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In vitro Antioxidant Activities and Effect of Hydroethanolic and Aqueous Extracts of *Terminalia avicennioides* (Combretaceae) on *Salmonella*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors LCNF and DG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BTT and RST managed the analyses of the study. Authors GTK, NK and STL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Today, Typhoid fever remains a public health problem in developing countries due to the poor quality of lifestyle associated with abusive and inappropriate use of antibiotics.
Aims: Considering the ethnopharmacological relevance of *Terminalia avicennioides* (*T. avicennioides*) (Combretaceae), this study was designed to investigate the *in vitro* antisalmonella and antioxidant activities of various extracts of this plant.
Methodology: The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *T. avicennioides* extract.

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These extracts were also subjected to *in vitro* antioxidant tests such as diphényl-2-picrylhydrazyle (DPPH) radical scavenging test, ferric reducing-antioxidant power (FRAP), hydroxyl radical (OH) nitric oxide (NO) and Hydrogen Peroxide Scavenging Capacity.
Results: *In vitro* antisalmonella activity reveals that *T. avicennioides* stem bark extracts presented MIC values ranging from 64 to 512 µg / mL on tested microorganisms. This extract exhibited a good ability to trap DPPH with an IC_{50} of 8.30 µg / mL. The iron reducing power obtained with this extract had ODs ranging from 0.96 to 1.63. Phytochemical screening showed the presence of alkaloids, flavonoids, saponins, phenols anthocyanin and anthraquinone in all the extracts.
Conclusion: The results suggest that stem extract of *T. avicennioides* contains antisalmonella and antioxidant substances, which could be used for the treatment of typhoid fever and another salmonellosis.

Keywords: Terminalia avicennioides; antisalmonella activity; antioxidant activity; phytochemical screening.

1. INTRODUCTION

Typhoid fever is a bacterial infectious disease with a digestive starting point and mandatory to declare. The bacteria responsible belongs to the genus, *Salmonella* enterica serotypes *Typhi* and Paratyphi, whose reservoir is strictly human [1]. Recent studies have shown that the annual global incidence of typhoid fever is 1120 million cases with deaths ranging between 128,000 and 161,000 [2]. In Cameroon, typhoid fevers affected about 124,526 to 154,103 people in between 2015 and 2016 [3]. After malaria, typhoid fever is the second most commonly reported disease in Cameroon by health personnels [3]. But the situation is all the more worrying as batrerial strain have been developing resistance to antibiotics (ampicillin and phenicolates) present on the market in recent years [4]. In addition, during *salmonella* infection, or following exposure of the body to exogenous toxins, the production of free radicals such as superoxide anion and nitric oxide (O_2^-, NO.), although controlled by antioxidant defence systems under normal physiological conditions, can increase and generate oxidative stress. This oxidative stress state is the direct cause of various pathological conditions such as aging and cancer and the indirect cause of the peroxidation of lipids in foodstuffs. In any case, the risk is increased with the accumulation of these molecules in the body resulting in a radical chain reaction that degrades vital biological molecules, namely DNA, lipids, proteins and carbohydrates [5]. Plants species belonging to the Combretaceae family have been tested for their antimicrobial activities against some pathogenic microorganisms that are prone to drug resistance [6]. Because of this, an update information on the properties and uses of any medicinal plant belonging to this group needs to be investigated. *Terminalia avicennioides* (Guill and Perr), has shown possess some medicinal values. It is used in the treatment of different types of ailments. The plant grows abundantly in the Savanna region of Africa as a shrub or small tree. It's popularly found growing in the west region of Cameroon. The common name of the plant; *T. avicennioides* is 'Indian laurel'. "In Nigeria, it is locally called 'baushe' in Hausa, 'Idi' in Yoruba, 'Edo' in Ibo, 'Kpace' in Nupe; 'Kpayi' in Gwari and 'Bodeyi' in Fulfulde [6]. In Cameroon, it is locally called 'Sahré' in Bamoun. This work was therefore aimed at evaluating the antisalmonella and antioxidant activities of crude extracts of *T. avicennioides* in order to ascertain their potential as antityphoid drugs.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

T. avicennioides stem barks were collected from Foumban, Noun Division, West Region of Cameroon, in July 2018. The plant was identified by Dr. TCHIENGUE Basthelemy (Botanist of Cameroon National Herbarium) and confirmed at the Cameroon National Herbarium (Yaoundé), using a voucher specimen registered under the reference HNC N°7908/SRF-Cam.

2.2 Preparation of Extracts

The fresh stem bark was air dried at room temperature under shade for three weeks, then mashed. The obtained powder was used for the preparation of hydroethanolic extracts (95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol) and aqueous extracts (infusion, decoction, maceration).

Aqueous extracts were prepared according to the methods described by Duke [7] while hydroethanolic extract were obtained by macerating 50 g of powder in 500 ml in hydroethanolic at defferent concentrations (95%, 70%, 50% and 30%). After 48 hours, these macerates were filtered using Whatman N°1 paper. The filtrates were dried at 45ºC in a ventilated oven (Memmert).

2.3 *In vitro* Antisalmonella Activity

2.3.1 Microorganisms and culture media

The test microorganisms including *Salmonella Typhi* (ST) and *Salmonella Typhi*murium (STM) isolates were obtained from Centre Pasteur, Yaoundé, Cameroon. One strain of *Salmonella Typhi* (ATCC 6539) obtained from the American Type Culture Collection (ATCC) was also used as reference strain. These microorganisms were maintained on agar slant at -4ºC and subcultured on a fresh colony approximately 24 h prior to any antimicrobial test. *Salmonella*-Shigella Agar (SSA) were used for the activation and maintenance of *Salmonella* strain/isolates whereas Mueller Hinton Broth (MHB) was used for susceptibility tests (Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs)).

