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In vitro Antisalmonellal and Antioxidant Properties of *Mangifera indica* L. Stem Bark Crude Extracts and Fractions

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Authors' contributions

This work was carried out in collaboration between all authors. Author LRN was the field investigator and drafted the manuscript; author DG designed the study and supervised the work; authors Fotso and JBS contributed to the evaluation of antioxidant properties; author NK contributed to the assessment of antimicrobial properties; author JRK assisted in the extraction, partitioning and phytochemical studies. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Typhoid fever is still a major public health problem in developing countries, where it remains endemic. In view of searching for new antityphoid substances, the antisalmonellal and antioxidant properties of extracts and fractions of *Mangifera indica* L. stem bark were assessed.
Study Design: Extraction, fractionation, antibacterial and antioxidant evaluation, phytochemical screening.
Place and Duration of Study: Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon, between October 2013 and May 2014.
Methodology: Different fractions, i.e. hexane, ethyl acetate and residual fractions, were obtained

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from methanol/methylene chloride ($MeOH/CH_2Cl_2$) (1:1) extract of *Mangifera indica* L. stem bark by partitioning. Various aqueous extracts were also prepared by decoction, infusion and maceration. All these extracts and fractions were subjected to antibacterial evaluation against *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Paratyphi B and *Salmonella* Typhimurium isolates, and a strain of *Salmonella* Typhi ATCC 6539, using the microdilution method. The evaluation of antioxidant properties, quantitative determination of total phenols and flavonoids, and phytochemical screening of these extracts and fractions were also performed.

Results: The antimicrobial results showed that *Mangifera indica* L. extracts and fractions exhibited minimum inhibitory concentrations (MICs) ranging from 64 to 1024 µg/mL. The extracts and fractions exhibited antioxidant properties, e.g. IC_{50} = 10.00 µg/mL for the $MeOH/CH_2Cl_2$ extract and fractions, and 11.99 µg/mL for the infused extract. Significant amounts of total phenols and flavonoids were detected. Phytochemical screening showed the presence of alkaloids, flavonoids, tannins, anthraquinones, triterpenes and steroids in all the extracts and fractions.

Conclusion: In the light of the foregoing, it was obvious that *Mangifera indica* L. stem bark contain 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 infection. These findings support the claim of the local community about the use of this plant for the treatment of typhoid fever.

Keywords: Typhoid fever; Mangifera indica L.; antisalmonellal; antioxidant; phytochemicals.

1. INTRODUCTION

Typhoid and paratyphoid fevers are systemic diseases common in humans, caused by a biotic factor *Salmonella enterica* serovar, Gram-negative bacteria. In 2000, the global burden of typhoid and paratyphoid fevers was estimated at 22 million new cases of typhoid fever, 210,000 typhoid fever-related deaths, and 5.4 million cases of paratyphoid fever [1]. Typhoid fever continues to be a marked public health problem in developing countries, especially the sub-Saharan Africa, where it is endemic [2]. In these countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics, and a rapid rise in multidrug resistance as was reported for the first line drugs chloramphenicol, ciprofloxacin and amoxicillin [2,3]. *Salmonella* infection cause the production of superoxide and nitric oxide which react together to form peroxinitrite, a strong biological oxidant [4]. Consequently, pathological conditions characterized by oxidative stress can greatly result from typhoid fever.

Since ancient times, people have used herbs and their derivatives as therapeutic medicines [5] but, with the advent of antibiotics, the use of the plants became rare. Plants constitute a slightly/lightly explored important source of antimicrobial agents. These medicinal plants can directly help to solve some health problems, especially in developing countries where the pharmaceutical products are costly and less accessible to the less privileged. With the increase in resistance to typhoid drugs, medicinal plants have gained popularity [3]. At present, despite the abundance and advancement of synthetic drugs, a significant proportion of developing countries' population still depend on traditional medicines for their health care needs [5]. According to the information obtained from traditional practitioners, *Mangifera indica* L. is one of the plants whose stem bark is frequently used for the treatment of typhoid fever and diarrhoea. This work was therefore aimed at assessing the antisalmonellal and antioxidant activities of the crude extracts and fractions of *Mangifera indica* L. stem bark.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Mangifera indica L. (Mango) stem barks were harvested in Bamendjou, situated in Hauts-Plateaux division, West region of Cameroon, in October 2013. This plant was identified at the National Herbarium of Cameroon, Yaoundé, where a voucher specimen was deposited under the reference Number 5734/HNC.

