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In vitro Antisalmonella and Antioxidant Activity of Hydroethanolic and Aqueous Extracts of *Bauhinia rufescens* Leaf and Stem Bark Extracts

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Authors' contributions

This work was done in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

In Chad, enteric fever remains a major public health problem where it is still endemic due to the precariousness of life hygiene combined with the abusive and inappropriate use of antibiotics. **Objective:** The aim of this work was to evaluate the *in vitro* antisalmonella and antioxidant activity of extracts from the leaves and stem bark of *B. rufescens*.
Methods: Phytochemical screening of these extracts was performed by standard methods to justify the observed activities. The antisalmonella activity was evaluated using the liquid microdilution method. Antioxidant activity of these extracts was determined by investigating their 1, 1-diphenyl-2 picrylhydrazyl ($DPPH^{\bullet}$) antiradical and iron reducing capacities.
Results: The Minimum Inhibitory Concentrations (MICs) were varied from 256 to 1024 µg/ml. The 95% hydroethanolic extract of the leaves exhibited higher $DPPH^{\bullet}$ antiradical activity than all extracts and IC50s ≤ 20 µg/ml for all extracts tested.

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Conclusion: These results showed that the 95% hydroethanolic extract of *B. rufescens* leaves possess *in vitro* antisalmonella and antioxidant activities and could be used for *in vivo* antisalmonella and antioxidant studies.

Keywords: Typhoid fevers; B. rufescens; phytochemical screening; antisalmonella; antioxidant.

1. INTRODUCTION

Typhoid and paratyphoid salmonelloses are usually found in areas with poor hygiene and are a serious public health problem worldwide [1]. They mainly affect Asia, Africa and Latin America. Estimates made by the World Health Organization in 2008 report 22 million cases and 600,000 deaths per year worldwide [2]. The high costs, as well as the toxicity of some of these antibiotics are commonly encountered, following the example of chloramphenicol whose use is limited due to its bone marrow toxicity [3]. All this confronts medicine with problems of anti-infective therapy. Furthermore, typhoid salmonellosis is often exacerbated by oxidative stress, which can be a consequence of microbial invasion or militate for its progression [4, 5]. These *Salmonella* infections produce superoxide ion and nitric oxide, which react together to form peroxynitrite, which is a potent biological oxidant [6]. It then becomes necessary to find a new, effective, low-cost therapy that in addition to anti-salmonella activity can reduce the level of free radicals produced during *Salmonella* infection. The traditional use of *B. rufescens* for the treatment of typhoid fever by the Chadian population and a retrospective study on this plant revealed that it is used in traditional medicine for the treatment of several pathologies such as diabetes, diarrhea, dysentery, fungus, fibrosis, jaundice and inflammations [7, 8]. Given its traditional use by the Chadian population in the treatment of typhoid fever and the results of numerous previous scientific researches on the plant species *B. rufescens*, its extracts could constitute a non-toxic alternative against typhoid fever. Hence the objective of this work which aims to provide concrete scientific evidence on the therapeutic efficacy of *B. rufescens* against typhoid fever through the *in vitro* antisalmonella and antioxidant study.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant material used for this work consisted the leaves and stem bark of *B. rufescens*, collected in December 2017 in Abeche, eastern Chad (13° 49' 0" North, 20° 49' 0" East.), and identified at the Botanical Unit of the Livestock Research Institute for Development (UBIRED) in N'djaména, Chad, under the reference IRED/LRVZ 1325.