2.3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The inhibitory potential of bacterial growth of *T. avicennioides* extracts was determined by the microdilution method as described by Mativandlela et al. [8]. In each well of a 96-well microplate, 100 µl culture broth (MHB) were introduced. Then, 100 µl of each extract were added to obtain an initial concentration (4096 µg/ml) respectively into the first 3 wells of the first line; then serial dilutions were performed to give final concentrations ranging from 2048 to 16 µg/ml. A volume of 100 µl of the inoculum was introduced into each well. The plates were incubated at 37ºC for 18 hours. Wells containing the inoculum as well as those containing only culture media and dimethylsulfoxide were drilled and represented the negative and neutral controls respectively. After this incubation time, 40 µl of para-iodonitrotetrazolium bromide chloride (INT) 0.2% were added to these wells. Thus, wells that turned pink after adding INT indicated bacterial growth [8]. All concentrations that prevented the pink colour from appearing were taken as inhibitory concentrations and the smallest was noted as the MIC. For each extract, three columns were made and the revelation was made on two columns. The third was used to determine the Minimum Bactericidal Concentrations. This test was performed three times. The MBC values were determined by adding 50 µL aliquots of content of each well (without INT which did not show any visible colour change after incubation during MIC assay), into 150 µL of fresh Mueller Hinton broth. These preparations were further incubated at 37ºC for 48 hours and MBCs were revealed by the addition of INT as above. All extract concentrations at which no colour changed were considered as bactericidal concentrations, and the smallest of these concentrations was considered as the MBC. These tests were carried out in triplicates at three different occasions.

2.4 Antioxidant Assay

2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

This test is based on the reduction of a free violet-coloured 1,1-diphenyl-2-picrylhydrazyl (DPPH.) radical to a stable yellow derivative in the presence of antioxidant compounds. The free radical scavenging activities of the crude extracts of *T. avicennioides* were evaluated using the DPPH (2, 2-diphenyl- 1-picrylhydrazyl) assay method as described by Mensor et al. [9]. Briefly, the extract (2000 µg/mL) was two-fold serially diluted with methanol. A hundred microliters (100 µL) of the diluted extract was mixed with 900 µL of DPPH (0.3 mM) methanol solution, to give a final extract concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). After 30 min of incubation in the dark at room temperature, the optical density was measured at 517 nm using a spectrophotometer “Jenway, model 1605”. Ascorbic acid (Vitamin C) was used as control. Each assay was done in triplicate and the results, recorded as the mean ± SD of the three findings, and were illustrated in a tabular form. The radical scavenging activity (RSA, %) was calculated as follows: RSA (%) = $\frac{\text{ADPPH}-\text{Asample}}{\text{ADPPH}}$ x 100(where A = Absorbance). The radical scavenging percentages were plotted against the logarithmic values of the concentration of test samples and a linear regression curve was established in order to calculate the RSA50 or IC_{50}, which is the amount of sample necessary to inhibit 50% of free radical DPPH.

2.4.2 Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing power was determined by the transformation of Fe^{3+} in to Fe^{2+} in the presence of the extracts. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Briefly, the extract (2090 µg/mL) was two-fold serially diluted with methanol. Four hundred microliters (400 µL) of the diluted extract were mixed with 500 µL of phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide and incubated at 50°C for 20 min. Then 0.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (0.5 mL) was diluted with 0.5 mL of water and mixed with 0.1 mL of freshly prepared ferric chloride (0.1%) to give a final extract concentration range of 12.5 – to 200 µg/mL. The absorbance was measured at 700 nm. All tests were performed in triplicates and the results were the average of the three observations. Vitamin C was used as the positive control. Increased absorbance of the reaction mixture indicated higher reduction capacity of the sample (extracts) [10].

2.4.3 Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [11]. Reagent mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.5 mL of extract at various concentrations. H_2O_2 was added to the mixture to start the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture was measured at 560 nm with the spectrophotometer "Jenway, model 1605". Vitamine C was used as standard and the hydroxyl radicals scavenging (HRS) activity was calculated as follow $\text{HRS\%} = \frac{\text{Acontrol}-\text{Asample}}{\text{Asample}} \times 100$ HRS (%) = $\frac{\text{Acontrol}-\text{Asample}}{\text{Asample}}$ x 100 (where A = Absorbance).

2.4.4 Nitric oxide radical scavenging (NO) assay

At physiological pH, Nitric oxide generated from sodium nitroprusside in aqueous solution interacts with oxygen to produce nitrite ions, which are measured using the Griess reaction [12]. The method reported by Chanda and Dave et al. [13] was used, with slight modification. To 0.75 mL of 10 mM sodium nitroprusside in phosphate buffer was added 0.5 mL of extract or reference compounds (Vitamin C) in different concentrations (62.5 - 1000 µg/mL). The resulting solutions were then incubated at 25ºC for 60 min. A similar procedure was repeated with methanol as blank, which served as negative control. To 1.25 mL of the incubated sample, 1.25 mL of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-napthylethylenediamine dihydrochloride in water) was added. A final concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL) was obtained. After 5 min of incubation in the dark at room temperature, the absorbance of the chromophore formed was measured at 540 nm. The percentage of inhibition of the nitrite oxide generated was measured by comparing the absorbance values of control and test samples. The percentage of inhibition was calculated according to the following equation: % inhibition= (1- (A_1/A_0)) x 100 Where, A_l = absorbance of the extract or standard and A_0 = absorbance of the negative control.