2.2 Preparation of Crude Extracts

The plant material was dried at room temperature for one month. Upon drying, the plant materials were pulverized. The plant extracts were prepared by the method described

by Masibo and He [3] with slight modifications. For the organic extract, the powder was completely submerged in methanol/methylene chloride (1:1) with a composition in a mass to volume ratio of 100 g: 1000 mL (w/v) and then covered with aluminium foil. Extraction was allowed to process for 48 hours with constant stirring. The mixture was then filtered using Whatman paper N°1 and the filtrate was concentrated by evaporating the solvent using a rotary evaporator (Buchi R-200).

The aqueous extract (maceration) 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 time per day). The decoction was prepared by dissolving 100 g of plant powder in 1000 mL of water and then boiling at 100°C for 15 min. The infusion 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 Whatman paper N°1 and the filtrates were concentrated by evaporating the solvent at 40°C in an oven for two days. The yield was calculated for each extracts using the formula below. Yield = [mass of extract / mass of dry powder] x 100.

The plant extracts were stored in the sterilized bottles at room temperature until usage.

2.3 Partitioning of Methanol/Methylene Chloride (1:1) Extract

The partitioning was done according to the method described by He et al. [6] with slight modification. Briefly, 30 g of methanol/methylene chloride (1:1) 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 procedure was repeated with methanol fraction until the complete extraction of hexane soluble compounds. The same procedure was repeated with ethyl acetate, and the residual fraction contained compounds soluble in methanol/water mixture.

2.4 *In vitro* Antimicrobial Tests

2.4.1 Test bacteria 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 the Medical Bacteriology Laboratory of the ''Centre Pasteur'', Yaoundé, Cameroon; and one strain of *Salmonella* Typhi (ATCC 6539), obtained from the American Type Culture Collection (ATCC). The culture media used on this study namely Salmonella-Shigella Agar (SSA) for activation and maintenance of *Salmonella* strain/isolates; and Mueller Hinton Broth (MHB) for the determination of the Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs).

2.4.2 Preparation of bacteria inocula

The bacterial cell suspensions were prepared at 1.5×10^8 Colony-Forming Unit/ml (CFU/mL) following 0.5 McFarland turbidity standard. For this purpose, 18 hours old bacterial cultures were prepared in SSA. A few colonies of bacteria were collected aseptically with a sterile loop and introduced into 10 mL of sterile 0.9% saline water. The concentration of the suspension was then standardized by adjusting the optical density to 0.13 at 600 nm wavelength [7]. These suspensions were diluted 100 times with MHB to yield about 1.5×10^6 before use.

2.4.3 Determination of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs)

The broth micro-dilution method was used for susceptibility testing of bacteria species. The extracts and fractions were tested against the bacteria species listed above. The tests were carried out in 96-miro well sterile plates as described by Newton et al. [8]. For this, the crude extracts and fractions were dissolved in 10% dimethylsulfoxide (DMSO) solution and serial two-fold dilutions of the test substances were made with Mueller Hinton Broth to yield a volume of 100 µL per well. 100 µL of each of 1.5×10^6 CFU/mL bacterial suspensions were added to respective wells containing the test samples and mixed thoroughly to give the final concentration ranging from 1024 to 16 µg/mL (for extracts and fractions) and 256 to 2 µg/mL (for antibiotics). The solvent control, 10% DMSO, did not show inhibitory effects on the growth of bacteria. The plates were covered and incubated at 37°C for 24 hours. The inhibitory concentrations of the extracts and fractions were detected after addition of 40 µL of 0.2 mg/mL of P-iodonitrotetrazolium chloride (INT) (Sigma-Aldrish, South Africa) and incubate at 37°C for 15 min. Viable bacteria change the yellow dye of P-iodonitrotetrazolium chloride to pink colour.

The lowest concentrations at which there were no visible colour changes were considered as MICs. The Minimal Bactericidal Concentrations were determined by adding 50 µL aliquots of the preparations (without INT), which did not show any visible colour change after incubation during MIC determination, into 150 µL of fresh broth. These preparations were further incubated at 37°C for 48 hours and Minimal Bactericidal Concentrations were revealed by the addition of INT as above. All extract concentrations at which no colour changes were observed were considered as bactericidal concentrations. The smallest of these concentrations was considered as the MBC. These tests were performed in triplicates at three different occasions. The ratio MBC/MIC was calculated to determine the bactericidal (MBC/MIC ≤ 4) and bacteriostatic (MBC/MIC > 4) extracts/fractions.

2.5 Phytochemical Screening

The phytochemical screening was performed using standard methods described by Harbone [9]. The extracts and fractions of *Mangifera indica* stem bark were screened for the following classes of phytochemicals: alkaloids, flavonoids, triterpenes, steroids, saponins, tannins, anthocyanins and anthraquinones.

