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Invitro Antioxidant and Anti-salmonellal activities of StemBark extracts of *Enantia chlorantha* (*Annonaceae*).

Merline Namekong Djimeli[1], Joseph M. Mendimi Nkodo[2], Guy Sedar Singor Njateng[1], Charles Fokunang[2], Norbert Kodjio[1], Serge Secco Atsafack[1], Antoine Honore Nkuete Lonfouo[3], Jean Baptist Sokoudjou[1], Magellan Guewo-Fokeng[4,5], and Donatien Gatsing[1]*

[1]Laboratory of Microbiology and Antimicrobial Substances, Department of Biochemistry, Faculty of Science, University of Dschang, P.O.Box 67 Dschang, Cameroon.
[2]Faculty of Medicine and Biomedical Sciences, University of Yaounde I, Yaounde, Cameroon.
[3]Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon.
[4]Department of Biochemistry, Faculty of Science, University of Yaoundé I, PO Box812 Yaounde, Cameroon.
[5]The Biotechnology Centre, University of Yaounde I, PO Box 3851 Yaounde, Cameroon.

ABSTRACT

The aim of the present study was to investigate the *in vitro* anti-salmonellal and antioxidant activities of the extracts of this plant against five *Salmonella* species. The anti-salmonellal activity was evaluated using broth microdilution method, whereas the evaluation of antioxidant properties, the quantitative determination of total phenols and flavonoids and phytochemical screening were performed by standard methods. This study showed that the *Enantia chloranta* extracts have a beneficial effect on all the tested pathogens (*Salmonella*) with minimum inhibitory concentrations (MICs) ranging from 64 to 512 µg/mL. MeOH, 95% EtOH and 70% EtOH extract have the highest antimicrobial activity on of the tested microorganisms. MeOH and 95% EtOH extracts exhibited the strongest antioxidant activities (IC_{50}<20 µg/mL) whereas water macerated extract presented among all extracts the lowest antioxidant activity (IC_{50}>20 µg/mL). Phytochemical screening of extracts revealed the presence of flavonoids and phenols in all the extracts and alkaloids, anthocyanins, anthraquinons, saponins, triterpenes and cardiac glycosids depending on the used extracts. *In vitro* antioxidant and anti-salmonellal activities of *Enantia chloranta* extracts support not only the local use of this plant for the treatment of typhoid fever, but also its use as possible source in the development of new drugs.

Keywords: *Enantia chlorantha*; anti-salmonellal activity; antioxidant activity

Corresponding author

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INTRODUCTION

Plants with proven medicinal value are traditionally used in the world and principally by Asian and African peoples to treat human diseases [1]. One of these interesting plants is *Enantia chlorantha* which belongs to Annonaceae family, and is found in dense forests of Nigeria, Angola, Zaire, Gabon and Cameroon. It is an African yellow wood presenting an attractive characteristic due to its intensive yellow color; it is an ornamental tree which may grow up to 30 m high with dense foliage and spreading crown [1]. This plant is used by African peoples in traditional medicine with a beneficial effect in the treatment of many diseases including tuberculosis, malaria [2], urinary tract infections, jaundice [3], diarrhea [4], cough and wounds [5], gastric ulcers [6], infective hepatitis, rickettsia fever and typhoid fever [7].

