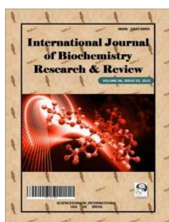

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In vitro Antisalmonellal and Antioxidant Activities of Extracts and Fractions of *Curcuma longa* L. Rhizomes (Zingiberaceae)

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Authors' contributions

This work was carried out in collaboration between all authors. Author NK was the field investigator and drafted the manuscript. Author SSA contributed to the evaluation of antioxidant activity. Author SPCF managed the analyses of the study and managed the literature searches. Author JRK revised the manuscript. Author DG designed the study and supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Typhoid fever is still a major public health problem in developing countries, where it remains endemic. With a view to searching for new antityphoid substances, *Curcuma longa* rhizome extracts and fractions were assessed for their antisalmonellal and antioxidant activities. The antisalmonellal efficacy in terms of minimum inhibitory concentrations (MICs) of the different extracts and fractions was determined using serial microdilution method. The antioxidant activity was determined by measuring FRAP (ferric reducing-antioxidant power), DPPH radical scavenging,

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nitric oxide (NO) radical scavenging, ferrous ion-chelating and hydroxyl radical scavenging activities. Total flavonoids and total phenolic contents were also evaluated. The results showed that the extracts and fractions of *Curcuma longa* were effective against all the bacteria tested with MICs ranging from 32 to 1024 µg/mL. These results also showed that $MeOH/CH_2Cl_2$ (v/v, 1:1) extract, residual and ethyl acetate fractions possessed strong antioxidant activities (IC_{50}< 20 µg/mL). Phytochemical screening showed the presence of alkaloids, flavonoids, saponins and phenols in all the extracts and fractions. In the light of the foregoing, it was obvious that *Curcuma longa* contains antisalmonellal and antioxidant principles which could be developed for the treatment of enteric fevers (typhoid and paratyphoid fevers) and the management of oxidative stress induced by the salmonellal infections. These findings support the claim of the local community about the use of this plant for the treatment of typhoid fever.

Keywords: Rhizome; Curcuma longa; antisalmonellal; antioxidant; phytochemical screening.

1. INTRODUCTION

Salmonella is one of the genera of the Enterobacteriaceae family. Among the Salmonellae of medical importance are *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Paratyphi B, which cause typhoid, paratyphoid A and paratyphoid B fevers respectively [1]. World-wide, there is an estimated 22 million episodes of typhoid fever causing 216 500 deaths each year, the overwhelming majority of infections and deaths occurring in developing countries where typhoid fever is endemic [2]. Classical antibacterial drugs are becoming more and more inaccessible to the common man in Africa due to augmented costs. In addition, there is a greater resistance to all the three first line antimicrobials (chloramphenicol, ampicillin and co-trimoxazol). Moreover, chloramphenicol which was used as reference drug against typhoid fever was removed from the market due to its medullary toxicity (medullary aplasia) [3]. *Salmonella* infection causes the production of superoxide and nitric oxide radicals which react together to form peroxinitrite, a strong biological oxidant [4]. Consequently, pathological conditions characterized by oxidative stress can greatly result from typhoid fever or other bacterial infections. Reactive oxygen species (ROS) are a class of highly reactive molecules derived from the metabolism of oxygen. Rapid production of free radicals may lead to oxidative damage of biomolecules and results in disorders such as degenerative diseases, cancer, diabetes, neural disorders and ageing [5]. These free radicals occur in the body during an imbalance between ROS and antioxidants. Many medicinal plants have large amount of antimicrobial and antioxidants compounds such as saponins, tannins and triterpenoids, polyphenols and others. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases like cancer [6].

Curcuma longa L. a perennial herb, is a member of Zingiberaceae family commonly known as saffron (India), turmeric (English name), djidja jaune (Cameroon) is widely distributed in the tropical region. In China, *C. longa* is used for diseases associated with abdominal pains. Turmeric (*Curcuma longa*) has a long tradition of use in the Chinese and Indian systems of medicine, particularly as an anti-inflammatory agent, treatment of many disorders such as flatulence, jaundice, menstrual difficulties, haematuria, haemorrhage and colic. Turmeric is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia [7]. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury. In recent times, Indian traditional healers uses turmeric powder for the treatment of biliary disorders, anorexia, hepatic disorders, rheumatism and sinusitis [8]. It is traditionally used in the West region of Cameroon for the treatment of malaria, yellow fever, bacterial infections like typhoid fever, diarrhoea and symptoms like stomach-ache. The plant grows to a height of three to five feet, and is cultivated extensively in India, China, and other countries with a tropical climate [9]. It has oblong, pointed leaves and bears funnel-shaped yellow flowers. The commonly used portion of this plant is its rhizome. Current research has focused on turmeric's hepatoprotective, anti-inflammatory and anti-carcinogenic. In addition, it is used in gastric ulcer, cardiovascular disease and gastrointestinal disorders [8]. This work was therefore aimed at evaluating the antisalmonellal and antioxidant activities of the crude extracts and fractions of *Curcuma longa* L. rhizomes in

order to ascertain their potential as antityphoid drug.

2. MATERIALS AND METHODS

2.1 Plant Material

The rhizomes of *Curcuma longa* L. were collected from Santchou Division, West region of Cameroon and were identified by Mr. NGANSOP Eric, a botanist at the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the reference No 42173/HNC.