2.4.5 Hydrogen peroxide scavenging capacity

The ability of the *T. avicennioides* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. [14]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). The absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging capacities of both *T. avicennioides* extracts and the standards were calculated:

$$\text{\% Scavenged [H2O2]} = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100$$

maximum absorbance at 500 nm.

2.4.6 Total phenols contents (TPC)

The amount of total phenols was determined by Folin-Ciocateu Reagent method [15]. The reaction mixture contained 20 µL of extract (2000 µg/mL), 1380 µL of distilled water, 200 µl (2N) of FCR (Folin Ciocalteu Reagent) and 400 µL (20%) of sodium carbonate solution. The mixture

was incubated at 40ºC for 20 min. After cooling, the absorbance was measured at 760 nm. In the control tube, the extract volume was replaced by distilled water. A standard curve was plotted using Gallic acid (0-0.2 µg/mL). The tests were performed in triplicates and the results were expressed in milligrams of Gallic Acid Equivalents (mgGAE) per gram of extract.

2.4.7 Total flavonoids content (TFC)

The total flavonoids was determined by the Aluminum chloride method [16]. Methanolic solution of extracts (100 µL, 2000 µg/ml) were mixed with 1.49 mL of distilled water and 30 µL of a 5% $NaNO_2$ solution. After 5 min, 30 µL of 10% $AlCl_3H_2O$ solution were added. After 6 min, 200 µl (0.1 M) of sodium hydroxide and 240 µl of distilled water were added. The solution was well mixed and absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoids content was calculated using a standard calibration curve. The results were expressed in milligrams of gallic acid Equivalents (mgGA) per gram of extract.

2.5 Qualitative Phytochemical Screening

The phytochemical screening was performed using standard methods described by Harbone [17]. The extracts of *T. avicennioides* stem bark were screened for the following classes of phytochemical compounds: Alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes.

2.6 Statistical Analysis

The data obtained in this study were analysed using one-way analysis of variance (ANOVA) and presented as mean ± standard deviation (SD) of the three replications. The levels of significance, considered at P = .05, were determined by Waller-Duncan test using the Statistical Package for the Social Sciences (SPSS) software version 22.0.

3. RESULTS

3.1 Antisalmonella Activities

The MICs/MBCs values are presented in (Table 1). All extracts showed activity against isolates and strain tested with MICs between 64 and 512 µg/ml. Hydroethanolic extracts showed the best activities with isolates and strains tested with MICs between 64 and 512 µg/ml compared to aqueous extracts. The 70% hydroethanolic extract was the most active extract (MIC of 64 µg/ml) with respect to the strain tested.

3.2 Antioxidant Activity

3.2.1 DPPH free-radical scavenging activity

The results of the DPPH antiradical activity of the different extracts are shown in Fig. 1. This figure shown that these extracts possess antiradical activities. In addition, these activities are concentration dependent for each extract tested. It is also noted that the antiradical activities of these extracts are not significantly different (p = .05) from the 50 µg/ml concentration and are compared to 25 to 200 µg/ml concentrations. However, the infusion showed a higher activity than the other extracts and that of Vitamin C. In addition, the 50% hydroethanolic extract significantly reduced the activity than the other extracts and that of Vitamin C except the decoction at the concentration of 12.5 µg/ml. The infusion has the lowest concentration that traps 50% DPPH compared to other extracts and vitamin C, and compared to the 50% hydroethanolic extract which has the highest concentration.

Concentrations that trap 50% of DPPH (IC_{50}) (Table 2) reveal that the IC_{50} of the infusion extract was the lowest compared to vitamin C. However, hydroethanolic extract, decoction and maceration extracts showed not significantly elevated IC_{50} (p= .05) compared to vitamin C.

3.2.2 Ferric reducing / antioxidant power determination

The reducing power of iron was determined by the transformation of Fe^{3+} into Fe^{2+} in the presence of the extracts. The results obtained are shown in Fig. 2, where it is noted that at concentrations of 0 to 12.5 µg/ml, the reducing power of iron for the 50% hydroethanolic extract is higher than other extracts, while that of vitamin C is lower than that of other extracts. However, the 70% hydroethanolic extract had a higher reducing power of iron than all other extracts and that of vitamin C at concentrations of 50 to 100 µg/ml.

Table 1. Antisalmonella activity of different extracts of *T. avicennioides*

Extracts		Strains/isolates		
		STS	ST	STM
EtOH 95%	MIC (µg/mL)	256	512	256
	MBC (µg/mL)	> 1024	1024	512
	MBC/MIC	/	2	2
EtOH 70%	MIC (µg/mL)	**64**	**128**	256
	MBC (µg/mL)	1024	1024	1024
	MBC/MIC	16	8	8
EtOH 50%	MIC (µg/mL)	512	**128**	256
	MBC (µg/mL)	> 1024	> 1024	> 1024
	MBC/MIC	/	/	/
EtOH 30%	MIC (µg/mL)	512	256	256
	MBC (µg/mL)	> 1024	> 1024	> 1024
	MBC/MIC	/	/	/
Decocted	MIC (µg/mL)	256	256	512
	MBC (µg/mL)	> 1024	1024	> 1024
	MBC/MIC	/	4	/
Infused	MIC (µg/mL)	512	256	256
	MBC (µg/mL)	> 1024	> 1024	> 1024
	MBC/MIC	/	/	/
Macerated	MIC (µg/mL)	256	**128**	**128**
	MBC (µg/mL)	> 1024	512	1024
	MBC/MIC	/	4	8
Ciprofloxacin	MIC (µg/mL)	8	4	4
	MBC (µg/mL)	64	16	32
	MBC/MIC	8	4	8
Oxytetracyclin	MIC (µg/mL)	1	2	1
	MBC (µg/mL)	8	4	16
	MBC/MIC	8	2	16