The vast majority of the world population, especially in developing countries, depends on the traditional systems of medicine for therapeutic agents for a variety of diseases. In Africa, 70%-80% of people consult traditional medical practitioners for their healthcare needs [8]. in order to protect these populations, it is therefore important to study the properties of the common use medicinal plants which may also be a potential source of natural antioxidant (with roles in cells protection from damage induced by oxidative stress) such as vitamins (E, C, A), phenolic diterpenes, flavonoids, alkaloids, stilbenes, tannins, quinones, coumarins, phenolic acids and other metabolites with antioxidant activity [9-11]. In the same line, concerning the stem bark of *Enantia chloranta*, berberine and protoberberine alkaloids with antimalarial [12,13], antibacterial [14,15], trypanosomicidal [16], anti HIV [17] and anti-hepatotoxic [18] properties were isolated. Recently, the cytoprotective and ulcer healing actions of the 7.8-dihydro-8-hydroxypalmatine from the stem barks of this plant were demonstrated as well as its in vitro and in vivo anti Helicobacter activities [19, 20]. Data on *Enantia chloranta* properties on typhoid fever, paratyphoid A and B fevers caused by *Salmonella* typhi, *Salmonella* paratyphi A and *Salmonella* paratyphi B respectively are very scare despite the fact that its stem bark are routinely sold in the open markets and concoctions prepared from it are widely used both by rural and urban communities in Cameroon for the management of typhoid fever. This latter is a serious global health problem and remains a major cause of morbidity and mortality in developing countries with approximately 33 million cases and 500,000 deaths occurring annually [21]. In view of this, the present study was designed to evaluate the in vitro antioxidant and anti-salmonellal activities of *Enantia chlorantha* Stem Barks.

MATERIALS AND METHODS

Plant Material: Collection and Identification

The stem barks of *Enantia Chlorantha* were collected in Lekie, Central region of Cameroon, in March 2014. Identification of the plant was done at the National Herbarium, in Yaounde-Cameroon, using a voucher specimen registered under the reference N°25918/SRFCAM. The stem bark were air-dried at room temperature (23±2°C) away from sunlight and milled to coarse particles at the Biotechnology Center, University of Yaounde 1.

Extracts preparation

Aqueous extracts (infusion, maceration and decoction) were prepared according to the methods described by Duke [22]. Then, the macerated extract was prepared by dissolving 100 g of plant powder into 1000 mL of water. Extraction was allowed to process for 48 h with constant stirring (three times per day). The decoction (decocted extract) was performed by dissolving 100 g of plant powder in 1000 mL of water and then boiling at 100°C for 15 min. The infusion (Infused extract) was prepared by boiling water and then immediately putting the plant powder in this boiled water for 15 min. These mixtures were then filtered using N°1 Whatman paper and the filtrates were concentrated by evaporating the solvent at 40 °C in an oven for 48 hours.

For the organic extracts (methanolic and hydro-ethanolic), 100 g of the powdered stem barks were macerated three times at room temperature in 1000 ml of methanolic and ethanolic solvent (95% EtOH; 70% EtOH; 50% EtOH; 30% EtOH) for 48 hours, and then filtered with N°1 Whatman paper. The filtrate was concentrated at 45 °C using a rotary evaporator (Büchi R200) and the obtained volume was later dried at 40 °C. The plant extracts were stored in sterilized bottles at room temperature until usage.

In vitro anti-salmonellal Tests

Tested bacteria and culture media

The tested bacteria were made up of four isolates named *Salmonella* typhi (ST), *Salmonella* paratyphi A (SPA), *Salmonella* paratyphi B (SPB) and *Salmonella* typhimurium (STM) obtained from the Medical Bacteriology Laboratory of the "Centre Pasteur" of Yaounde, Cameroon, and one strain of *Salmonella* typhi (ATCC 6539), obtained from the American Type Culture Collection (ATCC). *Salmonella Shigella* Agar (Italy Liofilchem) for activation and maintenance of *Salmonella* strain/isolates, and Mueller Hinton Broth (MHB) for the determination of the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were used as culture media.

Inocula preparation

Bacterial cell suspensions were prepared at 1.5×10^8 Colony-Forming Unit/mL (CFU/mL) following 0.5 McFarland turbidity. For this purpose, 18 hours old bacterial cultures were prepared in *SalmonellaShigella* Agar (SSA). From this culture, a few colonies of bacteria were collected aseptically with a sterile wire loop and introduced into 10 mL of sterile 0.9% saline water. These suspensions were diluted 100 times with MHB to yield about 1.5×10^6 CFU/mL before use.