2.2 Preparation of Plant Extracts and Fractions

Aqueous extracts (infusion, maceration and decoction) were prepared according to the methods described by Duke [10].

The methanol/methylene chloride (MeOH/ CH_2Cl_2) (v/v, 1:1) extract was prepared by macerating 1 kg of dried rhizomes powder of *Curcuma longa* at room temperature in 6 L of MeOH/CH_2Cl_2 (1:1) for 48 h. During that period, stirring was done twice daily. The mixture was filtered using Whatman N° 1 filter paper and the extract was concentrated under reduced pressure at 45℃ using a rotary evaporator (Buchi R-200). The plant extracts were stored in sterilized bottles at 4℃ until usage.

The splitting of the MeOH/CH_2Cl_2 (1:1) extract was done according to the method described by He et al. [11] with slight modification. Thirty grams of the extract was dissolved into 350 mL of methanol and equal volume of hexane was added. The mixture was shaken and then separated in the funnel. The same procedure was repeated with ethyl acetate, and the residual fraction contained compounds soluble in methanol/water mixture. In the last step, water was used to separate ethyl acetate phase from residual phase. The different extracts and fractions were concentrated by allowing them to stand in an oven (Memmert) set at 40℃.

2.3 Phytochemical Screening

Curcuma longa rhizomes extracts and fractions were screened for different classes of compounds, including alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes, using standard methods [12].

2.4 Antimicrobial Activity

2.4.1 Microorganisms and culture media

The test microorganisms including *Salmonella* Typhi (ST), *Salmonella* Paratyphi A (SPA), *Salmonella* Paratyphi B (SPB), *Salmonella* Typhimurium (STM) isolates were obtained from Pasteur Centre, Yaoundé, Cameroon. One strain of *Salmonella* Typhi (ATCC 6539) obtained from the American Type Culture Collection (ATCC) was also used. They were maintained on agar slant at 4℃ and subcultured on a fresh appropriate agar plates 24 h prior to any antimicrobial test. Culture media used were Salmonella-Shigella Agar (SSA) for activation and maintenance of *Salmonella* strain/isolates and Mueller Hinton Broth (MHB) for sensibility test (Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs)).

2.4.2 Bacterial susceptibility determinations

The minimum inhibitory concentrations (MICs) of extracts and fractions were determined using serial microdilution in the rapid p-Iodonitrotetrazolium chloride (INT) (Sigma-Aldrish, St Quentin Fallavier, France) colorimetric assay. The tests were carried out in 96-micro well sterile plates as previously described [13]. For this, the test extracts/fractions were dissolved in DMSO/MHB (v/v, 5%). This solution was then serially introduced in the different well of the microplate, which previously contains 100 µL/well of MHB. One hundred microliters of 10^6 CFU/ml bacterial suspensions prepared in MHB were added to the respective wells. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a shaker, and incubated at 37℃ for 18 h. Wells containing MHB, 100 µL of bacterial suspensions and DMSO at a final concentration of 2.5% served as a negative control. Ciprofloxacin and oxytetracyclin were used as reference antibiotics. The MICs of samples were detected after 18 h of incubation at 37℃, following addition of 40 µL of 0.2 mg/mL INT and incubation at 37℃ for 30 min [14]. Viable bacteria reduced the yellow dye of P-iodonitrotetrazolium to pink. MIC was defined as the lowest sample concentration that prevented this colour change and exhibited inhibition of microbial growth.

The Minimum Bactericidal Concentrations (MBCs) were determined by adding 50 µL aliquots of the preparations (without INT), which

did not show any visible colour change after incubation during MIC assays, into 150 µL of fresh Mueller Hinton broth. These preparations were further incubated at 37°C for 48 h and bacterial growth was revealed by the addition of INT as above. The lowest concentration at which no visible colour change was observed was considered as the MBC. These tests were performed in triplicates at three different occasions.

2.5 Antioxidant Assay

2.5.1 DPPH radical scavenging assay

The radical scavenging activities of crude extract/fractions were evaluated spectrophotometrically using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [15]. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour were measured at 517 nm under UV/Visible light spectrophotometer (Jenway, model 1605). Pure methanol was used to calibrate the counter. The extract/fraction (2000 µg/mL) was twofold serially diluted with methanol. One hundred microliters of the diluted extract/fraction were mixed with 900 µL of 0.3 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution, to give a final extract/fraction 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 densities were measured at 517 nm. Ascorbic acid (Vitamin C) was used as control. Each assay was done in triplicate and the results, recorded as the mean ± standard deviation (SD) of the three findings, were presented in tabular form. The radical scavenging activity (RSA, in %) was calculated as follows:

RSA = [(Absorbance of DPPH - Absorbance of sample) / Absorbance of DPPH] x 100

The radical scavenging percentages were plotted against the logarithmic values of concentration of test samples and a linear regression curve was established in order to calculate the RSA_{50} or IC_{50}, which is the concentration of sample necessary to decrease by 50% the total free DPPH radical [16].

2.5.2 Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing power was determined by the Fe^{3+} - Fe^{2+} transformation in the presence of the extracts/fractions. The Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Different volumes (400, 200, 100, 50, 25 µL) of methanolic extracts/fractions prepared at 2090 µg/mL 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 500 µL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (500 µL) was diluted with 500 µL of water and mixed with 100 µL of freshly prepared 0.1% ferric chloride. The absorbance 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 a positive control. Increased absorbance of the reaction mixture indicated higher reduction capacity of the sample (extracts/fractions) [17].

