0) was achieved by replacing methanol instead of the sample. The percentage inhibition of nitric oxide generated was calculated as follows:

% inhibition= (1- (A_1/A_0)) x 100.

A_l = absorbance of the extract or standard and A_0= absorbance of blank.

2.6.3 Hydroxyl radical scavenging activity assay

The activity of trapping hydroxyl radicals was determined by the method of Selvakumar *et al.* (2011) [24] using the Fenton reaction. For this purpose, 60 µl of iron chloride ($FeCl_2$) (1.0 mM) were mixed with 90 µl of 1,10-phenanthroline (1 mM), subsequently 2.4 mL of phosphate buffer (0.2 M, pH 7.4) were added. A volume of 150 µl of H_2O_2 (0.17 M) and 1.5 mL of extracts at different concentrations (from 12.5 to 200 µg/mL) were mixed. Addition of hydrogen peroxide (H_2O_2) to the reaction mixture made it possible to initiate the reaction, the mixture was incubated for 5 minutes at room temperature. After incubation, the optical density of the mixture was read at 560 nm. The optical densities were converted into percentages of hydroxyl radical scavenging activity (PHRSA) of the extracts tested according to the formula below:

PHRSA = [(Absorbance of control - Absorbance of sample)/ Absorbance of control] x 100

2.6.4 Ferric reducing antioxidant power (FRAP) assay

The reducing power of iron (Fe^{3+}) extracts was determined according to the method described by Padmaja *et al.* (2011) [25]. To 1 mL of extract at different concentrations (200; 100; 50; 25 and 12.5 µg/mL), 2.5 mL of a solution of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide solution $K_3Fe(CN)_6$ to 1% were added. The whole was incubated in a water bath at 50 °C for 20 minutes. Subsequently, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 minutes. A volume of 2.5 mL of supernatant were combined with 2.5 mL of distilled water and 0.5 mL of a methanolic solution of 0.1% $FeCl_3$. The absorbance of the mixture was read at 700 nm against the similarly prepared white, replacing the extract with distilled water. The positive control consisted of butylated hydroxytoluene (BHT). An increase in absorbance corresponds to an increase in the reducing power of the extracts tested.

2.6.5 Total phenols contents (TPC)

The total phenol content was determined by the method described by Ramde-Tiendrebeogo *et al.* (2012). The reagent consists of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMo12O40). It is reduced, during the oxidation of phenols, in a mixture of blue oxides of tungsten and molybdenum.

These blue pigments have maximum absorption depending on the qualitative and/or quantitative composition of phenolic mixtures in addition to the pH of the solutions, generally obtained by addition of sodium carbonate (Luis *et al.*, 2009). The reaction mixture in this test consisted of 0.02 mL of extract (2 mg/mL), 0.02 mL of Folin-Ciocalteu reagent (2N) and 0.4 mL of a 20% sodium carbonate solution. The mixture was stirred and incubated in a water bath at 40 °C for 20 minutes, then the absorbance was measured at 760 nm. The extract was replaced by distilled water in the blank tubes. A calibration curve was plotted using gallic acid (0-2 mg/mL); the results were expressed in milligrams equivalent of gallic acid per gram of extract.

2.6.6 Total flavonoids content (TFC)

The flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method (Padmaja *et al.*, 2011) [25]. To 100 µl of extract, 1.49 mL of distilled water and 0.03 mL of a solution of 5% $NaNO_2$ sodium nitrite were added. After 5 minutes, 0.03 mL of a solution of 10% aluminum chloride $AlCl_3$ was added. The mixture was allowed to stand for 6 minutes, then 0.2 mL of NaOH (1M) sodium hydroxide solution and 0.24 mL of distilled water was added. The whole was homogenized using a vortex and the absorbance measured at 510 nm. The total flavonoid content was calculated using the standard catechin calibration curve and the results were expressed in milligrams equivalent of catechin per gram of extract.

2.7 Statistical analyzes

The results of the various tests antioxidants were subjected to analysis of the variance (ANOVA) and expressed in the form of means ± ESM. Differences between averages where it existed were assessed using the Waller-Duncan test at the 5% probability level. The SPSS software version 21 for Windows was used for this purpose.