2.6 Antioxidant Assay

2.6.1 DPPH Radical scavenging assay

The free radical scavenging activities of the crude extracts and fractions of *Mangifera indica* L. were evaluated using the DPPH assay method as described by Mensor et al. [10]. Briefly, the extract/fraction (2000 µg/mL) was two-fold serially diluted with methanol. 100 µL 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 ± SD of the three findings, were illustrated 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 the concentration of test samples and a linear regression curve was established in order to calculate the RSA_{50}, which is the amount of sample necessary to inhibit by 50% the free radical DPPH.

2.6.2 Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was determined using Fenton reaction with respect to the method describes by Selvakumar et al. [11]. Briefly, 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 extracts and fractions with various concentrations (ranging from 12.5 to 200 mg/mL) were mixed together. H_2O_2 was added to the reaction mixture in order to initiate the reaction and the mixture was kept for incubation at room temperature for 5 min. Butylated hydroxytoluene (BHT) was used as standard antioxidant. After incubation the absorbance of the mixture was read at 560 nm using a spectrophotometer and the hydroxyl radicals scavenging (HRS) activity was calculated. HRS (%) = [(Absorbance of control - Absorbance of test sample) / Absorbance of control] x 100.

2.6.3 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. The method reported by Padmaja et al. [12] was used, with slight modification. Briefly, 400, 200, 100, 50, 25 µL of solution of methanolic extracts and fractions (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. Supernatant (500 µL) was diluted with 500 µL of water and shaken with 100 µL of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Vitamin C was used as a positive control. Increased absorbance of the reaction mixture indicates higher reduction capacity of the extracts.

2.6.4 The quantitative determination of total phenolic compounds

The total phenolic compounds were determined by the method described by Ramde-Tiendrebeogo et al. [13], with slight modification. It involves the oxidation of phenols in alkaline solution by the yellow molybdo-tungstophosphoric heteropolyanion reagent and colorimetric measurement of the resultant molybdo-tungstophosphate blue. These blue pigments have a maximum absorption depending on the qualitative and/or quantitative composition of phenolic mixtures besides the pH of solutions, usually obtained by adding sodium carbonate. The reaction mixture consisted of 0.02 mL of extract and fraction (2 µg/mL), 0.02 ml of 2N FCR (Folin Ciocalteu Reagent) and 0.4 mL of a 20% sodium carbonate solution. The mixture 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.

2.6.5 Determination of total flavonoids

Total flavonoids content of the extracts and fractions of *Mangifera indica* L. were determined according to a modified colorimetric method of Padmaja et al. [12] with slight modification. Methanolic solution of extracts and fractions (1 mL) were mixed with 1 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 mins, 0.03 mL of 10% $AlCl_3H_2O$ solution was added. After 5 mins, 0.2 mL of 1 M Sodium hydroxide and 0.24 mL of distilled water were added. The solution was well mixed and kept for 15 mins. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was calculated using standard catechin calibration curve. The results were expressed as milligrams of Catechin Equivalents (mgCE) per gram of extract/fraction.

2.7 Statistical Analysis

The experimental results were expressed as mean ± standard deviation of three replications. The data were subjected to 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) software version 12.0. The software Microsoft Excel 2007 was used to construct the graphs.

3. RESULTS

3.1. Yields of Extracts and Fractions

Following the extraction of *Mangifera indica* L. stem bark, marked differences were observed at the level of their outputs as well as their physical appearances. Table 1 shows the physical appearance and yields of the various extracts and fractions, which indicate the presence of polar compounds in *M. indica*.

3.2 Phytochemical Composition of Extracts and Fractions

Table 2 below shows the phytochemical composition of *Mangifera indica* L. stem bark with respect to the solvent used. The phytochemical screening of the extracts and fractions revealed the presence of different groups of secondary metabolites, including tannins, flavonoids, alkaloids, saponins, anthocyanin, triterpenes, steroids and anthraquinone. Saponins were absent in the hexane and ethyl acetate fractions, whereas anthocyanins were absent in the residual fraction.

Table 1. Physical appearance and yields of the extracts and fractions from the stem barks of *M. indica*

Plant extracts/fractions	Physical appearance	Yield (%)
Maceration	Dark brown tender	17.40
Infusion	Light brown crystal	22.60
Decoction	Dark brown tender	19.80
Methylene chloride/methanol (1:1)	Reddish brown tender	5.68
Hexane fraction	Yellowish liquid	10.33
Ethyl acetate fraction	Dark brown tender	34.83
Residual fraction	Dark brown crystal	53.03

Table 2. Phytochemical composition of the aqueous extracts and organic extracts/fractions of *Mangifera indica* stem bark

Phytochemical groups	Mac.	Inf.	Dec.	CH_2Cl_2-MeOH	Hex. F.	Et-Ac F	Re. F.
Tannins	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+
Saponins	+	+	+	+	-	-	+
Anthocyanin	+	+	+	+	+	+	-
Triterpenes and steroids	+	+	+	+	+	+	+
Anthraquinone	+	+	+	+	+	+	+