Minimum Inhibitory and Minimum Bactericidal Concentrations Determination

The susceptibility of *Salmonella* species was tested by broth micro-dilution method. They were used against the Salmonella species listed. As described by Newton and co-workers [23], the *E. chlorantha* crude extracts and fractions were dissolved in 2.5% dimethylsulfoxide (DMSO) solution and two-fold serial dilutions of the test substances were made with Mueller Hinton Broth to yield a volume of 100 µL per well. One hundred microliters (100 µL) of each of 1.5×10^6 CFU/mL bacterial suspensions were added to respective wells containing test samples (except extract and medium sterility control wells) and mixed thoroughly to give final concentrations ranging from 8 to 1024 µg/mL for extracts. Oxytetracycline and ciprofloxacin were used as standard antibiotics at concentrations ranging from 1 to 128 µg/mL and 0.5 to 64 µg/mL respectively, followed by 18 hours incubation at 37°C of these preparations. The inhibitory concentration of extracts was determined after addition of 40 µL of 0.2 mg/mL of p-iodonitrotetrazolium chloride (INT) (Sigma Aldrich, South Africa) and incubation at 37 °C for 30 min. Viable bacteria change the yellow dye (INT) to pink color. The lowest concentrations at which there was no visible color change were considered as MICs. The MBC values were determined by adding 50 µL aliquots of the preparations (without INT), which did not show any visible color change after incubation during MIC determination, into 150 µL of fresh broth. These preparations were further incubated at 37 °C for 48 hours and MBCs were revealed by the addition of INT as above. All extract concentrations at which no color change were considered as bactericidal concentrations, and the smallest of these concentrations was considered as the MBC. These tests were carried out in triplicates.

Phytochemical screening

According to the standard methods described by Harbone and co-workers, the phytochemical screening was performed [24]. The *Enantia chlorantha* stem bark extracts and fractions were screened for the presence of Alkaloids, anthocyanins, anthraquinons, flavonoids,phenols, saponins, tannins, steroids and triterpenes.

Antioxidant Assay

DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2 picrylhydrazyl) assay method was used to study the activities of the free radical scavenging of the crude extracts of *Enantia chlorantha* according to the technique described by Mensor and co-workers [25]. The extract (2000 µg/mL) was two-fold serially diluted with methanol. 100 µL of the diluted extract were mixed with 900 µL of 0.3 mM of DPPH methanol solution, for a final extract concentration of 12.5 - 200 µg/mL range (12.5, 25, 50, 100 and 200 µg/mL). Absorbance at 517 nm was measured by a spectrophotometer “Jenway, model 1605” following 30 min incubation at room temperature in the dark.

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 assays, were illustrated in tabular form (Table 3). The radical scavenging activity (RSA, in %) was calculated using the following formula: RSA (%) = [(OD $_{DPPH}$– OD $_{sample}$) / OD D_{PPH}] x 100; where OD = Optical Density. IC50 (quantity of sample needed to inhibit 50% of the free radical DPPH) was determined by plotting the percentages of RSA against the logarithmic values of the concentration of test samples.

Nitric oxide radical scavenging assay

Nitric oxide (NO) generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which are measured using the Griess reaction [26]. The method reported by Chanda and Dave [27] 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 and Butylatedhydroxytoluene (BHT)) in different concentrations (62.5 - 1000 µg/mL). The resulting solutions were then incubated at 25°C 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 formed chromophore was measured at 540 nm. Inhibition Percentage of the NO 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- (A1/A0)] x 100] where, Al = absorbance of the extract or standard and A0= absorbance of the negative control.