3. Results

3.1 Phytochemical Screening of leaves extracts of *S. torvum*

The phytochemical composition of *S. torvum* leaves extracts is presented in Table 1. It reveals the presence of several groups of secondary metabolites. Saponins, alkaloids, flavonoids, triterpenes and phenols are present in all extracts while steroids were only absent in the 50% ethanol extract. The 95° and 70% ethanol extracts reveal the presence of all phytochemicals tested except tannins and anthraquinones that were absent in the ethanol extract 70%. Anthocyanins, tannins and anthraquinones were absent in the ethanol extracts 30 and 50%, as well as in the aqueous extracts with the exception of the aqueous decoction which revealed the presence of tannins.

Table 1: Phytochemical composition of the different leaves extracts of *S. torvum*

Phyto Chemical metabolites	Extracts						
	95° ethanol	Ethanol 70%	Ethanol 50%	Ethanol 30%	Aqueous infused	Aqueous Decocted	Aqueous Macerated
Anthocyanin	+	+	–	–	–	–	–
Saponins	+	+	+	+	+	+	+
Steroids	+	+	–	+	+	+	+
Tannins	+	–	–	–	–	+	–
Anthraquinone	+	–	–	–	–	–	–
Alkaloids	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+
Phénols	+	+	+	+	+	+	+

+ : Present –: Absent.

3.2 *In vitro* antisalmonellal activities of leaves extracts of *S. torvum*.

Table 2 shows the antisalmonellal activities of various leaves extracts of *S. torvum*. These activities were variable on the bacterial isolates and tested strain. The 95° ethanol extract showed a MIC value of 32 µg/mL on the *Salmonella* Typhimurium, *Salmonella* Typhi and *Salmonella* Typhi ATCC6539 isolates. In addition, 70% and 30% ethanol extracts showed the same MIC values (32 µg/mL) only on the *Salmonella Typhi* isolate. Among the aqueous extracts, the most active was the aqueous decoction on all the isolates and tested strain with MIC values between 64 and 128 µg/mL. Extracts of *S. torvum* for those that existed showed MBC values varying between 256 and 1024 µg/mL. The aqueous and hydroethanol extracts (50 and 30%) of *S. torvum* showed MBC/MIC ratios ≤ 4 on almost all isolates and tested strains. However, 95% ethanol extract and 70% hydroethanol extract showed MBC/MIC ratios > 4 on all the tested pathogens.

Table 2: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of different leaves extracts of *S. torvum* on isolates and strain of *Salmonella*.

Extracts		Strain/isolates				
		STM	SPA	SPB	ST	STs
95° ethanol	MIC (µg/mL)	**32**	64	64	**32**	**32**
	MBC (µg/mL)	256	512	512	512	256
	MBC/MIC	8	8	8	16	8
Ethanol 70%	MIC (µg/mL)	128	64	64	**32**	64
	MBC (µg/mL)	1024	1024	1024	512	512
	MBC/MIC	8	16	16	16	8
Ethanol 50%	MIC (µg/mL)	512	256	512	512	1024
	MBC (µg/mL)	1024	1024	1024	/	/
	MBC/MIC	2	4	2	/	/
Ethanol 30%	MIC (µg/mL)	64	64	128	**32**	64
	MBC (µg/mL)	256	/	512	512	256
	MBC/MIC	4	/	4	16	4
Infused extract	MIC (µg/mL)	256	256	256	512	256
	MBC (µg/mL)	1024	/	1024	/	1024
	MBC/MIC	4	/	4	/	4
Decocted extract	MIC (µg/mL)	64	128	64	128	128
	MBC (µg/mL)	256	512	256	512	512
	MBC/MIC	4	4	4	4	4
Macerated extract	MIC (µg/mL)	256	256	256	512	512
	MBC (µg/mL)	/	1024	1024	1024	/
	MBC/MIC	/	4	4	2	/
Oxytetracycline	MIC (µg/mL)	4	4	8	8	8
	MBC (µg/mL)	32	64	64	64	32
	MBC/MIC	8	32	8	8	4

STM: *Salmonella* Typhimurium, SPA: *Salmonella* Paratyphi A, SPB: *Salmonella* Paratyphi B, ST: *Salmonella* Typhi, STs: strain of *Salmonella* Typhi (ATCC 6539); MIC= Minimal Inhibitory concentration; MBC= Minimal Bactericidal Concentration; /: more than 1024 µg/mL.