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

3.3 *In vitro* Antisalmonellal Activities

Antisalmonellal activities of *Mangifera indica* L. stem bark were assessed by the broth micro-dilution method and the results obtained for the crude extracts and fractions are presented in Table 3. Among the extracts and fractions used, the crude methylene chloride/methanol (1:1) extract had the values of MIC against *Salmonella* ranging from 128 µg/mL on ST, STM and STS to 256 µg/mL on SPA and SPB. The hexane fraction showed antisalmonellal activities with MIC ranging from 256 µg/mL on SPA to 1024 µg/mL on SPB. The decocted extract had the lowest activity among aqueous extracts, whereas the macerated and infused extracts had relatively the same activities. The macerated extract exhibited the greatest activity on STS, with MIC of 64 µg/mL. In general, all the extracts and fractions of *Mangifera indica* L. stem bark exhibited antisalmonellal activies, with MIC values ranging from 64 to 1024 µg/mL.

3.4 Antioxidant Activities

3.4.1 DPPH radical scavenging activity

The DPPH radical scavenging activity of different extracts and fractions was evaluated, and the results are shown in Table 4. Except the hexane fraction, all extracts and fractions of *Mangifera indica* L. stem bark exhibited stronger antioxidant activities, compared to that of the standard antioxidant molecule (Vitamin C) used. The hexane fraction showed the lowest activity with the value of 50% at the concentration 200 µg/mL, while the residual fraction showed the highest activity (91.74%) at the concentration 12.5 µg/mL, as far as the fractions were concerned. At the concentration 12.5 µg/mL, the activity of the standard (Vitamin C) was relatively lower than that of the organic crude extract, residue and ethyl acetate fractions, with a significant ($p < 0.05$) difference. At that concentration (12.5 µg/mL), the activity of the rest of extracts/fraction was lower than that of vitamin C, with significant ($p < 0.05$) difference. However, there was no significant ($p > 0.05$) difference between the activity of vitamin C and that of the infused extract.

3.4.2 IC_{50} of extracts and fractions of *Mangifera indica* L. stem bark

The concentrations which inhibited 50% of DPPH (IC_{50}) are presented in Table 5. These results show that the concentration which inhibited 50% of DPPH from all the extracts/fractions of *Mangifera indica* L. stem bark, except the hexane fraction and macerated aqueous extract, did not show any significant ($p>0.05$) difference. The decocted, infused, crude organic extracts, and the residual and ethyl acetate fractions had the lowest IC_{50} (i.e. had the highest activity). Hexane fraction had the highest IC_{50} (i.e. the lowest activity).

3.4.3 Hydroxyl radical scavenging activities

The result of the scavenging activity against hydroxyl radicals are presented on Fig. 1. The graph showed that the standard antioxidant (BHT) exhibited the highest activities, followed by hexane fraction. The activities of these two tests solution increased exponentially from the concentration 100 to 200 µg/mL. Infused and decocted extract showed relatively the same activity, while the activities of ethyl acetate and residual fractions and macerated extract were approximately the same at concentration 200 µg/mL. The crude organic extract showed the lowest activity at 200 µg/mL.

Table 3. MICs, MBCs, MBCs/MICs of different extracts and fractions of *Mangifera indica* L. stem bark on isolates and strain of *Salmonella*

Extracts/fractions		Strain/isolates				
		ST	**SPA**	**SPB**	**STM**	**STS**
Maceration	MIC (µg/mL)	256	**128**	256	256	**64**
	MBC (µg/mL)	1024	512	512	512	256
	MBC/MIC	4	4	2	2	4
Infusion	MIC (µg/mL)	256	256	256	**128**	256
	MBC (µg/mL)	1024	512	512	512	512
	MBC/MIC	4	2	2	4	2
Decoction	MIC (µg/mL)	512	**128**	512	**128**	256
	MBC (µg/mL)	1024	1024	1024	1024	1024
	MBC/MIC	2	8	2	8	4
Methylene chloride/ methanol (1:1)	MIC (µg/mL)	**128**	256	256	**128**	**128**
	MBC (µg/mL)	1024	1024	1024	1024	512
	MBC/MIC	8	4	4	8	4
Residual fraction	MIC (µg/mL)	256	**128**	256	512	**128**
	MBC (µg/mL)	512	1024	1024	/	512
	MBC/MIC	2	8	4	/	4
Ethyl acetate fraction	MIC (µg/mL)	512	512	256	512	1024
	MBC (µg/mL)	1024	512	512	512	1024
	MBC/MIC	2	1	2	1	1
Hexanic fraction	MIC (µg/mL)	512	256	1024	512	512
	MBC (µg/mL)	/	1024	/	1024	1024
	MBC/MIC	/	4	/	2	2
Ciprofloxacin	MIC (µg/mL)	8	4	8	2	2
	MBC (µg/mL)	16	16	32	4	4
	MBC/MIC	2	4	4	2	2
Oxytetracyclin	MIC (µg/mL)	32	32	64	64	32
	MBC (µg/mL)	256	256	256	128	128
	MBC/MIC	8	8	4	2	4