ST: Salmonella Typhi; STM: Salmonella Typhimurium; STS: Salmonella Typhi ATCC1369; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration EtOH: ethanol

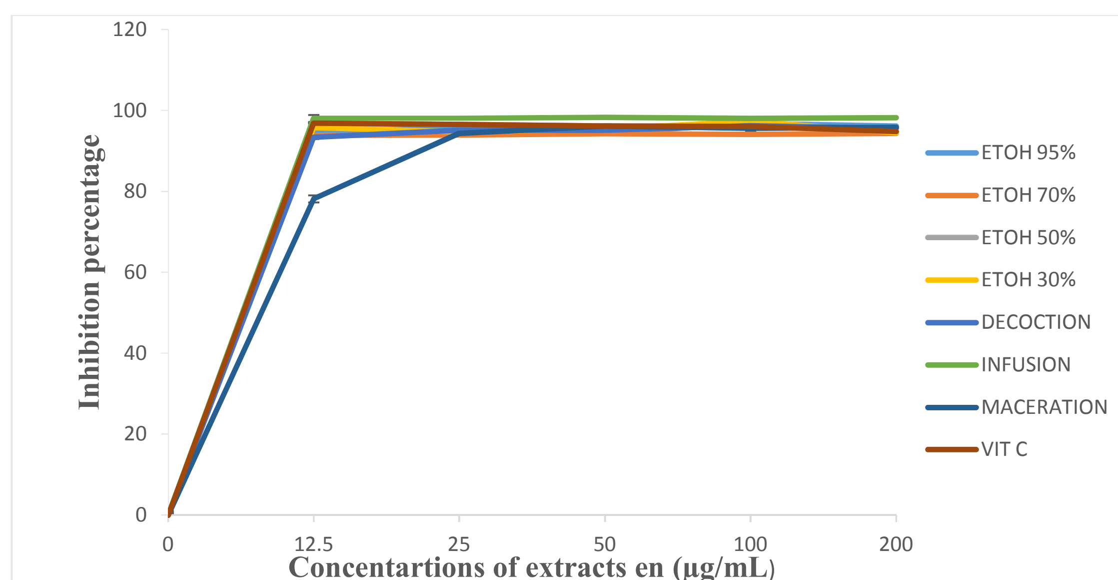

Fig. 1. Anti-radical effects of *T. avicennioides* extracts on DPPH at different concentrations
ETOH = ethanol; VIT C = Vitamin C

Table 2. IC_{50} values of DPPH of *Terminalia avicennioides* extracts

Extracts	IC50 (µg/ml)
EtOH 95%	$8{,}77 \pm 0{,}09^{b}$
EtOH 70%	$9{,}10 \pm 0{,}12^{bc}$
EtOH 50%	$10{,}43 \pm 0{,}49^{d}$
EtOH 30%	$8{,}80 \pm 0{,}22^{b}$
Decoction	$8{,}85 \pm 0{,}09^{b}$
Infusion	$8{,}30 \pm 0{,}01^{a}$
Maceration	$9{,}27 \pm 0{,}04^{c}$
VIT C	$8{,}73 \pm 0{,}02^{b}$

The values in the table are presented as averages ± standard deviation of 3 repetitions. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P = .05). VITC: Vitamin C, EtOH: Ethanol

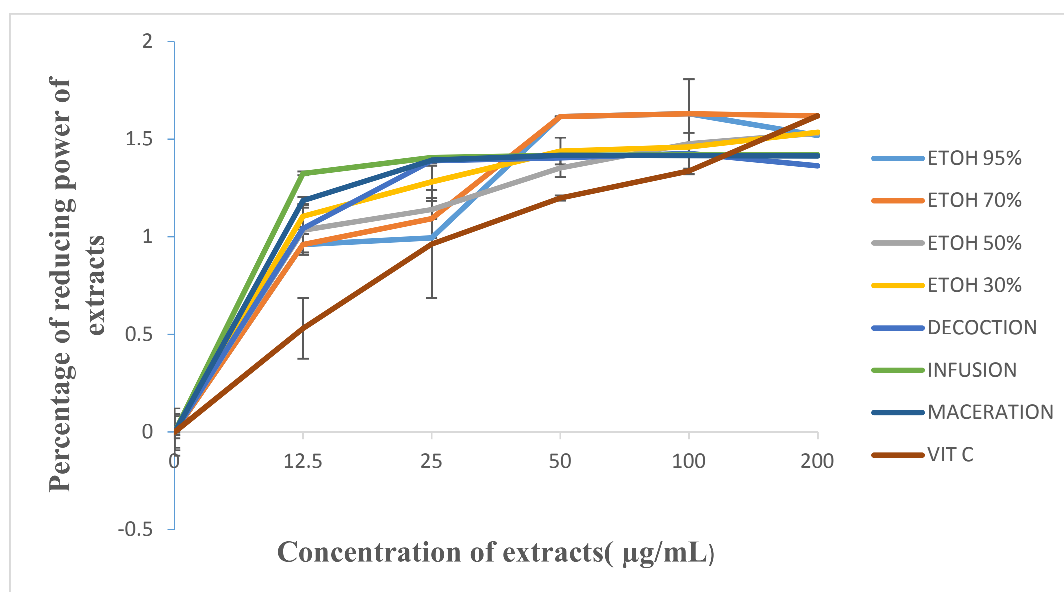

Fig. 2. Reducing power of extracts of *T. avicennioides*
ETOH = ethanol ; VIT C = Vitamin C

3.2.3 Hydroxyl radical scavenging activity

The comparative study presented in Fig. 3 shows that the antiradical activity of the decoction is higher than those of all the extracts except the 95% hydroethanolic extract at 25 to 200 µg/ml concentrations, unlike that of the maceration which had the lowest antiradical activity. As for the 70% hydroethanolic extract, its activity is comparable to that of the infusion extract as from the concentration of 25 µg/ml.