Ferric Reducing/Antioxidant Power (FRAP) assay

This power was determined by the Fe^{3+} to Fe^{2+} conversion in the presence of the extracts. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. The extract (2090 µg/mL) was two-fold serially diluted with methanol. 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. 0.5 mL of the supernatant was diluted with 0.5 mL of water and mixed with 0.1 mL of freshly prepared 0.1% ferric chloride, for a final extract/fraction concentration of 12.5 - 200 µg/mL range (12.5, 25, 50, 100 and 200 µg/mL). The optical density was measured at 700 nm. All the tests were performed in triplicate and the results were the average of three observations. Vitamin C was used as the positive control. Increased absorbance of the reaction mixture indicated higher reduction capacity of the sample (extracts/fractions) [28].

Total flavonoids content determination

The colorimetric Aluminum chloride method was used to have the total flavonoids content of the extracts of E. Chlorantha. Methanolic solution of extracts (0.1 mL, 2000 µg/mL) was mixed with 1.49 mL of distilled water and 0.03 mL of a 5% $NaNO_2$ solution. After 5 min, 0.03 mL of 10% $AlCl_3H_2O$ solution were added. After 6 min, 0.2 ml of 0.1 M sodium hydroxide and 0.24 mL of distilled water were added. The solution was well mixed and the increase in absorbance was measured at 510 nm using a UV- Visible spectrophotometer "Jenway, model 1605". The total flavonoids content was calculated using standard catechin calibration curve. The results were expressed as milligrams of Equivalents Catechin (mgECat) per gram of extract.

Total phenolic compounds determination

The Folin-Ciocateu method was used for this determination. The reaction mixture consisted of 0.02 ml of extract (2000 µg/ml), 1.38 mL of distilled water, 0.02 mL of 2N Folin-ciocalteu reagent and 0.4 mL of a 20% sodium carbonate solution. This was left to stand at room temperature for 20 min and then 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). Tests were performed in triplicate and the results were expressed as milligrams of Equivalents Gallic Acid (mgEGA) per gram of extract.

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STATISTICAL ANALYSIS

Data obtained were expressed as mean value ± standard deviation (SD). Significant difference between test and control groups was carried out using One-Way Analysis of Variance (ANOVA), and the significant differences between means at P<0.05 were determined by Waller-Duncan test using the Statistical Package for the Social Sciences (SPSS) computer software version 20.0 at 95% confidence intervals.

RESULTS

Anti-salmonellal Activity

The different organics and aqueous extracts of the *E. chlorantha* stem backs showed antibacterial activity with the MIC values ranging from 64 to 512 µg/ml (table 1). The best activities were observed in the methanolic, 95% and 70% ethanolic extracts with MIC values equal to 64 µg/mL respectively on STM, STM/SPA and STS.

Table 1: MICs, MBCs and MBCs/MICs of different extracts of *E. chlorantha* stem barks on isolates and strain of *Salmonella*

Extracts		Strain/isolates				
		STM	STS	ST	SPA	SPB
MeOH	CMI (µg/mL)	64	256	256	128	512
	CMB (µg/mL)	256	512	1024	256	>1024
	CMB/CMI	4	2	4	2	-
EtOH 95%	CMI (µg/mL)	64	64	256	64	128
	CMB (µg/mL)	256	512	1024	512	512
	CMB/CMI	4	8	4	8	4
EtOH 70%	CMI (µg/mL)	128	64	128	256	256
	CMB (µg/mL)	512	512	512	512	512
	CMB/CMI	4	8	4	2	2
EtOH 50%	CMI (µg/mL)	256	256	512	128	256
	CMB (µg/mL)	1024	512	>1024	512	512
	CMB/CMI	4	2	-	4	2
EtOH 30%	CMI (µg/mL)	512	256	256	512	512
	CMB (µg/mL)	1024	1024	1024	1024	>1024
	CMB/CMI	2	4	4	2	-
Maceration	CMI (µg/mL)	256	512	512	512	512
	CMB (µg/mL)	1024	1024	1024	1024	>1024
	CMB/CMI	4	2	2	2	-
Infusion	CMI (µg/mL)	256	256	128	512	256
	CMB (µg/mL)	512	512	1024	1024	1024
	CMB/CMI	2	2	2	2	4
Decoction	CMI (µg/mL)	128	512	256	512	512
	CMB (µg/mL)	512	>1024	1024	1024	1024
	CMB/CMI	4	-	4	2	2
Cip	CMI (µg/mL)	0,5	0,5	1	0,5	1
	CMB (µg/mL)	1	2	2	4	4
	CMB/CMI	2	4	2	8	4
Oxy	CMI (µg/mL)	1	2	4	2	2
	CMB (µg/mL)	2	4	8	8	4
	CMB/CMI	2	2	2	4	2