3.3 *In Vitro* Antioxidant Activities of Leaves Extracts of *S. torvum*

3.3.1 DPPH radical scavenging activity

The DPPH scavenging activity was evaluated on different extracts of *S. torvum*, and converted to inhibitory concentrations 50 (IC_{50}). The results are summarized in Table 3. It appears that the best IC_{50} values were obtained with the 95° and 70% ethanol extracts with insignificant activities ($p \geq 0.05$) compared to the other extracts. Apart from the 30% ethanol extract and the aqueous macerate, all extracts showed IC_{50} values of less than 50 µg/mL on DPPH entrapment. However, the IC_{50} of the 50% ethanol extract was significantly lower ($p<0.05$) than that of the aqueous infused and aqueous decoction.

Table 3: Free radical scavenging activity of extracts of *S. torvum* against DPPH radical.

Extracts	Concentration (µg/mL) and DPPH scavenging activity (%)					IC$_{50}$
	12.5	25	50	100	200	
Ethanol leaves extract	44.16 ± 1.37^{f}	54.69 ± 1.12^{e}	85.06 ± 0.86^{f}	96.04 ± 0.16^{f}	97.03 ± 0.59^{g}	13.62 ± 1.19^{b}
Ethanol 70%	55.88 ± 0.56^{g}	72.25 ± 1.03^{f}	80.46 ± 1.00^{e}	91.19 ± 0.47^{e}	94.56 ± 0.34^{f}	11.10 ± 1.20^{b}
Ethanol 50%	38.87 ± 0.82^{e}	41.89 ± 1.19^{d}	63.50 ± 1.31^{d}	88.82 ± 1.62^{d}	94.21 ± 0.14^{f}	20.3 ± 0.21^{c}
Ethanol 30%	11.87 ± 1.07^{a}	24.63 ± 0.78^{a}	29.92 ± 1.09^{a}	62.76 ± 1.27^{b}	81.65 ± 0.52^{c}	70.92 ± 2.68^{e}
Infused extract	29.92 ± 0.52^{d}	40.65 ± 1.70^{d}	48.71 ± 1.05^{c}	66.02 ± 1.17^{c}	77.69 ± 0.70^{b}	39.80 ± 0.34^{d}
Decocted extract	27.39 ± 0.37^{c}	35.36 ± 1.01^{c}	48.07 ± 1.12^{c}	68.10 ± 1.16^{c}	81.60 ± 1.12^{c}	40.12 ± 1.12^{d}
Macerated extract	24.33 ± 0.89^{b}	27.25 ± 0.51^{b}	33.78 ± 1.37^{b}	42.23 ± 1.11^{a}	68.15 ± 0.61^{a}	120.99 ± 4.86^{f}
BHT	72.61 ± 0.42^{h}	85.68 ± 0.33^{g}	88.65 ± 0.81^{g}	89.57 ± 0.73^{d,e}	90.92 ± 0.32^{e}	7.72 ± 0.01^{a}

3.3.2 NO radical scavenging activity

The ability of the extracts to trap nitric oxide was determined and presented as percentages of inhibition in Table 4. It appears that the 95° ethanol extract was the most active in the trapping of nitric oxide (12.5, 100 and 200 µg/mL). Furthermore, the 95° ethanol extract showed no significant difference ($p \geq 0.05$) compared to the 70% hydroethanolic extract at the 25 µg/mL concentration and at the aqueous decoction at the 50 µg/mL concentration. However, the 50% ethanol extract exhibited inhibition percentages significantly ($p<0.05$) lower than those of the other ethanolic and hydroethanol extracts. Among the aqueous extracts, the aqueous decoction was the most active on the trapping of nitric oxide at the concentration 25 µg/mL.

Table 4: Free radical scavenging activity of extracts of *S. torvum* against NO radical.