2.2 Preparation of the Extracts

The leaves and stem bark of *B. rufescens* were harvested and dried at room temperature 30±2°C away from the sun and ground using a Moulinex brand Zaiba (Super Blender, China). The powder obtained was stored in a cardboard box at room temperature, in a dry place and protected from humidity and light until use. The obtained powders were used for the preparation of different aqueous (infusion, maceration and decoction) and hydroethanolic (95%, 75% and 50%) extracts following the methods

Fig. 1. *B. rufescens* in its natural environment

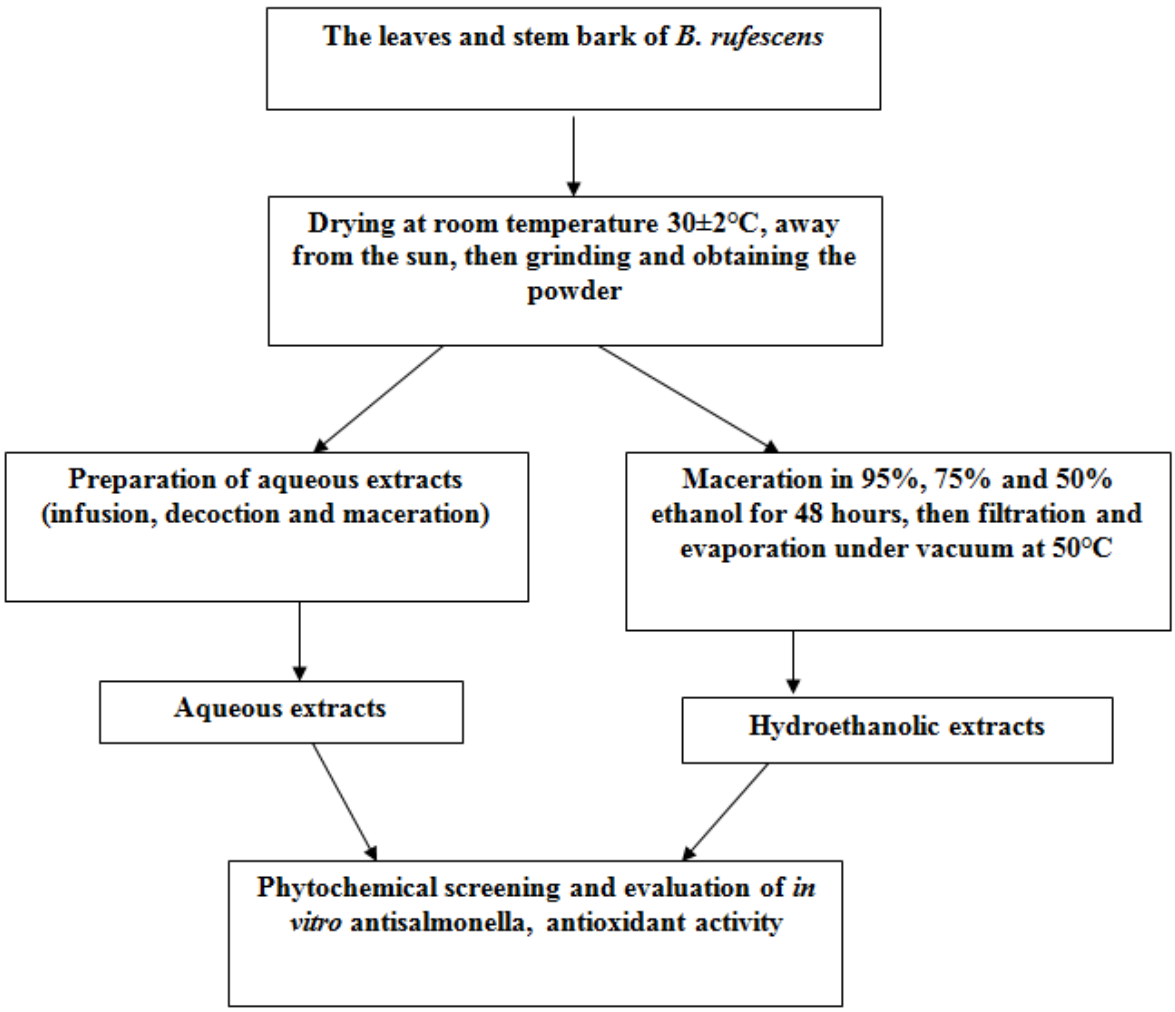

Fig. 2. General scheme

described by Kamsu et al. [9]. This protocol was scrupulously followed for the preparation of the extracts, using 250 g of powder previously obtained after desiccation of the plant material for 2500 ml of solvent at each time.