2.5.3 Nitric oxide radical scavenging (NO) assay

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 [18]. The method reported by Chanda and Dave [19] 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 Butylated hydroxytoluene (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 chromophore formed was measured at 540 nm. Percent 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:

$$\% \text{ inhibition} = (1 - (A_1/A_0)) \times 100$$

Where, A_l = absorbance of the extract or standard and A_0= absorbance of the negative control.

2.5.4 Ferrous ion-chelating assay

The ferrous ion chelating (FIC) activity was used to assay the antioxidants activity of the different extracts and fractions. It was appreciated by the increase of absorbance at 562 nm of the iron (II) and ferrozine complex. The extract/fraction (1000 µg/mL) was twofold serially diluted with methanol. 200 µL of the diluted extract/fraction were mixed with 740 µL of methanol and 20 µL (2 mM) $FeCl_2$. The reaction was initiated by the addition of 40 µL (5 mM) ferrozine. A final extract/fraction concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL) was obtained. The mixture was incubated at room temperature for 10 min and the absorbance at 562 nm was recorded. Methanol without sample was used as a control and methanol without ferrozine solution was used as a sample blank. Vitamin C and BHT were used as standard for the assay. Increased absorbance of the reaction mixture indicated higher reduction capacity of the samples.

2.5.5 Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was determined using Fenton reaction. To 60 µl (1.0 mM) $FeCl_2$, 90 µL (1.0 mM) 1,10-phenanthroline, 2.4 ml (0.2 M) phosphate buffer (pH 7.8), 150 µl (0.17 M) H_2O_2 and 1.5 ml of test solution with various concentrations (ranging from 12.5 to 200 mg/mL) were added and mixed together. H_2O_2 was added to the reaction mixture in order to initiate the reaction and the mixture was incubated at room temperature for 5 min. After incubation, the absorbance of the mixture was read at 560 nm using a spectrophotometer and the hydroxyl radical scavenging activity (HRS) was calculated as follows:

HRS (%) = [(Absorbance of control - Absorbance of test sample) /Absorbance of control] x 100

2.5.6 Total phenols contents (TPC)

The amount of total phenols was determined by Folin-Ciocateu Reagent method. The reaction mixture consisted of 20 µL of extract and fraction (2000 µg/mL), 1380 µL of distilled water, 200 µl of 2N FCR (Folin Ciocalteu Reagent) and 400 µL of a 20% sodium carbonate solution. The mixture was incubated at 40℃ 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 triplicate and the results were expressed as milligrams of Gallic Acid Equivalents (mgGAE) per gram of extract/fraction.

2.5.7 Total flavonoids content (TFC)

The amount of total flavonoids was determined by Aluminum chloride method. Methanolic solution of extracts and fractions (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 of 0.1 M sodium hydroxide and 240 µl 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. The total flavonoids content was calculated using standard catechin calibration curve. The results were expressed as milligrams of Catechin Equivalents (mgCE) per gram of extract/fraction.

2.6 Statistical Analysis

The data obtained were analysed using one-way analysis of variance (ANOVA) and presented as mean ± standard deviation (SD) of three replications. The levels of significance, considered at $P < 0.05$, were determined by Waller-Duncan test using the Statistical Package for the Social Sciences (SPSS) software version 12.0.

3. RESULTS

3.1 Phytochemical Composition of *Curcuma longa* L.

The results of qualitative analysis showed that each of extracts/fractions contains various phytochemicals compounds such as alkaloids, anthocyanins, anthraquinons, flavonoids, phenols, saponins, tannins, and triterpenes, as shown in Table 1.

3.2 Antisalmonellal Activities

The crude extracts and fractions obtained from the rhizomes of *Curcuma longa* L. were tested against four isolates of *Salmonella* species, namely *Salmonella* Typhi (ST), *Salmonella* Paratyphi A (SPA), *Salmonella* Paratyphi B (SPB), *Salmonella* Typhimurium (STM) and one strain of *Salmonella* Typhi ATCC 6539 (STs).

The data obtained from this test (Table 2) show that the extracts/fractions of plant exhibited *in vitro* antibacterial activity against the various *Salmonella* species, with the MIC values ranging from 32 to 1024 µg/mL. The crude methanol/methylene chloride (1:1, v/v) extract had the greatest activity with MIC values ranging from 32 µg/mL on STM to 64 µg/mL on the other pathogens. The decoction extract and ethyl acetate fraction showed antisalmonellal activities with MIC of 64 µg/mL on SPA and STM, and 128 µg/mL on SPB respectively. The infusion and decoction had the same activities on ST and ST_S, with MIC value of 128 µg/mL. The hexanic fraction (on ST_S) and macerated extract (on ST) had the lowest activity (MIC value of 1024 µg/ml). Except the methanol/methylene chloride (on ST, SPA and SPB) and the ethyl acetate fraction (on ST and STM), all extracts/fractions showed MBC/MIC ration ≤ 4.