3.2.4 Nitric oxide scavenging capacity assay

The bark extracts of *T. avicennioides* have shown potential antioxidant properties against nitric oxide. The results are presented in Fig. 4, which shows that the decoction had the highest activity at concentrations ranging from 12.5 to 100 µg/ml, although there is no significant difference (p = .05) from that of ascorbic acid (control) at 25 µg/ml. In addition, the 70% hydroethanolic extract shows low activities at concentrations of 50 to 100 µg/ml.

3.2.5 Hydrogen peroxide scavenging capacity

The stem bark extracts of *T. avicennioides* have shown potential antioxidant properties against hydrogen peroxide. The results are presented in Fig. 5. It appears that the 95% hydroethanolic extract had the highest activity compared to the other extracts and was significantly different (p = .05) from that of ascorbic acid (control). In addition, maceration wine showed low activity.

3.2.6 Total phenolic content (TPC)

Phenol levels in *T. avicennioides* extracts were determined in this study and the results are presented in Fig. 6. The concentration of phenolic compounds is high in the decoction compared to all other extracts. We note that there is no significant difference between (p = .05) these extracts with the exception of the decoction. However, it is noted that the 30% hydroethanolic extract has the lowest concentration of phenolic compounds.

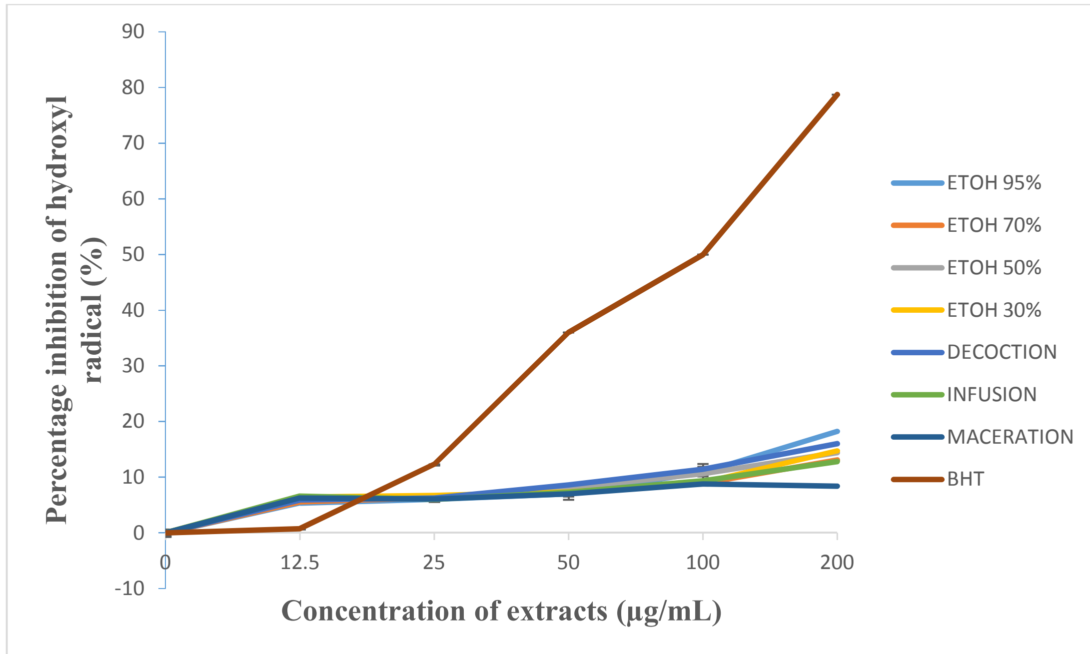

Fig. 3. Hydroxyl radical scavenging activity of *T. avicennioides*
ETOH = ethanol; BHT = bultylhydroxytoluen

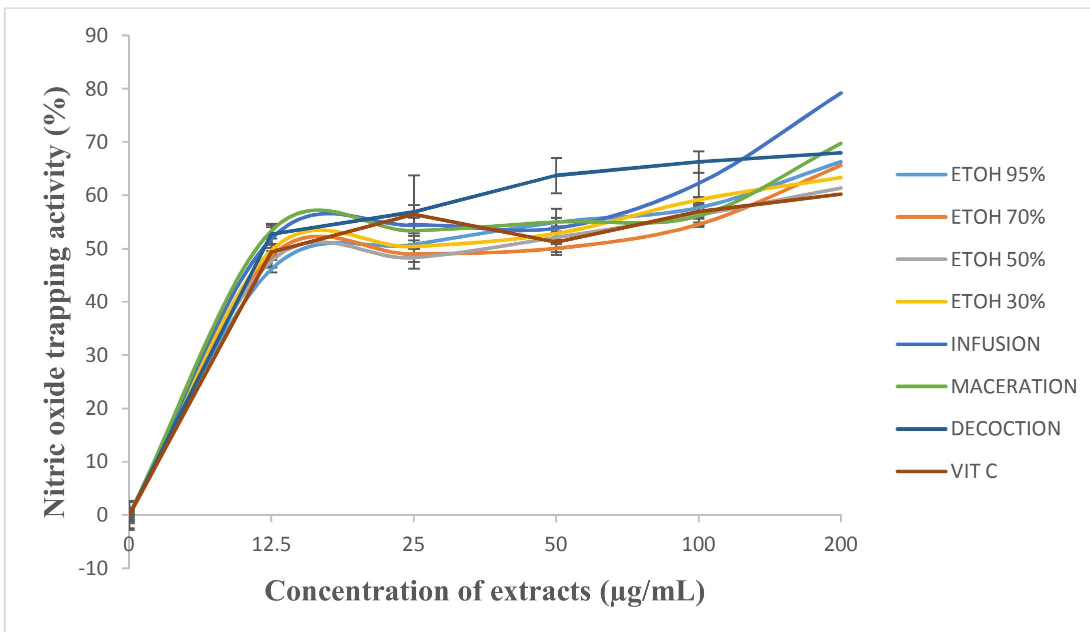

Fig. 4. Nitric oxide trapping activity of *T. avicennoides* extracts
EtOH : ethanol ; VIT C : Vitamin C