The extraction yield of the different extracts was calculated according to the following formula [10].

$$R = (m \times 100)/m°$$

R: yield of the crude extract in percentage (%), m: the mass of the crude extract obtained after extraction (g), m°: the mass of the dry plant material (g).

The different extracts obtained were stored at -20°C until their use.

2.3 Phytochemical Screening

The determination of the different classes of potential bioactive compounds present in the extracts of *B. rufescens* was carried out following the standard method of Harbone [11].

2.4 Microorganisms and Culture Media

The microorganisms used for the determination of the antibacterial activities of the extracts were Gram-negative bacteria: *Salmonella enterica* serovar Typhi (ST), *Salmonella enterica* serovar Paratyphi A (SPA), *Salmonella enterica* serovar Paratyphi B (SPB) and *Salmonella enterica* serovar Typhimurium (STM) obtained at the Centre Pasteur du Cameroun and a *Salmonella enterica* serovar Typhi ATCC6539 strain. Microorganisms were stored at -20°C on

Salmonella-Shigella agar (SSA) (Liofilchem, Italia) and subcultures were freshly prepared before use. Mueller Hinton broth (MHB) (Liofilchem, Italia) was used as a basic enrichment medium for aerobic culture at 37°C with agitation at 150 rpm in the different tests.

2.5 Reference antibiotic and bacterial growth indicator reagent

Ciprofloxacin was used as a positive control during the determination of MICs and BMCs. Its choice is justified by the fact that it is used as a first-line treatment for typhoid fevers (typhoid and paratyphoid fevers) in Central Africa [12]. Para-iodonitetrazoliumchloride (INT) was used as an indicator of bacterial growth.

2.6 Antisalmonella Activity of *B. rufescens* Extracts

The bacterial growth inhibitory potential of *B. rufescens* extracts was determined by the microdilution method as described by Mativandlela et al. [13]. In each well of a 96-well microplate, 100 µl of culture broth (MHB) was introduced. Then, 100 µl of each extract was introduced to obtain an initial concentration (4096 µg/ml) respectively in the first three wells of the first row; subsequently serial dilutions were performed to obtain final concentrations ranging from 2048 to 16 µg/ml. A volume of 100 µl of broth plus inoculums at the concentration of 1.5×10^6 CFU/ml bacterial was introduced into each well. Plates were brought to incubation at 37°C for 18 h. Wells containing the inoculums as well as those containing only the culture media were made and constituted the negative controls and the positives control with the antibiotic. After this incubation time, 40 µl of a 0.2% aqueous para-iodonitrotetrazolium chloride solution was added to these wells and incubated at 37°C for 30 min. Thus, wells that turn pink after addition of INT indicate bacterial growth [13]. All concentrations that prevented the appearance of pink color were taken as the inhibitory concentrations and the smallest was scored as Minimal Inhibition Concentration (MIC). For each extract, three columns were made and the revelation was done on two columns.

The third column was used to determine the Minimum Bactericidal Concentrations (MBC). After reading the different MICs, 150 µl of newly prepared Mueller-Hinton Broth was introduced into the wells of the new plates, and then 50 µl of the contents of each well where there was inhibition of bacterial growth (absence of pink staining) was withdrawn with a micropipette and introduced into the corresponding wells of the new plate. These plates were again covered with a sterile lid. Negative control wells, containing only Mueller-Hinton broth and those containing the inoculums without extract or antibiotics were made. The new incubation was also done at 37°C, for 48 h. The revelation was done as for MIC determination (40 µl of an aqueous solution of INT was added to each well). All extract concentrations for which the absence of bacterial growth was noted (no appearance of pink coloration) were considered as bactericidal concentrations and the smallest was noted as MBC. This test was repeated independently three times.