Table 1. Phytochemical composition of the different extracts/fractions

Phytochemical groups	Extracts and fractions						
	Mac	Inf	Dec	$MeOH/CH_2Cl_2$	Hex F	Et-Ac F	Re F
Tannins	-	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Anthocyanin	-	+	-	-	-	+	-
Triterpenes and steroids	-	+	+	+	+	+	-
Anthraquinone	-	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+

Mac: Maceration; Inf: Infusion; Dec: Decoction; Hex F: Hexanic Fraction; $MeOH/CH_2Cl_2$: methanol/Methylene chloride; Et-Ac F: Ethyl Acetate Fraction; Re F: Residual Fraction, + = Presence; - = absence

Table 2. MICs, MBCs, MBCs/MICs of different extracts and fractions of *Curcuma longa* L. on isolates and strain of *Salmonella*

Extracts and fractions		ST	ST_S	SPA	SPB	STM
Decoction	MIC (µg/mL)	128	128	64	128	64
	MBC (µg/mL)	512	512	256	512	256
	MBC/MIC	4	4	4	4	4
Infusion	MIC (µg/mL)	128	128	128	256	128
	MBC (µg/mL)	512	256	512	512	512
	MBC/MIC	4	2	4	2	4
Maceration	MIC (µg/mL)	1024	256	256	128	64
	MBC (µg/mL)	-	512	1024	512	256
	MBC/MIC	-	2	4	4	4
Methanol/ methylene chloride (1:1)	MIC (µg/mL)	64	64	64	64	32
	MBC (µg/mL)	512	256	512	512	128
	MBC/MIC	8	4	8	8	4
Hexanic fraction	MIC (µg/mL)	512	1024	128	512	512
	MBC (µg/mL)	1024	1024	512	-	1024
	MBC/MIC	2	1	4	-	2
Ethyl acetate fraction	MIC (µg/mL)	32	64	64	128	64
	MBC (µg/mL)	256	256	256	256	512
	MBC/MIC	8	4	4	2	8
Residual fraction	MIC (µg/mL)	256	256	128	128	128
	MBC (µg/mL)	1024	512	512	512	256
	MBC/MIC	4	2	4	4	2
Ciprofloxacin	MIC (µg/mL)	1	2	0,5	1	2
	MBC (µg/mL)	8	8	2	64	4
	MBC/MIC	8	4	4	32	2
Oxytétracyclin	MIC (µg/mL)	8	8	4	8	4
	MBC (µg/mL)	32	64	64	64	32
	MBC/MIC	4	8	32	8	8

ST: Salmonella Typhi, SPA: Salmonella Paratyphi A, SPB: Salmonella Paratyphi B, STM: Salmonella Typhimurium, STs: strain of Salmonella Typhi (ATCC 6539), MIC= Minimal Inhibitory concentration. MBC= Minimal Bactericidal Concentration

3.3 Antioxidant Activities

3.3.1 DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts and fractions was evaluated, and the results are shown in Table 3. The macerated extract showed the lowest activity at any concentrations with inhibition percentage of 52% at 200 µg/mL. As far as the fractions are concerned, the residual fraction showed the highest activity (71.26%) at 12.5 µg/mL. At the concentration of 12.5 µg/mL, the activity of the standard (Vitamin C) was higher than that of the extracts/fractions. However, there was no significant difference ($p > 0.05$) between the activity of vitamin C and that of the residual fraction (at 12.5 µg/mL), ethyl acetate fraction and residual fraction (at 25 µg/mL), ethyl acetate fraction, residual fraction and hexanic fraction (at 50 µg/mL), and ethyl acetate fraction, residual fraction, hexanic fraction and infusion extract (at 100 µg/mL). However, hexanic fraction exhibited the strongest antioxidant activities at 200 µg/mL, compared to that of the standard antioxidant molecule ($p < 0.05$).

The concentrations which inhibited 50% of DPPH (IC_{50}) are presented in Table 3. These results show that all aqueous extracts (decoction, infusion and maceration) had high IC_{50} (low activity). The highest value of IC_{50} (lowest activity) was obtained with aqueous maceration extract. The organic extract and its fractions did not showed any significant difference ($p>0.05$). However, the fractions showed the lowest IC_{50} (i.e. the highest activity).

3.3.2 Ferric reducing / antioxidant power determination

The reducing power was determined by the Fe^{3+} - Fe^{2+} transformation in the presence of the extracts and fractions, and the results obtained are shown in Table 4. The hexane fraction showed the lowest reducing power, while the standard (Vitamin C) exhibited the highest reducing power at the concentrations of 100 and 200 µg/ml. At 12.5 µg/mL, the reducing power of the maceration (0.057±0.008) was superior to that of Vitamin C (0.028±0.009). However, hexanic fraction showed the lowest optical density (i.e. lowest reducing power) at every concentration. The remaining extract/fraction exhibited varied activities from one extract/fraction to another at each concentration.

3.3.3 Nitric oxide scavenging capacity assay

The results of the scavenging capacity against nitric oxide were recorded in terms of % inhibition as presented in Table 5. The *Curcuma longa* extracts and fractions showed considerable antioxidant potential. Infusion and maceration extracts revealed the highest % inhibition indicating best nitric oxide scavenging activity. However, crude organic extract showed the lowest scavenging activity at every concentration. Moreover, residual and ethyl acetate fractions had moderate activity at every concentration and there was no significant difference ($p > 0.05$) between the above fractions as far as nitric oxide scavenging activity is concerned.