Extracts	Concentration (µg/mL) and NO scavenging activity (%)				
	12.5	25	50	100	200
Ethanol leaves extract	24.10 ± 0.10^{f}	24.52 ± 0.02^{d}	36.93 ± 0.35^{d}	47.20 ± 1.04^{f}	62.43 ± 2.21^{g}
Ethanol 70%	17.96 ± 0.30^{d}	24.46 ± 0.20^{d}	33.83 ± 0.75^{c}	40.93 ± 0.85^{d}	48.53 ± 0.35^{d}
Ethanol 50%	15.43 ± 0.11^{c}	18.30 ± 1.00^{b}	19.80 ± 0.20^{a}	21.03 ± 0.58^{a}	31.53 ± 0.87^{a}
Ethanol 30%	19.56 ± 0.86^{e}	22.73 ± 0.97^{c}	27.16 ± 0.49^{b}	44.26 ± 1.25^{e}	58.80 ± 1.01^{f}
Infused extract	12.33 ± 0.45^{a}	17.60 ± 0.60^{b}	20.50 ± 0.52^{a}	26.60 ± 0.62^{b}	45.30 ± 0.43^{c}
Decocted extract	11.60 ± 0.45^{a}	23.66 ± 0.25^{c}	36.66 ± 0.15^{d}	41.30 ± 0.63^{d}	53.3 ± 0.40^{e}
Macerated extract	13.66 ± 0.40^{b}	15.10 ± 0.20^{a}	26.43 ± 0.41^{b}	37.50 ± 0.10^{c}	40.60 ± 0.36^{b}
BHT	27.80 ± 1.15^{g}	44.20 ± 1.31^{e}	54.56 ± 1.83^{e}	66.66 ± 3.51^{g}	83.43 ± 1.25^{h}

3.3.3 Hydroxyl radical (OH) scavenging activity

The antiradical potential of extracts of *S. torvum* on the inhibition of the production of the hydroxyl radical is presented as a percentage inhibition in Table 5. It follows that the percentage of inhibition of the extract to the 95° ethanol was significantly ($p<0.05$) elevated from the 50 µg/mL concentration than those of the other extracts. Furthermore, the 95° ethanol extract showed no significant difference ($p \geq 0.05$) compared to the ethanol extract 70% and the aqueous macerate at the concentration of 12.5 µg/mL and, at extracted with 30% ethanol at a concentration of 25 µg/mL. However, the aqueous macerate was the most active among the aqueous extracts to trap the hydroxyl radical.

Table 5: Free radical scavenging activity of extracts of *S. torvum* against Hydroxyl radical.

Extracts	Concentration (µg/mL) and OH scavenging activity (%)				
	12.5	25	50	100	200
Ethanol leaves extract	2.77 ± 0.15^{c}	5.97 ± 1.12^{d}	13.77 ± 2.06^{f}	24.07 ± 3.59^{d}	49.97 ± 0.93^{f}
Ethanol 70%	2.30 ± 0.42^{c}	3.57 ± 0.20^{b}	7.03 ± 0.29^{d}	10.57 ± 0.75^{b}	19.73 ± 0.35^{d}
Ethanol 50%	1.30 ± 0.02^{b}	3.10 ± 0.30^{b}	5.60 ± 0.17^{c}	7.67 ± 0.75^{a}	15.30 ± 1.11^{bc}
Ethanol 30%	3.77 ± 0.25^{d}	5.77 ± 0.64^{d}	8.37 ± 0.38^{e}	10.37 ± 0.25^{b}	14.50 ± 0.44^{b}
Infused extract	1.00 ± 0.10^{a}	1.90 ± 0.10^{a}	3.80 ± 0.17^{b}	6.53 ± 0.45^{a}	10.50 ± 1.65^{a}
Decocted extract	1.40 ± 0.10^{b}	2.33 ± 0.31^{a}	2.83 ± 0.21^{a}	7.00 ± 0.30^{a}	16.60 ± 0.53^{c}
Macerated extract	2.73 ± 0.21^{c}	3.97 ± 0.11^{c}	10.20 ± 0.70^{e}	19.50 ± 0.70^{c}	22.07 ± 1.19^{e}
BHT	0.77 ± 0.21^{a}	12.57 ± 0.60^{e}	36.63 ± 0.55^{g}	50.00 ± 4.36^{e}	78.20 ± 0.95^{g}

3.3.4 Ferric Reducing activity (FRAP)

The reducing power of iron was determined by the conversion of Fe^{3+} to Fe^{2+} in the presence of *Extracts of S. torvum*. The results obtained are summarized in Table 6. It shows that the 95° ethanol extract possessed the greatest iron reducing power at the concentration of 50 µg/mL compared with BHT (standard) and the other extracts. On the other hand, the iron reducing power of the ethanol extract 70% was significantly ($p<0.05$) higher than that of the other extracts at low concentrations (12.5 and 25 µg/mLL). At concentrations of 100 and 200 µg/mL, the iron reducing power of aqueous decoction was found to be higher than that of all other extracts. However, the aqueous macerate exhibited weak chelating powers compared to other extracts.