2.7 Antioxidant Activity of *B. rufescens* Extracts

2.7.1 DPPH• radical assay

DPPH• radical scavenging by the DPPH• (1, 1-diphenyl-2 picrylhydrazyl) assay was used to investigate the radical scavenging activities of *B. rufescens* extracts according to the technique described by Mensor et al. [14]. The extract (2000 µg/ml) was serially diluted twice with methanol. 100 µl of diluted extract was mixed with 900 µl of 0.3 mM DPPH methanol solution, for a final extract concentration of 12.5 to 200 µg/ml (12.5, 25, 50, 100 and 200 µg/ml). The absorbance at 517 nm was measured using a spectrophotometer, after 30 min of incubation at room temperature in the dark. Ascorbic acid (vitamin C) was used as a control.

$$\text{Radical reduction percentage} = \frac{[\text{Absorbance of DPPH} - \text{Absorbance of sample}]}{\text{Absorbance of DPPH}} \times 100$$

The IC_{50} (amount of sample required to inhibit 50% of the free radical DPPH) was determined by plotting the percentages of radical scavenging activity against the log values of the test sample concentration.

2.7.2 Ferric Reduction/Antioxidant Power assay (FRAP)

The iron (Fe^{3+}) reducing power of the extracts was determined according to the method described by Padmaja et al. [15]. For this purpose, one milliliter of extract at different concentrations (200; 100; 50; 25 and 12.5 µg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 ml of 1% potassium ferricyanide $K_3Fe(CN)_6$ solution. The whole set was incubated in a water bath at 50°C for 20 min; then 2.5 ml of 10% trichloroacetic acid was added to stop the reaction and the tubes were centrifuged at 3000 rpm for 10 min. An aliquot (2.5 ml) of supernatant was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% $FeCl_3$ ethanol solution. The absorbance of the reaction medium was read at 700 nm against a similarly prepared blank, where the plant extract was replaced with distilled water.

The positive control was represented by a solution of a standard antioxidant (L- ascorbic acid or vitamin C) whose absorbance was measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the tested extracts [16].

2.7.3 Determination of total phenols

The content of total phenols was determined by the method described by Ramde-Tiendrebeogo et al. [17].The reagent was a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). It was reduced upon oxidation of the phenols to a mixture of blue oxides of tungsten and molybdenum. These blue pigments have a maximum absorption depending on the qualitative and or quantitative composition of phenolic mixtures in addition to the pH of the solutions, usually obtained by adding sodium carbonate [18]. The reaction mixture in this assay consisted of 20 µl of extract (2 mg/ml), 20 µl 2N of Folin-Ciocalteu reagent and 40 µl of a 20% sodium carbonate solution. The mixture was stirred and incubated in a water bath at 40°C for 20 min, and then the absorbance was measured at 760 nm. The extract was replaced with distilled water in the blank tubes. A calibration curve was plotted using gallic acid (0-2 mg/ml); results were expressed as milligrams of gallic acid equivalent per gram of extract (mgGAE/g).

2.7.4 Determination of flavonoid content

The flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method of Padmaja et al. [15]. 100 µl of the extract was mixed with 1.49 ml of distilled water, and then 30 µl of a 5% sodium nitrite $NaNO_2$ solution was added. After 5 min, 30 µl of a 10% aluminum chloride solution $AlCl_3$ was added. The mixture was left to stand for 6 min, and then 200 µl of 1M NaOH solution and 240 µl of distilled water were added. The whole mixture was homogenized with a vortex and the absorbance was measured at 510 nm. The total flavonoid content was calculated using a calibration curve with catechin and the results were expressed as milligrams of catechin equivalent per gram of extract (mgCE/g).

2.8 Statistical Analysis

Statistical analyses were done using SPSS (22.0) for Windows software by ANOVA analysis of variance followed by Waller-Duncan test for comparison between the parameters of the control groups and those of the test groups. All results obtained were expressed as means ± standard deviation. Probability values $P < 0.05$ were considered significant.

3. RESULTS

3.1 The extraction Yield of the Different Extracts

Table 1 shows the extraction yield of hydroethanolic and aqueous extracts of *B. rufescens* leaves and stem bark. The extraction yield varies with the solvent used. The yields of hydroethanolic extracts were higher than those of aqueous extracts. Regardless of the plant part, the best extraction yield was obtained with the 95% hydroethanolic solvent system.