ETOH: Ethanol,MeOH: methanol, Cip: ciprofloxacine, Oxy: oxytetracycline.

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Antioxidant Activities

DPPH radical scavenging activity

The results of the DPPH radical scavenging activity of *E. chlorantha* extracts are given in Table 2. From this table, it appears that at 200µg/mL, methanolic extract showed the highest antioxidant activity (98.52%) followed by 95% ethanolic extract (95.45%) which presented almost the same activity as BHT (control). This latter was significatively low ($p > 0.05$) compared to that of methanolic extract. Among all the tested extracts, the extract obtained by decoction showed the lowest activity (41.16%) at the same concentrations. This is more explicit through the determination of IC_{50} (i.e. concentrations which inhibited 50% of DPPH) of extracts presented in Table 2. These results show that aqueous extracts (decoction, infusion and maceration) presented lowest antioxidant activities (high IC_{50}) compared to organic and hydroethanolic extracts.

Table 2: DPPH radical-scavenging activities of the extracts of *E. chlorantha* stem barks

Extracts	Extract Concentrations (µg /ml)					IC50 (µg/ml)
	12.5	25	50	100	200	
MeOH	71.17±5.217^{c}	86.10±1.097^{g}	86.54±1.507^{d}	90.83±2.892^{d}	98.52±0.342^{e}	3.093
EtOH 95%	73.53±4.909^{d}	85.21± 0.678^{fg}	88.98±0.127^{d}	93.93±0.462^{d}	95.49±1.507^{de}	4.980
EtOH 70%	40.35±2.119^{b}	58.61±3.781^{d}	73.02±2.044^{c}	80.33±0.254^{c}	91.79±3.705^{de}	16.580
EtOH 50%	63.33±1.276^{c}	73.09±1.337^{e}	80.41±1.637^{cd}	79.82±3.899^{c}	80.92±0.588^{c}	18.142
EtOH 30%	34.73±1.666^{b}	44.12±0.588^{c}	58.16±1.097^{b}	75.01±1.139^{c}	90.46±4.485^{d}	15.484
Maceration	28.45±2.106^{b}	39.98±5.140^{ab}	48.11±2.905^{a}	47.82±6.749^{a}	48.52±5.126^{a}	178.400
Infusion	27.56±3.590^{b}	41.76±2.817^{b}	44.78±7.882^{a}	54.17±3.694^{ab}	64.89±7.891^{b}	40,142
Decoction	15.10±2.184^{a}	35.25±3.413^{a}	41.16±8.982^{a}	60.23±4.217^{b}	70.51±3.266^{b}	31.431
BHT	79.05±17.853^{d}	79.82±1.235^{f}	85.73±0.894^{d}	89.06±2.898^{d}	95.42±0.713^{de}	4.176

BHT: Butylatedhydroxytoluene, ETOH: Ethanol, MeOH: methanol. Along each column, values with the same superscripts are not significantly different, Waller Duncan ($P > 0.05$).

Nitric oxide scavenging capacity assay

The stem bark extracts of *E. chlorantha* showed potential antioxidant properties against nitric oxide at different concentrations (Table 3). Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which are measured using the Griess reaction [37]. MeOH and 95% EtOH extracts showed the highest nitric oxide scavenging activities although not significatively different ($p > 0.05$) from that of ascorbic acid, the control while water macerated extract and 30% EtOH extract showed the lowest scavenging activity at all concentrations.