3.3.4 Ferrous ion-chelating assay

The Ferrous ion-chelating activity of different extracts and fractions was evaluated, and the results are shown in Table 6. From this table, the standard antioxidants (Vitamin C and BHT) showed the highest activities, followed by ethyl acetate fraction and crude organic extract respectively. The hexanic fraction showed the lowest chelating activity. The other extracts and fractions showed relatively the same chelating activity.

3.3.5 Hydroxyl radical scavenging activity

The results of the scavenging activity against hydroxyl radicals are presented on Fig. 1. This figure showed that all aqueous extracts had low hydroxyl radical scavenging activities. Crude organic extract ($MeOH/CH_2Cl_2$), hexanic fraction and ethyl acetate fractions exhibited high activities. The standard antioxidant (BHT) exhibited the highest activity.

3.3.6 Total phenolic content (TPC)

The total phenolic content of extracts and fractions of *Curcuma longa* L. were determined in this study using Folin-Ciocateu Reagent method and the results are presented in Table 7. The concentration of phenolic compounds in crude methanol/methylene chloride (1:1) extract (20.30 mgGAE/mg) was higher than in all other extracts and fractions. Decoction and maceration had relatively the same concentration ($p > 0.05$) and the lowest concentration of phenolic compounds was observed in the hexanic fraction (2.00 mgGAE/mg).

Table 3. DPPH radical-scavenging activities of the extracts and fractions of the *Curcuma longa*

Extracts/fractions	Concentrations (µg/mL) and scavenging activity (%)					IC_{50} (µg/ml)
	12.5	25	50	100	200	
Decoction[c]	43.438±3.779[ab]	53.092±1.814[c]	65.158±0.815[c]	78.129±0.522[b]	86.727±1.069[bc]	21.079±1.388[b]
Infusion[d]	42.081±2.296[ab]	68.024±0.569[d]	74.132±1.306[cd]	81.749±0.794[bcd]	86.123±0.345[b]	21.417±3.167[b]
Maceration[a]	21.573±1.769[a]	25.778±1.590[a]	37.850±4.972[a]	48.130±1.421[a]	52.803±1.070[a]	234.369±38.816[c]
$MeOH/CH_2Cl_2$[b]	37.227±1.722[a]	43.925±4.053[b]	52.336±1.070[b]	78.348±1.369[bc]	88.862±2.687[c]	15.119±3.709[ab]
Residual fraction[f]	71.261±12.142[d]	86.059±14.840[e]	78.894±18.501[de]	84.501±18.015[bcd]	91.666±1.104[d]	11.621±1.598[a]
Et-Ac fraction[ef]	49.376±2.697[bc]	80.841±1.421[e]	83.567±1.286[de]	90.732±0.944[d]	92.445±2.397[d]	14.844±0.888[a]
Hexanic fraction[e]	52.611±1.480[c]	57.462±0.493[c]	84.328±1.119[de]	88.930±2.533[cd]	98.694±0.186[e]	16.960±5.090[ab]
Vitamin C[g]	76.178±6.690[d]	86.186±0.624[e]	87.262±0.758[e]	90.157±1.033[d]	93.465±0.379[d]	10.614±0.277[a]

MeOH/ CH_2Cl_2: Methanol /Methylene chloride (1:1); Et-Ac F: Ethyl acetate. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05)

Table 4. Ferric reducing power activities of the extracts and fractions of the *Curcuma longa*

Extracts/fractions	Concentrations (µg/mL) and absorbance at 700 (nm)				
	12.5	25	50	100	200
Decoction [d]	0.046±0.011[bc]	0.075±0.014[c]	0.131±0.024[c]	0.208±0.027[b]	0.356±0.027[b]
Infusion [b]	0.039±0.008[bc]	0.057±0.008[b]	0.075±0.008[ab]	0.117±0.004[a]	0.296±0.072[b]
Maceration [cd]	0.057±0.008[c]	0.080±0.018[c]	0.105±0.013[bc]	0.148±0.015[ab]	0.338±0.165[b]
CH2Cl2/MeOH [f]	0.030±0.024[ab]	0.075±0.016[c]	0.202±0.011[d]	0.399±0.005[c]	0.847±0.090[d]
Residual fraction [bc]	0.050±0.011[bc]	0.043±0.006[ab]	0.102±0.015[bc]	0.129±0.008[a]	0.271±0.041[ab]
Et-Ac fraction [e]	0.036±0.008[abc]	0.156±0.008[d]	0.291±0.065[e]	0.412±0.052[c]	0.507±0.067[c]
Hexanic fraction [a]	0.013±0.011[a]	0.033±0.004[a]	0.094±0.021[abc]	0.093±0.031[a]	0.139±0.032[a]
Vitamin C [g]	0.028±0.009[ab]	0.044±0.0005[ab]	0.056±0.021[a]	2.183±0.089[d]	3.339±0.098[e]

MeOH/ CH_2Cl_2: Methanol /Methylene chloride (1:1); Et-Ac F: Ethyl acetate. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05)