3.2 Phytochemical study of *B. rufescens* Extracts

The qualitative phytochemical screening of the extracts of the leaves and stem barks of *B. rufescens* revealed the presence of several classes of secondary metabolites (Table 2).

From this table, it appears that tannins, alkaloids, anthraquinones, phenols and flavonoids are present in all extracts tested. All the secondary metabolisms tested are present in the 50%, 75% hydroethanolic extracts and the decoction of the stem barks. The triterpenes and saponins are present in the 95% and 75% hydroethanolic extracts of the leaves; they are also present in the decoction, the infusion and the 50% and 75% hydroethanolic extracts of the stem barks.

3.3 *In vitro* Antisalmonella activity of *B. rufescens* Leaf and Stem Bark Extracts

The values of the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) obtained in the evaluation of the *in vitro* antisalmonella activity of the extracts of the leaves and stem barks of *B. rufescens* are represented in Table 3. From this Table, it appears that all the hydroethanolic extracts showed MICs between 256 and 1024 µg/ml. The macerated and decocted leaves showed no activity up to the threshold tested (1024 µg/ml). The 95% hydroethanolic extract of the leaves exhibited a MIC of 512 µg/ml on *Salmonella* Typhi (ST), *Salmonella* Typhi ATCC6539, *Salmonella* Typhimurium (STM) and *Salmonella* Paratyphi A (SPA).The hydroethanolic extracts (95%, 75% and 50%) and aqueous extracts (decocted, macerated and infused) of stem barks inhibited the growth of *Salmonella* Typhi, *Salmonella* Paratyphi B, and *Salmonella* Paratyphi A with concentrations ranging from 256 to 512 µg/ml. Regarding the hydroethanolic extracts of leaves and stem barks, MBCs/MICs ratios < 4 were observed on all extracts that presented MBC value.

3.4 Study of *in vitro* Antioxidant Activity of Extracts from leaves and Barks of *B. rufescens*

3.4.1 Effects of the extracts on the DPPH$^{\bullet}$ radical

The free radical scavenging activity of the different extracts from the leaves of *B. rufescens* was determined after 30 minutes of reaction in the presence of DPPH$^{\bullet}$ radical. The ability of the extracts of *B. rufescens* leaves and vitamin C (reference antioxidant) to inhibit the DPPH$^{\bullet}$ radical is presented in Table 4. From this Table, it can be seen that all these extracts possess significant activity against the DPPH$^{\bullet}$ radical. The most active extracts are the 95% hydroethanolic extract of leaves and the macerated stem bark, which inhibit more than 90% of the DPPH$^{\bullet}$ radical at the concentration of 200 µg/ml. All extracts significantly ($p<0.05$) inhibited DPPH less than vitamin C at all concentrations tested.

3.4.2 IC_{50} of the extracts of the leaves and stem barks of *B. rufescens*

The IC_{50} of the different extracts of the tested leaves and barks of *B. rufescens* in relation to that of ascorbic acid are presented in Table 5. We find that all the extracts present IC_{50} values between 9,780 and 19,531 µg/ml. The lowest IC_{50} value of the extracts was obtained with the 95% hydroethanolic extract of the leaves (IC_{50} = 9.780±0.125). However, the 50% hydroethanolic extract of the leaves showed the highest IC_{50} (IC_{50} = 19,531±2,502). Vitamin C was the most active substance with an IC_{50} (8,570 mg/ml) significantly lower ($p < 0.05$) than that of all extracts (Table 5).

3.4.3 Iron reducing capacity (FRAP) of *B. rufescens* leaf and stem bark extracts

The results of iron reducing power of *B. rufescens* extracts are presented in Table 6. From this table, it can be seen that the 95% hydroethanolic extract of *B. rufescens* leaves and stem bark showed the highest reducing power ($p<0.05$) compared to all extracts followed by the 50% hydroethanolic extract of stem bark from the concentration 50 µg/ml. In general, the activity of L-ascorbic acid was significantly ($p<0.05$) higher than that of all extracts at all concentrations.