ST= *Salmonella* Typhi. SPA= *Salmonella* Paratyphi A. SPB= *Salmonella* Paratyphi B. STM= *Salmonella* Typhimirium, STS= *Salmonella* Typhi strain. *MIC= Minimal Inhibitory concentration. MBC= minimal Bactericidal Concentration*

Table 4. DPPH radical-scavenging activities of the crude extracts and fractions of *Mangifera indica* L. stem barks

Extracts and Fractions	Concentration of extract/fraction (µg/mL) and scavenging activity (%)				
	12.5	**25**	**50**	**100**	**200**
Dec.	47.52±1.34^{b}	81.74±1.24^{b}	83.00±2.39^{b}	89.85±1.61^{b}	97.26±1.45^{b}
Inf.	61.78±3.64^{c,d}	85.11±1.92^{c,d}	93.48±4.08^{c,d}	95.26±1.48^{c,d}	97.78±0.55^{c,d}
Mac.	72.38±0.71^{c}	82.76±0.78^{c}	86.28±3.71^{c}	91.50±0.99^{c}	97.61±0.13^{c}
CH_2Cl_2-MeOH	92.78±1.79^{e}	93.68±0.34^{e}	94.79±1.27^{e}	95.46±0.46^{e}	96.43±0.39^{e}
Re. F.	91.74±0.81^{e}	93.60±0.26^{e}	93.64±1.07^{e}	94.57±0.46^{e}	95.16±2.39^{e}
Et-Ac F.	89.78±0.93^{e}	93.78±0.92^{e}	94.71±1.41^{e}	95.69±0.48^{e}	97.39±0.13^{e}
Hex. F.	27.86±0.24^{a}	29.55±1.60^{a}	32.04±3.89^{a}	35.43±0.73^{a}	51.88±3.01^{a}
Vit C	79.59±1.21^{d}	86.19±0.6^{d}	87.26±0.76^{d}	89.91±1.03^{d}	93.47±0.38^{d}

Along each column, values with the same superscripts are not significantly different. Waller Duncan ($P > 0.05$). Mac: Maceration; Inf: Infusion; Dec: Decoction; Hex F: Hexane Fraction; CH_2Cl_2-MeOH: Methylene chloride/methanol; Et-Ac F: Ethyl Acetate Fraction; Re. F.: Residual Fraction; Vit C: Vitamin C

Table 5. IC_{50} values of extracts/fractions of *Mangifera indica* L. stem bark

Extracts/fractions	IC_{50} (µg/mL)
Decoction	17.12±0.19[a]
Infusion	11.99±0.71[a]
Maceration	43.29±4.98[b]
MeOH/CH_2Cl_2 (1:1)	10.00±0.00[a]
Residual fraction	10.00±0.00[a]
AcEt fraction	10.00±0.00[a]
Hexane fraction	565.20±30.92[c]
Vitamine C	10,02±0.01[a]

Values with the same superscripts are not significantly different. Waller Duncan (P > 0.05)

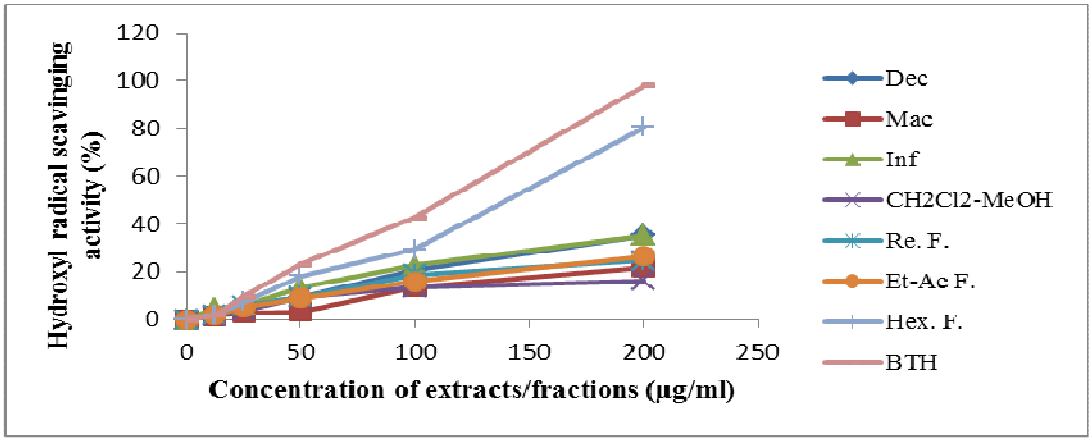

Fig. 1. Hydroxyl radical scavenging activities of extracts/fractions of *Mangifera indica* L. stem bark