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Table 3: Nitric oxide (NO) radical scavenging of the extracts of *E. chlorantha* stem barks

Extracts	Concentrations (µg/mL) and scavenging activity (%)				
	12.5	25	50	100	200
MeOH	96.52±0.42^{f}	94.14±0.11^{de}	95.23±1.46^{de}	97.63±0.74^{e}	98.84±0.09^{e}
EtOH 95%	95.60±1.79^{f}	95.98±1.83^{d}	96.22±0.43^{e}	96.57±0.20^{e}	97.10±1.85^{e}
EtOH 70%	88.85±0.28^{e}	93.25±0.23^{de}	95.07±0.26^{de}	96.54±0.17^{e}	95.44±1.39^{de}
EtOH 50%	81.98±0.96^{d}	90.90±0.67^{d}	92.73±0.03^{d}	93.81±0.15^{d}	94.56±0.02^{d}
EtOH 30%	58.13±1.15^{b}	67.94±0.58^{a}	70.42±1.45^{a}	71.53±0.18^{a}	72.99±0.092^{a}
Maceration	53.33±0.29^{a}	65.65±0.22^{a}	69.26±0.12^{a}	69.89±0.06^{a}	71.37±0.39^{a}
Infusion	72.81±1.98^{c}	76.60±4.73^{b}	81.13±5.89^{b}	83.33±2.22^{b}	84.55±0.09^{b}
Decoction	76.48±1.49^{cd}	82.60±0.32^{c}	85.23±1.52^{c}	87.67±1.53^{c}	89.42±0.29^{c}
AsA	95.51±3.62^{f}	96.76±1.33^{d}	95.74±0.29^{de}	96.05±0.64^{e}	97.99±2.13^{e}

AsA = Ascorbic Acid, ETOH: Ethanol, MeOH: methanol. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05).

Reducing power activity

Table 4: Ferric reducing power activities of the extracts of *E. chlorantha* stem barks

Extracts	Extract Concentrations (µg /ml)				
	12.5	25	50	100	200
MeOH	0.83±0.142^{c}	1.22±0.156^{f}	1.36±0.107^{e}	1.69±0.022^{f}	1.87±0.047^{e}
EtOH 95%	0.57±0.016^{b}	0.66±0.046^{de}	0.86±0.049^{d}	1.35±0.071^{e}	1.68±0.014^{de}
EtOH 70%	0.51±0.082^{b}	0.74±0.047^{e}	0.79±0.069^{d}	1.07±0.030^{d}	1.42±0.042^{c}
EtOH 50%	0.16±0.064^{a}	0.33±0.081^{e}	0.51±0.020^{bc}	0.76±0.046^{b}	1.09±0.031^{b}
EtOH 30%	0.30±0.026^{a}	0.47±0.073^{ab}	0.39±0.047^{ab}	0.94±0.040^{c}	1.15±0.132^{b}
Maceration	0.20±0.054^{a}	0.21±0.016^{bc}	0.32±0.064^{a}	0.38±0.096^{a}	0.62±0.022^{a}
Infusion	0.45±0.026^{b}	0.58±0.040^{a}	0.84±0.084^{d}	1.02±0.094^{cd}	1.52±0.228^{cd}
Decoction	0.60±0.081^{b}	0.60±0.022^{cd}	0.63±0.028^{c}	0.79±0.033^{b}	1.02±0.053^{b}
AsA	0.65±0.026^{b}	0.79±0.125^{g}	1.25±0.063^{f}	1.45±0.062^{d}	1.55±0.020^{d}

AsA = Ascorbic Acid, ETOH: Ethanol, MeOH: methanol. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P>0.05).