Table 6: Ferric reduction ability of leaves extracts of *S. torvum.*

Extracts	Concentrations (µg/mL) and absorbance at 700 (nm)				
	12.5	25	50	100	200
Ethanol leaves extract	0.653 ± 0.012[c]	1.079 ± 0.019[e]	1.935 ± 0.016[e]	2.093 ± 0.008[e]	2.120 ± 0.018[c]
Ethanol 70%	0.839 ± 0.008[e]	1.159 ± 0.036[f]	1.772 ± 0.008[d]	1.964 ± 0.044[d]	1.967 ± 0.011[b]
Ethanol 50%	0.577 ± 0.027[b]	0.745 ± 0.006[b]	1.336 ± 0.027[c]	1.848 ± 0.013[c]	1.970 ± 0.020[b]
Ethanol 30%	0.441 ± 0.011[a]	0.679 ± 0.016[a]	1.320 ± 0.027[c]	1.897 ± 0.023[d]	2.101 ± 0.005[c]
Infused extract	0.746 ± 0.003[d]	0.815 ± 0.003[c]	1.143 ± 0.058[b]	1.552 ± 0.129[b]	1.940 ± 0.011[b]
Decocted extract	0.765 ± 0.015[d]	0.917 ± 0.042[d]	1.227 ± 0.082[bc]	2.204 ± 0.043[f]	2.869 ± 0.083[d]
Macerated extract	0.423 ± 0.007[a]	0.661 ± 0.017[a]	0.936 ± 0.030[a]	1.141 ± 0.039[a]	1.353 ± 0.075[a]
BHT	0.978 ± 0.049[f]	1.165 ± 0.016[f]	1.649 ± 0.133[d]	2.320 ± 0.057[g]	2.927 ± 0.051[d]

3.3.5 Total phenolic and flavonoids contents of *S. torvum*

The concentration of total phenols and flavonoids of the *Extracts of S. torvum* was determined, and the results are shown in Table 7. It appears that the 95° and 70% ethanol extracts showed levels of statistically identical phenolics ($p \geq 0.05$) and are significantly higher ($p<0.05$) than those of the other extracts. In contrast, the aqueous macerate exhibited the lowest phenolic compound content compared to that of the other extracts. Moreover, the 30% ethanol extract and the aqueous decoction, comparable to each other, had a significantly higher content ($p<0.05$) than those of the 50% ethanol extract and the aqueous infusion.

The flavonoid content is very high in the 95° ethanol extract followed by the ethanol extract 70%, and very low in the macerated compared to the other extracts. In addition, the 30% ethanol extract and the aqueous decoction had a flavonoid content that was statistically identical ($p \geq 0.05$), and significantly ($p<0.05$) higher than the 50% ethanol extract and the aqueous infusion.

Table 7: Total phenolic and flavonoids contents of leaves extracts of *S. torvum.*

Extracts	Total phenolic (mgEAG/g of extract)	Flavonoids (mgECat/g of extract)
Ethanol leaves extract	29.64 ± 0.59[d]	1.67 ± 0.13[e]
Ethanol 70%	28.80 ± 0.43[d]	1.24 ± 0.01[d]
Ethanol 50%	15.65 ± 0.69[b]	0.59 ± 0.05[b]
Ethanol 30%	22.60 ± 0.97[c]	1.15 ± 0.02[c]
Infused extract	16.82 ± 0.78[b]	0.49 ± 0.06[b]
Decocted extract	21.08 ± 2.42[c]	1.09 ± 0.05[c]
Macerated extract	9.05 ± 0.80[a]	0.34 ± 0.02[a]

4. Discussion

4.1 Antibacterial activities

The results of antisalmonellal tests of *S. torvum* leaves extracts (MIC and MBC) showed that *Salmonella* Typhimurium and *Salmonella* Typhi were more sensitive (MIC = 32 µg/mL) compared to other bacteria. By following the classification scale of the antimicrobial activity of plant extracts as presented by Kuete (2010) [29], significant activities (MIC less than 100 µg/mL) were found with the 95° extract on all isolates and strains tested. The ethanol extracts 30 and 70% also showed significant activities on 4/5 of the tested bacteria. However, moderate inhibitory activities (MIC between 100 and 625 µg/mL) were found with 50% ethanol extract, infused and aqueous macerated on all bacteria tested. The aqueous decoction was the most significant (MIC < 100 µg/mL) of the aqueous extracts on the *Salmonella* Typhimurium and *Salmonella* Paratyphi B isolates. The genetic and structural composition of the isolates and strain could justify the differences in activity observed with the same extract. On the other hand, the quantitative and qualitative variations of the active metabolites of each extract could explain the differences of activity observed for the same isolate or strain with the different extracts (Takeo *et al.*, 2004) [30].