Mac: Maceration; Inf: Infusion; Dec: Decoction; Hex F: Hexane Fraction; CH_2Cl_2-MeOH: Methylene chloride/methanol; Et-Ac F: Ethyl Acetate Fraction; Re F: Residual Fraction

3.4.4 Ferric reducing/antioxidant power (FRAP)

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 on Fig. 2. The hexane fraction showed the lowest reducing power, while the standard (Vitamin C) exhibited the highest reducing power at the concentration of 200 µg/ml. All the remaining extract/fraction exhibited varied activities from one extract/fraction to another at each concentration.

3.4.5 Total phenolics

The total phenolic content of extracts and fractions of *Mangifera indica* L. stem bark were determined in this study and the results are presented on Fig. 3. The concentration of phenolic compounds in crude methanol/methylene chloride (1:1) extract (0.099 mgGAE/mg) was higher than in all other extracts and fractions. The lowest concentration was observed in the hexane fractions (0.010 mgGAE/mg).

3.4.6 Total flavonoid content

The total flavonoid contents of the various extracts and fractions are presented on Fig. 4. The result obtained showed that the crude organic extract had the highest flavonoid content (0.086 mgCE/mg). Ethyl acetate and residual fractions had relatively the same concentration, while hexane fraction showed the lowest value of flavonoid content (0.035 mgCE/mg). The aqueous extracts did not show any significant ($p > 0.05$) difference in flavonoid concentrations. The same result was observed with the organic extracts/fractions where there was also no significant ($p > 0.05$) difference in flavonoid contents. The organic extract/fractions, except hexane fraction, showed considerable flavonoid content.

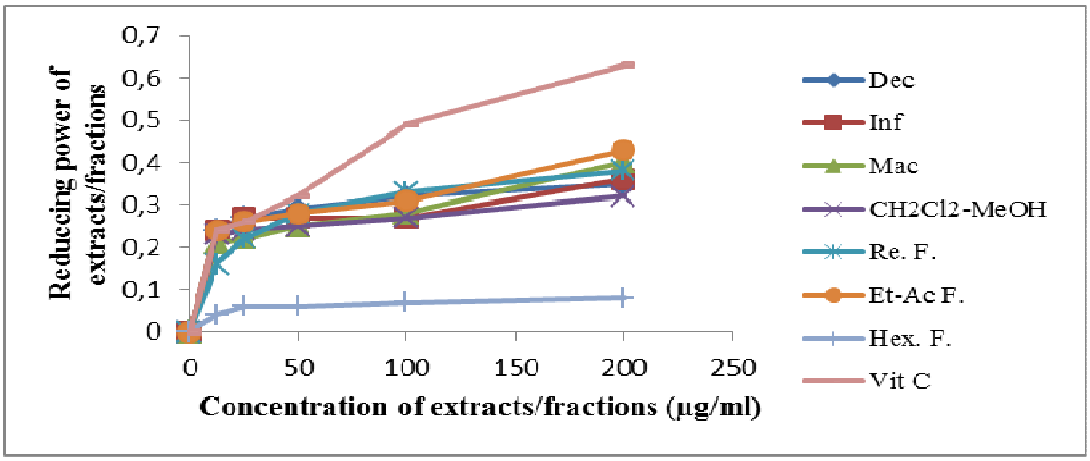

Fig. 2. Reducing power activities of the crude extracts and fractions of *Mangifera indica* stem L. bark as well as vitamin C

Mac: Maceration; Inf: Infusion; Dec: Decoction; Hex F: Hexane Fraction; CH_2Cl_2-MeOH: Methylene chloride/methanol; Et-Ac F: Ethyl Acetate Fraction; Re F: Residual Fraction