The reducing power was determined through the transformation of Fe^{3+} to Fe^{2+} in the presence of the extracts. The results obtained are presented in Table 4. With the extract concentration of 12.5 µg/ml, the reducing power of methanolic extract (0.83±0.142) was higher than that of Ascorbic Acid (0.65±0.026) statistically comparable to those obtain with decoction extract, infusion extract and 70% ethanolic extract (p >

0.05). The extract obtains by maceration showed the lowest reducing power at all concentrations (12.5, 25, 50,100 and 200 µg/ml) whereas methanolic extract showed the highest reducing power at the same concentrations.

Total phenolic content

The Folin-Ciocateu method was used in this investigation for the determination of the total phenolic content of *E. chlorantha* stem bark extracts. As presented in Table 5, the concentrations of these phenolic compounds were very higher in methanolic extract follow by 50% ethanolic extract (690.53 ± 23.597 and 636.07 ± 30.434 mgEAG/g of extract respectively). There was no statistical difference among those obtained with 30% ethanolic, 70% ethanolic, macerated, infused and decocted extracts ($p > 0.05$) which are significantly low compared to 95% ethanolic extract.

Total flavonoids content

As shown in Table 5, the methanolic and 95% ethanolic extract of *E. chlorantha* stem bark had the highest flavonoids content (57.86 ± 3.086 and 49.36 ± 16.586 mgECat/g of extract respectively) followed by decocted extract (33.48 ± 6.141mgECat/g of extract) compared to the rest which showed the lowest value of flavonoid content with a statistical difference ($p < 0.05$).

Table 5: Total phenolic and flavonoid contents of extracts of *E. chlorantha* stem barks

Extracts	Total phenols (mgEAG/g of extract)	Flavonoids (mgECat/g of extract)
MeOH	690.53±23.597^{c}	57.86±3.086^{e}
EtOH 95%	551.59±44.000^{b}	49.36±16.586^{e}
EtOH 70%	386.65±37.076^{a}	19.32±1.090^{abc}
EtOH 50%	636.07±30.434^{c}	7.21±2.180^{a}
EtOH 30%	317.54±46.970^{a}	8.70±3.922^{ab}
Maceration	320.84±17.329^{a}	24.98±3.814^{cd}
Infusion	331.48±52.812^{a}	22.39±5.670^{bcd}
Decoction	349.15±7.515^{a}	33.48±6.141^{d}

ETOH: Ethanol, MeOH: methanol. Along each column, values with the same superscripts are not significantly different, Waller Duncan ($P > 0.05$).

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Table 6: Phytochemical composition of the different extracts of *E. chlorantha* stem barks

Compounds	Extracts							
	MeOH	EtOH 95%	EtOH 70%	EtOH 50%	EtOH 30%	Maceration	Infusion	Decoction
Alkaloids	+	+	–	–	–	–	–	+
Anthocyanins	–	+	+	+	–	–	+	+
Anthraquinons	+	+	+	+	–	–	–	+
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Saponins	+	+	–	–	–	–	+	+
Steroids	+	–	–	+	+	+	+	+
Tannins	–	–	–	–	–	–	–	–
Triterpens	+	–	–	–	+	+	+	+
Cardiac glycosids	–	+	–	–	–	+	–	+

ETOH: Ethanol, MeOH: Methanol, (–) Absent, (+) present.

Phytochemical Composition of *E. chlorantha* stem barks

Qualitatively, as presented in Table 6, the phytochemical screening of the extracts of *E. chlorantha* stem barks revealed the presence of secondary metabolites in the extracts such as alkaloids, anthocyanins, anthraquinons, flavonoids, phenols, saponins, triterpenes and cardiac glycosids.