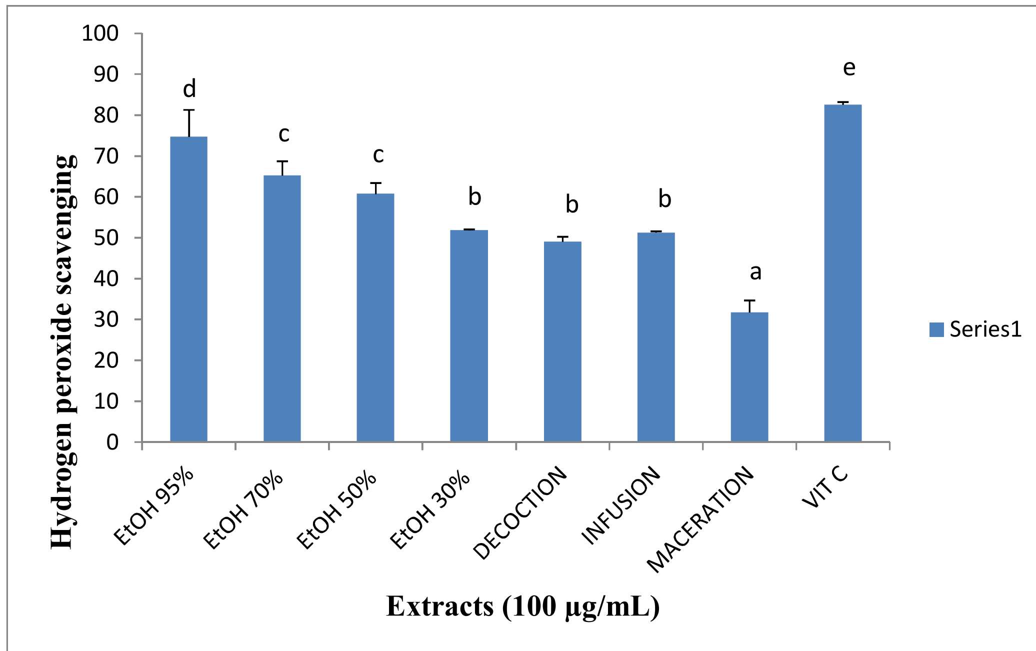

Fig. 5. Percentage hydrogen peroxide scavenging activities of *T. avicennioides* extracts

a, b, c, d, e: Figures with the same letter are not significantly different at the 5% alpha value. The values in the figure are presented as averages ± standard deviation of 3 repetitions.
EtOH: ethanol; VIT C: Vitamin C

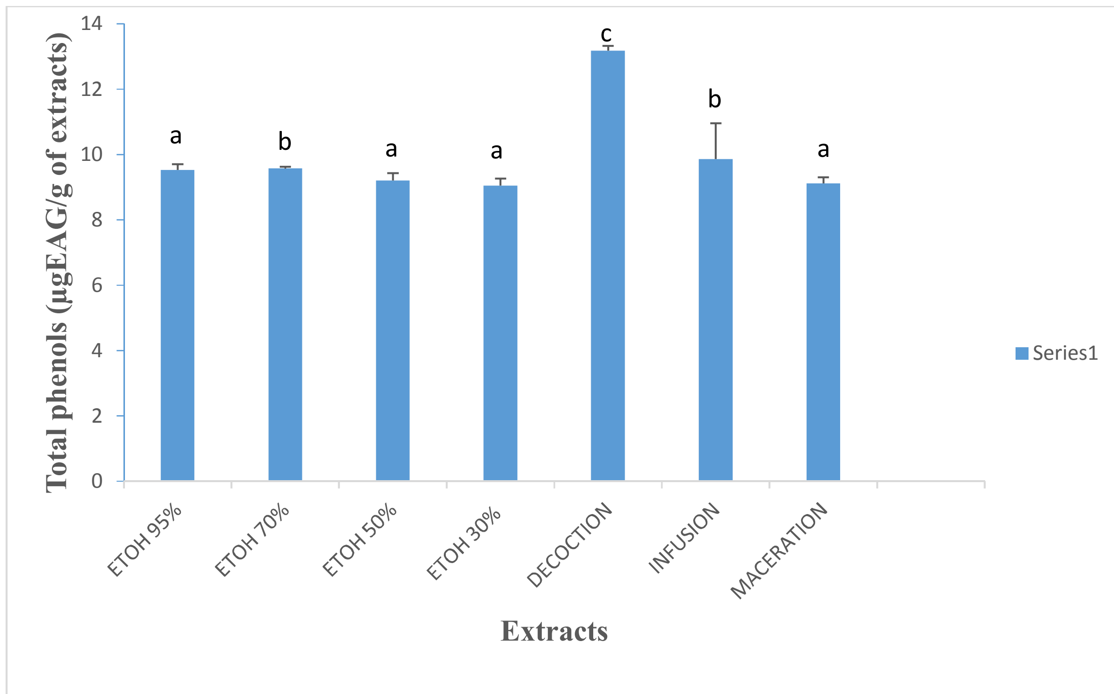

Fig. 6. Total phenol content of *T. avicennioides* extracts

a, b, c : Figures with the same letter are not significantly different at the 5% alpha value. The values in the figure are presented as averages ± standard deviation of 3 repetitions