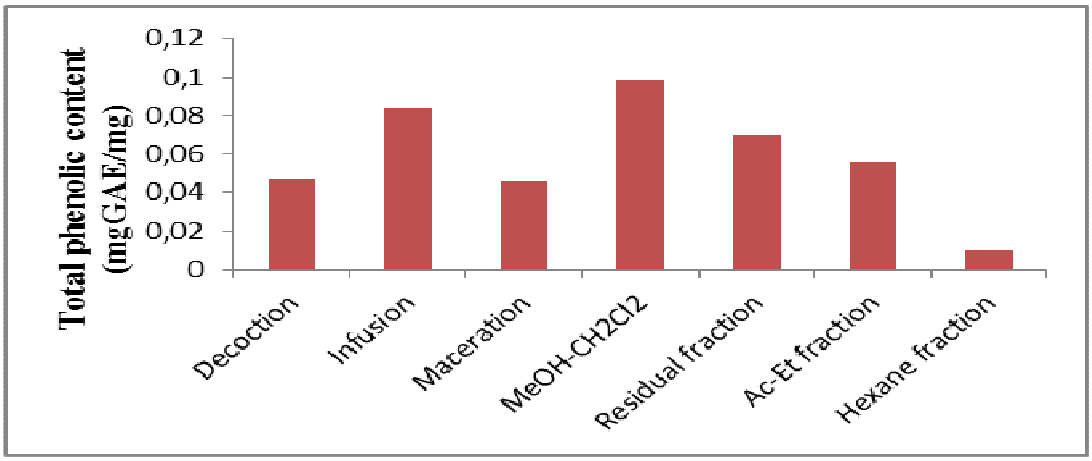

Fig. 3. Total phenolic content of extracts/fractions of *Mangifera indica* L. stem bark

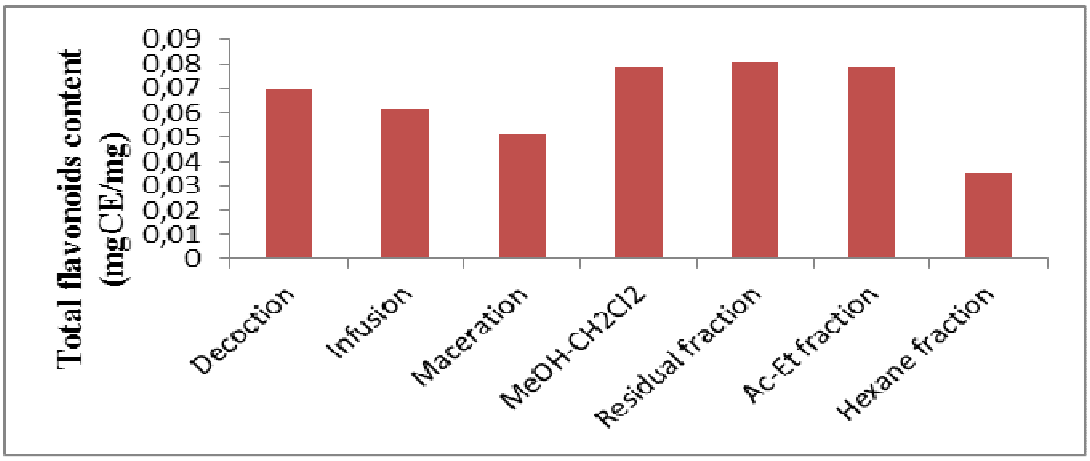

Fig. 4. Total flavonoid content of extracts/fractions of *Mangifera indica* L. stem bark

4. DISCUSSION

4.1 Antimicrobial Activities

The results of antimicrobial tests (MICs, MBCs) showed that *M. indica* L. stem barks contain substances with antisalmonellal activities which may be used in the treatment of typhoid and paratyphoid fevers. The wide range of antisalmonellal properties can be explained by the presence of various groups of potentially active secondary metabolites in them. The antimicrobial activities suggest that the *M. indica* L. stem barks may contain several antibacterial active principles with different polarities, acting in a synergistic way. Indeed, the existence of these antimicrobial substances was confirmed by the phytochemical screening which revealed the presence of certain classes of compounds whose members have already been shown to exhibit antimicrobial activities. Tannins have been found to form irreversible complexes with proline-rich proteins [14] resulting in the inhibition of cell protein synthesis. Tannins prevent bacterial growth by precipitating their proteins [15]. Some flavonoids have shown several pharmacological activities including antibacterial and antifungal [16]. Alkaloids have been reported to exhibit antisalmonellal activities [15]. Many saponins are known to be antimicrobial, to inhibit mould [17]. Ciprofloxacin was about 10-fold more active than the CH_2Cl_2/MeOH (1:1) extract. This may result from the fact that ciprofloxacin is a pure compound, unlike the extract, which is a mixture of compounds that tend to dilute the activity of the active principle(s).

Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC ≤ 4 and bacteriostatic when the ratio MBC/MIC > 4 [2,15,18]. For most of the various extracts/fractions used, the ratio MBC/MIC was ≤ 4 against most of the bacteria strain/isolates, suggesting that these extracts/fractions may be classified as bactericidal agents against these bacteria.