Phytochemical screening of leaves extracts of *S. torvum* has revealed in various ways the presence of bioactive compounds such as anthocyanins, saponins, steroids, tannins, anthraquinone, alkaloids, flavonoids, triterpenes, phenols. Some of these compounds had already been described in the literature (Jaiswal, 2012; Karmakar *et al.*, 2015) [5, 4]. These antimicrobial activities could be justified by the presence of methyl caffeate in these extracts. Methyl caffeeate compound isolated in the methanolic extract of *S. torvum* fruits whose antibacterial activity was demonstrated by Chandrasekhar *et al.* (2012) [6]. The presence of tannins is also important in the antimicrobial activity of these extracts (Cowan, 1999) [31].

Many studies indicate that substances are considered bacteriostatic agents when the MBC/MIC ratio is > 4 and as bactericidal agents when the MBC/MIC ratio ≤ 4 (Gatsing and Adoga, 2007) [32]. In this study, 95° and 70% ethanol extractswere bacteriostatic on all bacteria tested. While, aqueous and hydroethanol extracts (50 and 30%) were bactericidal on almost all isolates and strain tested.

4.2 Antioxidant Activities

Microbial infection increases the formation of highly reactive molecules during oxygen metabolism, which causes significant cell and tissue damage, resulting in the formation of free radicals (KamLesh *et al.*, 2007) [33]. To check whether, in addition to antisalmonellal activities, extracts of *Solanum torvum* could counteract the formation of free radicals, the evaluation of antioxidant activities has been studied *in vitro* on the trapping of DPPH, nitric oxide, the OH° radical and on the reducing power of iron.

In the presence of free radical scavengers (extract), the DPPH (1, 1-diphenyl-2-picrylhydrazyl) purple color is reduced to yellow 1,1-diphenyl-2-picrylhydrazyl hydrazine (Maataoui *et al.*, 2006) [34]. In the present study, DPPH radical scavenging activity was converted to inhibitory concentrations (IC_{50}) and following the antioxidant potential classification scheme of plant extracts as presented by Souri *et al.* (2008) [35], the high antioxidant potential (IC_{50} < 20 µg/mL) was observed on 95° and 70% ethanol extracts. The moderate antioxidant potential (20 µg/mL ≤ IC_{50} ≤ 75 µg/mL) was observed on the aqueous infusion, the aqueous decoction and the hydroethanol extracts 30% and 50%. Only the aqueous macerate has a low antioxidant potential (IC_{50} > 75 µg/mL). Some authors have already shown the ability of the fruit of this plant to trap DPPH (Waghulde *et al.*, 2011; Abdulkadir *et al.*, 2016) [3, 2].

In biological systems, one of the most unstable and reactive free radicals is the hydroxyl radical. Its limited diffusion and instability allow it to react with many nearby biological structures (proteins, lipids, DNA), resulting in multiple

damage (Delattre *et al.*, 2005) [36]. In the present study, the 95° ethanol extract exhibited the highest percentage of hydroxyl radical inhibition, which is in agreement with the result obtained on the DPPH° test as well as, in the nitric oxide trapping test.

Transition metals in their reduced state may participate in the Fenton reaction. This is the case of Fe^{2+}, which in the free State is a powerful electron donor, reacting notably with hydrogen peroxide to generate highly reactive hydroxyl radicals (Haleng *et al.*, 2007) [37]. The reducing power is evaluated by the conversion of Fe^{3+} to Fe^{2+} in the presence of plant extracts. Indeed, the reducing capacity of a compound can serve as an indicator of its potential antioxidant activity (Meir *et al.*, 1995) [38]. Thus, extracts of *S. torvum* showed varying iron reducing powers according to the extracts. This could be justified by the presence of antioxidant compounds in quantity and quality in each extract and also by the fact that each oxidant corresponds its mechanism of antioxidant action. The richness of phenolic compounds and flavonoids in the 95° and 70% ethanol extracts could justify their strong *in vitro* antioxidant properties to inhibit DPPH, nitric oxide and hydroxyl radical. Phenolic compounds (isoquercetin and quercetin) isolated from *S. torvum* by Lu *et al.* (2011) [7] confirm our results on the antioxidant potential of this plant. Note that quercetin is generally used as a reference antioxidant.