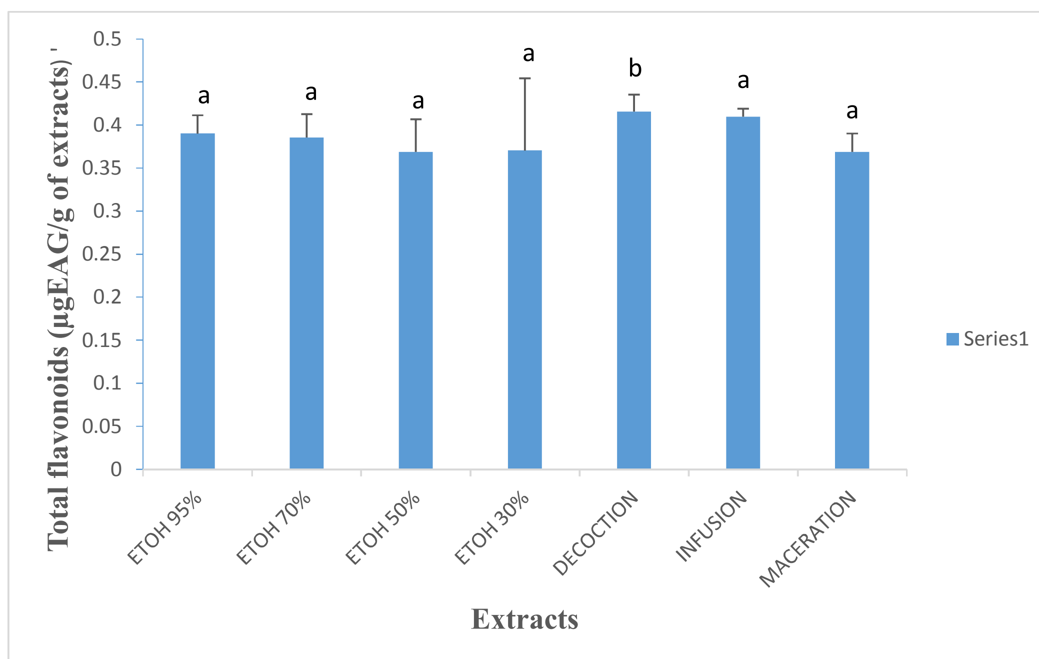

Fig. 7. Total flavonoids content of *T. avicennioides* extracts

a,b : Figures with the same letter are not significantly different at the 5% alpha value. The values in the figure are presented as averages ± standard deviation of 3 repetitions

Table 3. Phytochemical composition of the different extracs

Secondary metabolic	Extracts						
	EtOH 95%	EtOH 70%	EtOH 50%	EtOH 30%	Decoction	Infusion	Maceration
Alkaloids	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Stéroids	-	-	-	-	-	-	-
Triterpenes	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-
Saponins	+	+	+	+	+	+	+
Anthocyanin	+	+	+	+	+	+	+
anthraquinone	+	+	+	+	+	+	+

EtOH = Ethanol ; + = Presence ; - = absence

3.2.7 Total flavonoids content (TFC)

Flavonoid levels in *T. avicennioides* extracts have been determined and the results are presented in Fig. 7. The flavonoid concentration of the extracts is not significantly different (p = .05) except for maceration. In addition, the decoction had the highest concentration of total flavonoids, while the maceration had the lowest concentration.

3.3 Phytochemical Composition of *Terminalia avicennioides*

The qualitative phytochemical screening of *T. avicennioides* extracts revealed several classes of secondary metabolites (Table 3). It appears from this screening that alkaloids, phenols, flavonoids, saponins, anthocyanins and anthraquinones are present in all extracts. Steroids, triterpenes and tannins are absent in all extracts.

4. DISCUSSION

4.1 Antisalmonella Activities

In this study, we evaluated the antityphic activity of aqueous and hydroethanolic extracts of *T. avicennioides* on the *Salmonella* strain and

isolates. The analysis of experimental data shows that hydroethanolic extracts showed the best activities on pathogens tested with MICs between 64 and 512 µg/ml compared to aqueous extracts. Hydroethanolic solvents would concentrate the active antibacterial ingredients contained in the plant better than aqueous solvents. These results are similar to those of Bolou et al. [18] who showed that the hydroethanolic extract of *Terminalia glaucescens* was more active than the aqueous extract on *Salmonella Typhi* and *Salmonella Typhi*murium. It also appears from the results obtained that, the 70% hydroethanolic extract was found to be more active (MIC ≤ 128 µg/ml) with respect to the strain tested, therefore its antisalmonella activity is more important than that of the other *T. avicennioides* extracts studied in this work. The other extracts showed moderate activities with MIC between 128 and 512 µg/ml. Indeed, according to Kuete [19], the antibacterial activity of plant extracts is considered significant when MIC < 100 µg/ml, moderate when 100 µg/ml ≤ MIC ≤ 625 µg/ml and low when the MIC > 625 µg/ml. These antitiphic results could be explained by the presence of secondary metabolites such as alkaloids, anthocyanins, anthraquinones, flavonoids, phenols and saponins in these extracts but, not in equal amounts to induce the same activity, and that these active compounds are more concentrated in the hydroethanolic extract. (30:70; v/v). These secondary metabolites have several pharmacological properties, including antibacterial properties [20,21], which corroborate those of Foutse et al. [22] and Musa et al. [23] which have shown that extracts from *T. avicennioides* bark are highly rich in alkaloids, anthraquinones, flavonoids, phenols and saponins which are compounds with such properties.