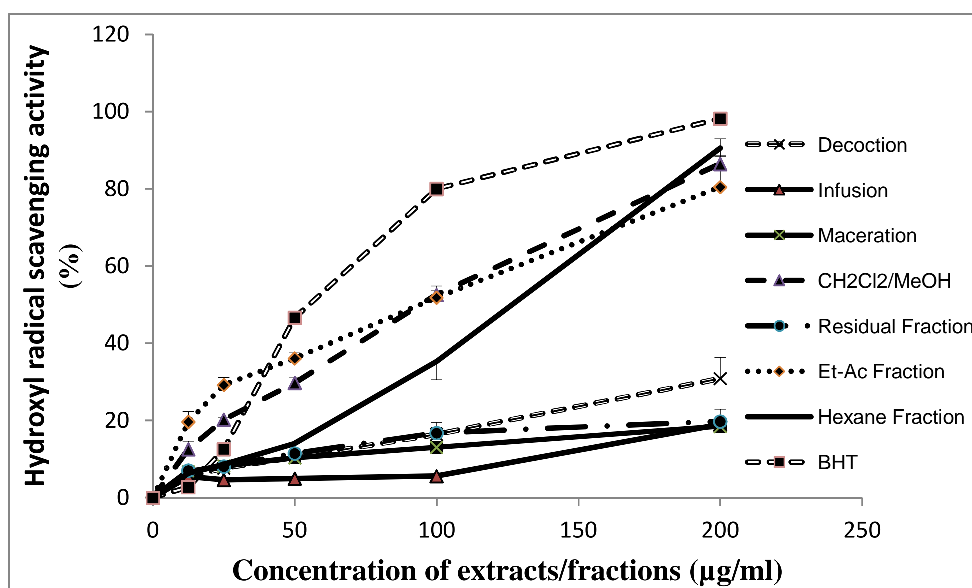

Fig. 1. Hydroxyl radical scavenging activities of extracts/fractions of *Curcuma longa* L

Table 5. Nitric oxide (NO) radical scavenging of the extracts and fractions of the *Curcuma longa*

Extracts/fractions	Concentrations (µg/mL) and scavenging activity (%)				
	12.5	**25**	**50**	**100**	**200**
Decoction[d]	83.405±1.014[d]	87.517±1.493[d]	89.733±0.490[d]	91.120±0.073[d]	92.810±1.076[d]
Infusion[e]	92.491±1.095[ef]	94.707±1.053[e]	93.910±0.386[e]	92.555±0.506[e]	92.810±1.076[d]
Maceration[e]	92.220±0.292[e]	92.842±0.358[e]	94.006±0.554[e]	94.006±0.842[f]	94.771±0.895[e]
MeOH/CH2CL2[a]	2.120±1.203[a]	6.775±6.750[a]	27.642±5.440[a]	75.912±0.120[a]	78.829±0.345[a]
Residual fraction[c]	77.044±1.168[c]	83.197±0.055[c]	85.525±0.027[c]	87.804±0.095[c]	89.701±0.138[c]
Et-Ac fraction[c]	78.080±1.410[c]	83.580±0.765[c]	85.397±0.138[c]	88.601±1.022[c]	88.649±0.193[c]
Hexanic fraction[b]	66.156±2.421[b]	77.012±1.251[b]	78.622±1.127[b]	82.145±0.444[b]	83.405±0.047[b]
Vitamin C[f]	92.427±3.627[ef]	94.595±1.339[e]	96.556±0.298[ef]	96.556±0.895[g]	94.595±2.032[e]
BHT[g]	94.946±0.800[f]	96.429±0.110[e]	97.274±0.526[f]	97.624±0.027[h]	99.410±0.055[f]

MeOH/ CH_2Cl_2: Methanol /Methylene chloride (1:1); Et-Ac F: ethyl acetate. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05)

Table 6. Ferrous ion-chelating of the extracts and fractions of the *Curcuma longa* L

Extracts/ fractions	Concentrations (µg/mL) and absorbance at 562 (nm)				
	12.5	**25**	**50**	**100**	**200**
Decoction [b]	0.086±0.005[b]	0.110±0.009[b]	0.151±0.019[ab]	0.190±0.010[a]	0.415±0.035[a]
Infusion [bc]	0.104±0.004[b]	0.120±0.008[fb]	0.182±0.026[b]	0.206±0.010[a]	0.452±0.024d[a]
Maceration [cd]	0.112±0.009[b]	0.139±0.014[bc]	0.170±0.004[b]	0.238±0.032[a]	0.471±0.120d[a]
MeOH/CH2Cl2 [e]	0.172±0.003[c]	0.229±0.012[d]	0.326±0.008[c]	0.393±0.085[b]	0.918±0.137[c]
Residual fraction [d]	0.128±0.008[b]	0.159±0.011[c]	0.206±0.011[b]	0.273±0.020[a]	0.512±0.030[b]
Et-Ac fraction [f]	0.198±0.028[d]	0.264±0.035[e]	0.425±0.012[d]	0.498±0.026[b]	1.144±0.062[c]
Hexanic fraction [a]	0.087±0.012[a]	0.092±0.022[a]	0.096±0.008[a]	0.100±0.002[a]	0.207±0.030[a]
Vitamin C [h]	0.507±0.036[e]	0.771±0.125[g]	1.039±0.086[f]	1.223±0.062[d]	1.298±0.020[d]
BHT [g]	0.366±0.016[e]	0.539±0.005[f]	0.740±0.091[e]	0.960±0.054[c]	1.358±0.030[e]