These results suggest that the leaves extracts of *Solanum torvum* have a high antioxidant activity thanks to the phenolic compounds present in each of these extracts.

Conclusion

The results obtained in this work showed that the ethanolic and hydroethanol leaves extracts of *Solanum torvum* have the interesting antisalmonellal activities, and can prevent the oxidative stress caused by the infection. However, further studies should be conducted to study the antisalmonellal properties in a living organism and the state of oxidative stress generated by the infection.

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References

1. George K, Patrick A, Terrick A, Immunomodulatory and erythropoietic effects of aqueous extract of the fruits of *Solanum torvum* Swartz (Solanaceae), Pharmacognosy Res. 2011; 3(2):130-134.
2. Abdulkadir AR, Mata N, Hasan MM, Jahana MS. *In vitro* antioxidant activity of the ethanolic extract from fruit, stems, and leaves of *Solanum torvum.* Science Asia. 2016; 42:184-189.
3. Waghulde H, Kamble S, Patankar P, Jaiswal B, Pattanayak S, Bhagat C, Mohan M. Antioxidant Activity, Phenol and Flavonoid Contents of Seeds of *Punica granatum* (Punicaceae) and *Solanum torvum* (Solanaceae). Pharmacologyonline. 2011; 1:193-202.
4. Karmakar K, Islam AM, Chhanda SA, Tuhin TI, Muslim T, Rahman MA. Secondary Metabolites from the Fruits of *Solanum torvum* SW. J Pharm Phyto chem. 2015; 4(1):160-163.
5. Jaiswal BS. *Solanum torvum*: A review of its traditional uses, phytochemistry and pharmacology. Int J Pharm Bio Sci. 2012; 3(4):104-111.
6. Chandrasekhar B, Veeramuthu D, Naif Abdullah AD, Balakrishna K, Nitin PK, Vikrant SR *et al*. Antimicrobial and antimycobacterial activities of methyl caffeate isolated from *Solanum torvum* swartz. Fruit. Ind J Micro biol. 2012; 52(4):676-681.
7. Lu YY, Luo JG, Yijong L. Chemical Constituents from *Solanum torvum.* Chin J Nat Med. 2011; 9(1):30-32.
8. WHO. Typhoid fever surveillance and vaccine use, South-East Asia and Western Pacific Regions, 2009-2013. Weekly Epidemiological Record. 2014; 89:429-40.
9. Gordana M, Bogdanka A, Dragica T, Milena L, Brankica D. Antibiotic susceptibility of *Salmonella* spp.: a comparison of two surveys with a 5 years interval. J InterMed Asso Bulg - Ann Proceed. 2012; 18(1):216-219.
10. Ashok R, Peter K, Joselyne N, Emma N. Antimicrobial susceptibility patterns of *Salmonella* Typhi from Kigali, Rwanda. Shiraz E-Med J. 2010; 11(3):117-121.
11. Shukla P, Bansode WF, Singh KR. Chloramphenicol toxicity: A Review. J Med Medic Sci. 2011; 2(13):1313-1316.
12. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, *et al*. Nitricoxide and cardiac function. Life Sci. 2007; 81:779-793.
13. Sanogo R. Le rôle des plantes médicinales en médecine traditionnelle. Développement, Environnement et Santé. Faculté de médecine et de pharmacie et d'onto-Stomatologie, Université de Bamako, 2006, 53.
14. Ndebia EJ, Kamga R, Nchunga ANB. Analgesic and anti-inflammatory properties of aqueous extract from leaves of *Solanum torvum* (Solanaceae). AJTCAM. 2007; 4:240-244.
15. Nguelefack TB, Mekhfi H, Dimo T, Afkir S, Nguelefack-Mbuyo EP, Legssyer A, *et al*. Cardiovascular and anti-platelet aggregation activities of extracts from *Solanum torvum* (Solanaceae) fruits in rat. JCIM. 2008; 5(7):180-192.
16. Lalitha V, Raveesha K, Kiran B. Antimicrobial Activity of *Solanum torvum* Swart. Against Important Seed Borne Pathogens of Paddy. IJEE. 2010; 1(2):160-164.
17. Sivapriya M, Dinesha R, Harsha R, Gowda SST, Srinivas L. Antibacterial activity of different extracts of Sundakai (*Solanum torvum*) fruit coat. Int J Biol Chem. 2011; 10:3923.
18. Gandhi GR, Ignacimuthu S, Paulraj MG, Sasikumar P. Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from *Solanum torvum* Swartz. Fruit in streptozotocin induced diabetic rats. Eur J Pharmacol. 2011; 30:623-31.
19. Duke JA. Le pouvoir des plantes. Encyclopédie des Plantes Médicinales du Département de Phytothérapie de Bobigny, France, 2000.
20. Mativandlela SPN, Lall N, Meyer JJM. Antimicrobial, antifungal, antitubercular activity of *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. South Africa Jour B. 2006; 72:232-237.
21. Mensor LL, Menezes FS, Leitao GG, Reis ASO, Dos Santos TC, Coube CS, *et al*. Screening of Brazilian plant extracts for antioxidant activity by the used DPPH free radical method. Phyto Research. 2001; 15:127-130.
22. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and ^{15}N nitrate in biological fluids. Anal. Bio chem.