4.2 Antioxidants Activities

The anti-oxidative profile of various solvent extracts/fractions of *Mangifera indica* L. stem bark as a prelude to finding agent(s) that could be used to ameliorate oxidative stress-associated complications. We found that some of the *Mangifera indica* L. extracts/fractions possess high anti-oxidant activities, at least with the experimental models used. For this reason, studying the complex antioxidant activities often needs a multi-method approach [19]. Antioxidants can be reductants, and inactivation of oxidants by reductants can be described as oxido-reduction reactions [19]. 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 [12], therefore, provides a reliable method to study the antioxidant activity of various extracts/fractions. In this study, the high reducing power of the infused and decocted extracts, EtOAc and residual fractions of *M. indica* stem bark suggested that the phytochemical constituents with higher redox potential were more extractable with these solvents. These data suggest that the *Mangifera indica* L. stem barks may contain several antioxidants with different polarities, acting in a synergistic way. As far as the partitioning is concerned, the highest activity was obtained with ethyl acetate fraction, while the lowest activity was obtained with the hexane fraction. There is a significant difference among the various fractions as far as the activity is concerned. At the concentration 200 µg/mL, the activity of the crude organic extract was lower than the activity of ethyl acetate and the residual fractions, but significantly different from the activity of the hexane fraction. The reducing power of these extracts/fractions is relatively low compared to the standard (Vitamin C). This may account for the observation that plant extracts tends to have activity at higher concentrations [20]. The reducing activity of these plant extracts/fractions may mostly be due to polar compound(s). This can be explained by the fact that low reducing power was observed for the hexane fraction. Hexane is a solvent which extract generally non polar compounds due to its non-polar nature.

The effect of antioxidants on DPPH has been thought to be due to their hydrogen donating ability. Hence, DPPH is usually used as a substrate to evaluate anti-oxidative or free radical scavenging activity of antioxidants agents *in vitro* [21]. 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, MeOH/CH_2Cl_2 extract, EtOAc and residual fractions from *M. indica* L. demonstrated a more

remarkable anti-radical activity with IC_{50} values lower than those of ascorbic acid. These results corroborate those of Núñez-Sellés [22] where the aqueous extract of *M. indica* L. exhibited a stronger scavenging capacity than Vitamin C and vitamin E, but the activity was relatively the same when vitamins C and E were combined. Previous studies reported that the antioxidant activity of *M. indica* L. stem bark is related to the phenolic compounds such as mangiferin, catechins, galloyl, and benzoyl esters derivatives acting in synergy [23].

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [22]. From the study results, the infused extract had the highest activity among all aqueous extracts, while the hexane fraction showed the best activity among all extracts and fractions. Therefore, compounds exhibiting hydroxyl radical scavenging activities could be lipid soluble compounds. In regard to phytochemical screening, these compounds could belong to triterpenoids or steroids, which are lipid soluble compounds. The partitioning had a remarkable impact on the activity. The organic crude extract (CH_2Cl_2/MeOH extract) which had low activities has its fractions showing activities increasing gradually as the solvent polarity decreased. The hexane fraction exhibited very low anti-radical scavenging ability and antimicrobial activities, but showed a hydroxyl radical scavenging activity similar to the standard BTH molecule. Meanwhile in the course of typhoid fever treatment, each part of the extracts/fractions may play it own role by one process or another. 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.

Flavonoids protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reduce radicals of alpha-tocopherol or to inhibit oxidases [13]. Phenolic compounds are very important plant constituents because they exhibit antioxidant activity [24] by inactivating free radicals or preventing decomposition of hydroperoxide into free radicals [13]. The concentration of phenolic compounds in crude methanol/methylene chloride extract was higher than in other extracts and fractions. This result corroborates the DPPH free radical scavenging assay where this extract exhibited the best antioxidant 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, which probably indicates that this solvent system extracts phenolics with more pronounced antioxidant properties. This result suggests that the phenolic compounds contribute significantly to the antioxidant capacity of the investigated plant species. In addition, this result is consistent with the finding of many researchers who reported such positive correlation between total phenolic content and antioxidant activity [13,24]. However, Bajpai et al. [25] disproved the correlation between phenolic compounds and antioxidant activity. The results of antioxidants assay further suggest that these extracts/fractions contain powerful free radical scavenging phytochemicals that could be used to fight against free radical upsurge, as well as oxidative stress, and consequently might ameliorate oxidative stress-associated metabolic disorders.

5. CONCLUSION

In the light of the foregoing, it is clear that the crude extracts and fractions of *Mangifera indica* L. stem bark contains bioactive substances which are active against salmonellosis pathogens. This supports the claim of the local communities for its potential use as the therapeutic agents for the treatment of typhoid fever. All the extracts and fractions exhibited antioxidant activity, suggesting that Mangifera *indica* L. could be used to manage oxidative stress caused by typhoid fever (or other salmonellal infections). The study result further suggested that the extracts/fractions 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 ameliorate oxidative stress-associated metabolic disorders. However, further studies should be carrying out in order to investigate the antimicrobial and antioxidant properties of this plant *in vivo,* and its toxicological profile.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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