BHT: (Butylated hydroxytoluen); Et-Ac: ethyl acetate; MeOH/ CH_2Cl_2: methanol /Methylene chloride (v/v); Along each column, values with the same superscripts are not significantly different. Waller Duncan (P > 0.05)

Table 7. Total phenolic and flavonoid contents of extracts/fractions of *Curcuma longa* L

Extracts/Fractions	Phenols (mgGAE/mg)	Flavonoids (mgCE/mg)
Decoction	9.60±0.54[b]	1.21±0.12[c]
Infusion	17.08±1.14[e]	1.07±0.04[b]
Maceration	9.32±1.01[b]	0.61±0.09[a]
$MeOH/CH_2Cl_2$	20.30±0.75[f]	1.48±0.04[e]
Residual fraction	14.20±0.60[d]	1.39±0.08[de]
Et-Ac fraction	11.48±0.94[c]	1.36±0.04[d]
Hexanic fraction	2.00±0.36[a]	0.60±0.02[a]

$MeOH/CH_2Cl_2$: Methanol /Methylene chloride (v/v) extract; Et-Ac: ethyl acetate. Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05)

3.3.7 Total flavonoids content (TFC)

As shown in Table 7, the crude organic extract had the highest flavonoid content (1.48 mgCE/mg) followed by residual and ethyl acetate fractions, while hexanic fraction showed the lowest value of flavonoid content (0.60 mgCE/mg). However, there was no significant difference (p > 0.05) between residual fraction and crude organic extract ($MeOH/CH_2Cl_2$) as far as flavonoid content is concerned.

4. DISCUSSION

4.1 Antimicrobial Activities

The extracts/fractions of *Curcuma longa* showed *in vitro* antibacterial activities against the various *Salmonella* species used, with the MIC values ranging from 32 to 1024 µg/mL. In fact, according to the criteria described by Kuete [20], activities of plant extracts are significant when MIC ≤ 100 µg/mL, moderate when 100< MIC≤625 µg/mL, weak when MIC > 625 µg/mL. Therefore, the extracts/fractions of *C. longa* are active and the crude organic extract possesses significant inhibitory potential vis-à-vis all tested *Salmonella* species.

Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC ≤ 4, and bacteriostatic when the ratio MBC/MIC > 4 [1,21]. For most of the various extracts/fractions used, the ratio MBC/MIC was ≤ 4 against most of the bacteria tested, suggesting that these extracts/fractions may be classified as bactericidal agents against these bacteria.

The wide range of antisalmonellal properties can be explained by the presence of various groups of potentially active secondary metabolites in each of the extracts/fractions as shown by the phytochemical studies. Indeed, several alkaloids, flavonoids, phenols, saponins, anthocyanins, anthraquinones, sterols, tannins, and triterpenes have been found active against pathogenic microorganisms [20,22-24]. Some of these compounds were found to be present in the extracts and fractions of *C. longa* during this study, and they could contribute to the observed antisalmonellal activities.

4.2 Antioxidants Activities

The antioxidative profile of various extracts/ fractions of *C. longa* rhizomes as a prelude to finding agent(s) that could be used to reduce oxidative stress associated with typhoid and paratyphoid fevers. Since multiple characteristic reactions and mechanisms are involved in the so-called oxidative stress, using a single test is not sufficient to evaluate the antioxidant potential of plant natural compounds or extracts [25]. Therefore, many antioxidant assays such as, DPPH radical scavenging activity, ferric reducing/antioxidant power, nitric oxide scavenging capacity, ferrous ion-chelating activity and hydroxyl radical scavenging activity methods were chosen in order to evaluate the antioxidant properties of *C. longa* extracts/fractions.

The effect of the antioxidants on DPPH radical has been thought to be due to their hydrogen donating ability. Hence, DPPH is usually used as a substrate to evaluate antioxidative or free radical scavenging activity of antioxidant agents. In our experiment, the high DPPH radical scavenging activities of the various extracts/fractions which were comparable to the standard antioxidant, vitamin C, suggested that the extracts have some compounds with high proton donating ability and could therefore serve as free radical inhibitors. However, crude $MeOH/CH_2Cl_2$ extract and its fractions demonstrated a more remarkable anti-radical activity with IC_{50} < 20 µg/mL. In fact, according to Souri et al. [26], the antioxidant activities of plant

extracts are significant when IC_{50} < 20 µg/mL, moderate when 20 µg/mL ≤ IC_{50} ≤75 µg/mL, weak when IC_{50} > 75 µg/mL. There was no significant difference ($p > 0.05$) between IC_{50} values of the crude extract and fractions, and ascorbic acid. Maceration presented a weak scavenging capacity (IC_{50} = 234.36 µg/mL). These results corroborate those of Noghogne et al. [17], where the macerated extract of *M. indica* L. exhibited a weak scavenging capacity.