1982; 126:131-138.

23. Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Microb. Res. 2009; 3:981-996.
24. Selvakumar K, Madhan R, Srinivasan G, Baskar V. Antioxidant Assays in Pharmacological Research. A J Pharm Techn. 2011; 1(4):99-103.
25. Padmaja M, Sravanthi M, Hemalatha KPJ. Evaluation of antioxidant activity of two Indian medicinal plants. Jour Phytology. 2011; 3(3):86-91.
26. Ramde-Tiendrebeogo A, Tibiri A, Hilou A, Lompo M, Millogo-Kone H, Nacoulma OG, *et al*. Antioxidative and antibacterial activities of phenolic compounds from Ficus sue Forssk. Int J Biol Chem Sci. 2012; 6(1):328-336.
27. Luis A, Domingues F, Gil C, Duarte AP. Antioxidant activity of extracts of Portuguese shrubs: *Pterospartum tridentatum, Cystisus scoparius* and Erica spp. J Med Plants Res. 2009; 3(11):886-893.
28. Harbone JB. Phytochemical Methods: a Guide to Modern Techniques of Plants Analysis. Chapman and Hall Ltd: London, 1973, 50-116.
29. Kuete V. Potential of cameroonian plants and derived products against microbial infections. Planta Medica. 2010; 76:1-13.
30. Takeo O, Masato K, Keiko S, Rika O, Junko M, Hiroshi I, *et al*. *In vitro* and *in vivo* antibacterial activities of tricyclic ketolide Te-802 and its analogs. Journ Antibio. 2004; 57:518-527.
31. Cowan MM. Plant products as antimicrobial agent. Clin Microbio Rev. 1999; 12(4):564-582.
32. Gatsing D, Adoga GI. Antisalmonellal activity and phytochemical screening of the various parts of Cassia *petersiana Bolle* (Caesalpiniaceae). Res J Micro biol. 2007; 2(11):876-880.
33. KamLesh D, Yogest S, Ajit P. Evaluation *in vitro* of antioxidant activity of *Sida rhombifolia* (L.) Ssp retusa (L.). J Med Food. 2007; 10:683-688.
34. Maataoui BS, Hmyene A, Hilali S. Activités antiradicalaires d'extraits de jus de fruits du figuier de barbarie (*Opuntia ficus indica*). Lebanese Sci. J. 2006; 7(1):3-8.
35. Souri E, Amin G, Farsam H, Barazandeh TM. Screening of antioxidant activity and phenolic content of 24 medicinal plants. J Pharm Sci. 2008; 16(2):83-87.
36. Delattre J, Beaudeux JL, Bonnefont-Rousselot D. Radicaux libres et stress oxydant, aspects biologiques et pathologiques Première édition. Edn. Tec et Doc, Lavoisier, Paris. 2005; 547:1708-1714.
37. Haleng J, Pincemail J, Defraigne JO, CHarlier C, Chapelle JP. Le stress oxydant. Rev Med Liege. 2007; 62(10):628-638.
38. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agri Food Chem. 1995; 43:1813-1817.