4.2 Antioxidants Activities

The antioxidant activity of *T. avicennioides* extracts was evaluated *in vitro* by the DPPH-test, the ferric reducing antioxidant power (FRAP) iron reduction technique. The hydroxyl radical reduction test, the nitric oxide trapping test and the hydrogen peroxide reduction test.

Phenolic compounds such as phenolic acids, flavonoids and tannins are considered to be the major contributors to the antioxidant capacity of plants [24]. These compounds also possess various biological activities such as anti-inflammatory, antibacterial, antiviral, antiallergic, antithrombotic and vasodilator activities that can be related to their antioxidant activity [25] For this reason, the determination of total polyphenols and total flavonoids of *T. avicennioides* extracts was performed in this study.

The results of the DPPH antiradical test showed that the inhibition percentages range from 78.16% to 98.28% depending on the concentration and the extracts. These inhibition percentages indicate that the extracts contain an anti-free radical power. The antiradical activity of these extracts is explained by the presence of different secondary metabolites such as flavonoids [23] contained in *T. avicennioides* extracts. Indeed, flavonoids have been shown to discolour DPPH- because of their ability to yield hydrogen, and protective effects in biological systems are linked to their ability to transfer electrons to free radicals [15,25]. This is in line with the work carried out by Ćetković et al. [26] which showed that the antioxidant activity of certain plant extracts is linked to their richness in phenolic compounds. The antiradical activity (DPPH-) of the extracts was also expressed as IC_{50}. IC_{50} is defined as the effective concentration of the substrate that inhibits 50% of the DPPH radicals present in the solution [27]. The IC_{50} values of *T. avicennioides* extracts show that they have a high antioxidant potential because the IC_{50} values range from 8.30 to 10.43 µg/ml. Indeed, according to Souri et al. [27], the antioxidant potential of a plant is divided into three groups: high when IC_{50} < 20 µg/ml, moderate when 20 µg/ml ≤ IC_{50} ≤ 75 µg/ml and low when IC_{50} > 75 µg/ml. These results suggest that the extracts tested contained free radical scavenging agents that act as primary antioxidants.

In addition, the results obtained in the test of the reducing power of iron show a strong reducing power of the infusion, which corroborates the results of the DPPH. In this case, the extract would reduce the iron, thus preventing the reaction of Fenton, and the formation OH^- radical. This hypothesis corroborates the results obtained by Palash et al. [28] on the efficacy of *Drymania diandra* leaves and bark to stabilize the OH^- radical. This high antioxidant power is believed to be due to the high presence of phenolic compounds in *T. avicennioides*.

The reducing capacity of a compound can be used as an indicator of its potential antioxidant activity [29]. The presence of reducing compounds results in a reduction of

Fe^{3+}/ferricyanide to ferrous ion (Fe^{2+}) Sousa et al. [29]. Numerous studies have shown that there is a direct correlation between antioxidant activities and the reducing power of certain plant extracts [30,31]. Reducing properties are generally associated with the presence of reducers, whose antioxidant action has been demonstrated by reducing chain reactions through the gain of one hydrogen atom. It should also be noted that reducers react with some peroxide precursors, thus preventing the formation of peroxide [32].

The results of the NO antiradical test show inhibition percentages ranging from 46.13% to 79.16% depending on the dose and extracts. These inhibition percentages indicate that the extracts contain an anti-free radical power. This activity is explained by the presence of different secondary metabolites such as flavonoids and phenols [23,26] contained in bark extracts of *T. avicennioides*. In addition, the difference in activity between the extracts would be due to the quantitative and qualitative variation of the secondary metabolites content. The 50% hydroethanolic extract showed low activity, which shows that it would contain fewer antioxidant compounds than the other extracts. The trapping potential of nitric oxide may be due to the antioxidant principle of the extract which competes with oxygen to react with nitric oxide and thus inhibits the production of nitrite anions.

The trapping activity of H_2O_2 by the extracts can be attributed to their phenolic compounds, which can give electrons to H_2O_2 and neutralize it in water [33]. Hydrogen peroxide is a weak oxidant and can directly inactivate some enzymes, usually by the oxidation of essential thiol groups (-SH). It rapidly crosses the cell membrane and once inside the cell, H_2O_2 can probably react with Fe^{2+}. And possibly Cu^{2+} ions to form a hydroxyl radical, which could cause several of its toxic effects [34,35]. Thus, the elimination of H_2O_2 is very important for antioxidant defense in cellular or food systems.

There is a very positive relationship between total phenols and antioxidant activity in many plant species, due to the trapping capacity of their hydroxyl groups [36]. Phenolic compounds have also been reported to be effective hydrogen donors, making them very good antioxidants [37].

5. CONCLUSION

In this work, we have investigated the *in vitro* antisalmonella and antioxidant effects of *T. avicennioides* extracts on the germs responsible of typhoid fevers. According to the results obtained, the stem bark extracts of *T. avivennioides* contain several compounds, namely alkaloids, flavonoids, saponins, phenols, anthraquinones and anthocyanins. *T. avicennioides* extracts have anti-*salmonella* and antioxidant activities. Macerated and infused presented interesting antioxidant activities compared to other extracts. *T. avicennioides* extracts also contain powerful free radical scavenging phytochemicals that could have the ability to inhibit a free radical upsurge, as well as oxidative stress, and consequently might reduce oxidative stress associated metabolic disorders. However, further studies should be carried out in order to investigate the antisalmonella and antioxidant properties of this plant *in vivo*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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