Antioxidants can be reductants, and inactivation of oxidants by reductants can be described as oxido-reduction reactions [27]. The presence of reductants, such as antioxidant substances, in the samples causes the reduction of the ferric to the ferrous form, which can be monitored by measuring the formation of Perlis prussian blue at 700 nm. The FRAP assay, therefore, provides a reliable method to study the antioxidant activity of various extracts/fractions. In this study, the aqueous extracts had moderate reducing power; the highest activity was obtained with the crude $MeOH/CH_2Cl_2$ extract and the lowest activity was obtained with the hexanic fraction. These data suggest that the *Curcuma longa* L. rhizomes may contain several compounds with intermediate polarity. As far as the partitioning is concerned, the highest activity was obtained with ethyl acetate fraction; the residual fraction had moderate reducing power, while the lowest activity was obtained with the hexanic fraction. It was observed that the activity increased with the polarity until it reached its maximum with the $MeOH/CH_2Cl_2$ extract then started to decline. This can be explained by the fact that low reducing power was observed for the hexanic fraction and the infusion extract. Hexane is a solvent which extract generally non polar compounds due to its non-polar nature [17].

Nitric oxide (NO) is a very unstable species reacting with oxygen molecule to produce stable nitrate and nitrite which can be estimated using Griess reagent. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal messenger, vasodilation and antimicrobial and anti-tumour activities [28,29]. Excess concentration of nitric oxide is associated signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. NO is a mediator generated by endothelial cells and macrophages. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals [30]. Table 5 illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The increased nitric oxide radical scavenging activity was observed in every extracts/fractions of the tested plant. Residual and ethyl acetate fractions showed better scavenging capacity compare to $MeOH/CH_2Cl_2$ extract. The nitric oxide scavenging potential may be due to antioxidant principle in the extract which competes with oxygen to react with nitric oxide and thus inhibits the generation of nitrites.

Ferrous ions are one of the most effective pro-oxidants; their interaction with hydrogen peroxide in biological systems can lead to formation of highly reactive hydroxyl radicals. Ferrozine is a ferroin compound that can quantitatively form stable magenta-coloured complexes with ferrous ion (Fe^{2+}). In the presence of other chelating agents, the complex formation is disrupted and the colour of the complex decreases. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [31]. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The most *C. longa* rhizomes active extracts interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. Some extracts/fractions with highest flavonoids contents showed highest chelating of Fe^{2+}. For example, $MeOH/CH_2Cl_2$ extract and ethyl acetate fraction that contained highest flavonoid contents showed the best chelating activity.

Hydroxyl radical is one of the potent reactive oxygen species in the biological system and has a short half-life. It can react with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause lipid peroxidation and cellular damage [28]. From this study the organic crude extract ($MeOH/CH_2Cl_2$ extract), hexanic and ethyl acetate fractions showed the best hydroxyl radical scavenging activity. The aqueous extracts and the polar fraction (residual fraction) showed the lower scavenging activity. Therefore, compounds exhibiting hydroxyl radical scavenging activities could be lipid soluble

compounds. In fact, according to Noghogne et al. [17], the compounds exhibiting hydroxyl radical scavenging activities are lipid soluble compounds such as triterpenoids or steroids. The effectiveness of an antioxidant in the body depends on which free radical is involved, how and where it is generated, and where the target of damage is present.

Plant polyphenols, a diverse group of phenolic compounds (flavones, flavonols, anthocyanins, phenolic acids, etc.), possess a powerful free radical scavenging activity [32]. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) [33]. Phenolic compounds exhibit antioxidant activity by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [34]. Flavonoid's protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes and reduce radicals of alpha-tocopherol or to inhibit oxidases [34]. The results obtained in this study showed that the antiradical scavenging activity was related to the phenolic content. Then, the organic crude extract ($MeOH/CH_2Cl_2$ extract) was found to have high phenolic contents with 20.30±0.75 (mgGAE/mg) and which may be one the reason explaining its high antioxidant activity with an IC_{50} of 15.119±3,709 (DPPH radical-scavenging activity), absorbance of 0.847±0.090 at 200 µg/mL (Ferric reducing power activity). There was a positive linear correlation between antioxidant activity index and total phenolic content for all the extracts and fractions. It can be observed that the concentration of the phenolics in the extracts/fractions correlates with their antiradical activity; this fact is more pronounced in the methanol/methylene chloride (1:1) extract.

In a stressful situation such as infection, the organism may produce several types of pro-oxidants and oxidants; this explains why a single test may not be sufficient to demonstrate the real antioxidant capacity of plant extracts. The hydroxyl radicals produced during stress is the primary cause of cell death by DNA damage. The extracts/fractions of *Curcuma longa* showed variable antioxidant potential according to the tests carried out. This suggests that the various extracts and fractions reduce iron (FRAP) and chelate iron, thereby preventing the Fenton reaction i.e. the formation of the $OH^{\bullet}$ radical. These results also show that, the extracts and fractions are proton or electron donors. The activities obtained showed that the $OH^{\bullet}$ radicals produced during the stress can be transformed into other molecules (e.g. H_2O) which can be excreted by the body.

5. CONCLUSION

From the findings of this work, it can be concluded that *Curcuma longa* rhizomes have diverse compounds including alkaloids, flavonoids, phenols, saponins, anthocyanins, anthraquinones, sterols, tannins, and triterpenes. These compounds are not evenly distributed in the various extracts/fractions. *C. longa* extracts/fractions demonstrated antibacterial activity against *Salmonella* species and antioxidant activities; the antioxidant potency depending on the type of extract. The study results further suggested that the extracts/fractions contain substances with antibacterial activities which may be used in the treatment of typhoid and paratyphoid fevers. *C. longa* extracts/fractions 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 antimicrobial and antioxidant properties of this plant *in vivo*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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