DISCUSSION

Anti-salmonellal Activity

The extracts of *E. chlorantha* stem barks showed variable antibacterial activities on the five tested microorganisms depending on the type of extract. According to the scale of Kuete and co-workers (significant activity: MIC ≤ 100 µg/mL; moderate: 100<MIC≤625 µg/mL and weak: MIC > 625 µg/mL) [29], these activities was significant or moderate depending on the tested microorganism. This finding might be explained by the presence of secondary metabolites such as alkaloids, anthocyanins, anthraquinons, flavonoids, phenols, saponins, and triterpenes in these extracts. The difference in the sensibility of the tested Salmonella may be due to their structural variability at the cytoplasmic membrane level or by the presence of mutations (single nucleotide polymorphism, addition, deletion or others) in some Salmonella species conferring resistance to the active principle contained in the sample [30]. The majority of extracts exhibited bactericidal effects on the tested Salmonella species (MBC/MIC≤4). This may be ascribed to the presence of secondary metabolites which are proven to have antibacterial properties [31, 32, 33]. In fact, according to Bruneton, flavonoids and alkaloids have already shown several pharmacological properties including antibacterial properties [30].This can also explain the activities obtained by those stem bark extracts.

Antioxidants Activities

In order to know and master the mechanisms related to the antioxidant activities of *E. chlorantha* stem bark extracts, different methods were used [42].

The free radical scavenging activity of different extracts of *E. chlorantha* was studied by its ability to reduce the DPPH. The methanolic and the 95% ethanolic extracts presented radical scavenging activities against stable DPPH free radical in a concentration-dependent manner. The effect of the antioxidants on DPPH radical has been thought to be due to their hydrogen donating ability [41]. According to Souris [34], an extract is considered as having significant antioxidant property when IC_{50}< 20 µg/mL, moderate when 20 µg/mL ≤ IC_{50} ≤75 µg/mL and weak when IC_{50}> 75 µg/mL. Based on this scale, all the extracts from *E. chlorantha* possessed a significant antioxidant activity except the macerated extract with a weak antioxidant activity.

NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity [43]. It is a

diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities [38]. The scavenging of NO by the extracts increased with the dose of extracts meaning that NO radical decrease could be due to the scavenging ability of *E. chlorantha* extracts. These results corroborated those of Kodjio and collaborators which showed that NO scavenging activities of extracts/fractions of Curcuma longa increased in a dose dependant manner [39]. The antioxidant principle which in the extract competes with oxygen to react with nitric oxide and then inhibits the formation of nitrites, could explain the nitric oxide scavenging potential [39].

Ferrous ions are one of the most effective prooxidants; their interaction with hydrogen peroxide in biological systems can lead to formation of highly reactive hydroxyl radicals [39]. The iron reduction power was high in presence of methanol compared to iron reduction power of extracts obtained by decoction, infusion, 70% ethanol, and maceration suggesting that second metabolites with such an activity were more concentrated in the Methanolic extract. In fact, ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals. Ferrozine can make complexes with ferrous ions [22]. *E. chlorantha* extracts possessing Fe^{2+} chelating activities may play a protective role against oxidative damage induced by metal catalyzed decomposition reactions.

The antioxidant power of these extracts may be due to the presence of phenolic compounds which were proved to having antioxidant activities [35]. Methanolic extract and 95% ethanolic extracts, which present the higher antioxidant properties, have shown the highest contain in total phenols. Phenolic compounds are ubiquitous to the plant kingdom and possess several classes of compounds including flavonoids, anthocyanins and catechins [40]. They possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron [44].

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation [36]. Flavonoids are able to scavenge practically all known ROS. The methanol and 95% ethanolic extracts of E. chlorantha showed the highest flavonoids even than ascorbic acid which is the control (Table 5). These phytochemicals in the plant could be due to its high radical scavenging activity.

CONCLUSION

The results of this study provide an important basis for the use of *Enantia chlorantha* stem back extracts to eradicate infectious diseases caused by salmonella species. They also provide information on a number of plants extracts which show promising antioxidant activity. This plant is a good source of natural antioxidants and can be useful in treatment of the diseases associated with oxidative stress.

Competing interests

The authors declare that this work was absolutely free from all issues related to conflicts of interest.

Availability of data and materials

Data will be made available by the corresponding authors upon request.

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