3.4.4 Total phenol and flavonoids content of *B. rufescens*

The analysis of total phenols and flavonoids content revealed that most of these types of metabolites are present in each extract of *B. rufescens* as presented in Table 7. From this table, it can be seen that the total phenol content of the 95% hydroethanolic extract of the leaves and stem barks were significantly ($p<0.05$) higher than that all of extracts. However, the flavonoid content of the 50% hydroethanolic extract of leaves and stem bark were significantly ($p<0.05$) higher than all extracts. The leaf infusions showed the lowest phenol and flavonoid contents.

Table 1. Extraction yield of leaves and stem bark of *B. rufescens*

Plant extracts		Sample mass in (g)		Mass of extracts (g)		Yields %	
		leaves	Barks	leaves	Barks	leaves	Barks
Hydro	95%	250		45.96	39.23	18.387	15.695
Ethanolic extracts	75%			43.83	35.49	17.544	14.196
	50%			41.82	28.45	16.728	11.38
Aqueous extracts	Infused			25.13	25.97	10.052	10.39
	Decocted			27.66	27.50	11.064	11.00
	Macerated			26.25	23.75	10.50	9.50

Table 2. Phytochemical composition of leaf and bark extracts of *B. rufescens*

Extracts	Chemical classes	Hydroethanolic extracts			Aqueous extracts		
		95%	75%	50%	Decocted	Infused	Macerated
Leaves	Alkaloids	+	+	+	+	+	+
	Phenols	+	+	+	+	+	+
	Flavonoids	+	+	+	+	+	+
	Anthocyanins	−	−	−	−	−	−
	Anthraquinones	+	+	+	+	+	+
	Tannins	+	+	+	+	+	+
	Steroids	+	+	−	−	−	−
	Triterpenes	+	+	−	−	−	−
	Saponins	+	+	−	−	−	−
Stem barks	Alkaloids	+	+	+	+	+	+
	Phenols	+	+	+	+	+	+
	Flavonoids	+	+	+	+	+	+
	Anthocyanins	−	+	+	+	−	+
	Anthraquinones	+	+	+	+	+	+
	Tannins	+	+	+	+	+	+
	Steroids	−	+	+	+	+	−
	Triterpenes	−	+	+	+	+	−
	Saponins	−	+	+	+	+	−

-: Absence; +: presence.

Table 3. MICs values, MBCs and MBCs/MICs ratios of *B. rufescens* leaf and stem bark extracts on the four *Salmonella* isolates and *Salmonella* Typhi ATCC6539

Extracts	Concentrations in (µg/ml)	Leaves					Stem bark				
		STS	ST	STM	SPB	SPA	STS	ST	STM	SPB	SPA
95% Hydroethanolic extract	CMI	512	512	512	1024	512	1024	512	512	512	512
	CMB	1024	1024	512	−	1024	1024	1024	512	1024	1024
	CMB/CMI	2	2	1	−	2	1	2	1	2	2
75% Hydroethanolic extract	CMI	1024	1024	−	1024	−	512	512	256	512	256
	CMB	−	1024	−	−	−	512	1024	512	512	512
	CMB/CMI	−	1	−	−	−	1	2	2	1	2
50% Hydroethanolic extract	CMI	1024	1024	1024	1024	1024	512	512	1024	512	512
	CMB	1024	1024	−	−	−	−	−	−	512	1024
	CMB/CMI	1	1	−	−	−	−	−	−	1	2
infused	CMI	1024	1024	1024	1024	--	1024	512	512	512	256
	CMB	−	1024	−	−	−	−	−	1024	−	1024
	CMB/CMI	−	1	−	−	−	−	−	2	−	4
Decocted	CMI	−	−	−	−	−	1024	256	512	512	254
	CMB	−	−	−	−	−	−	1024	−	512	512
	CMB/CMI	−	−	−	−	−	−	4	−	1	2
Macerated	CMI	−	−	−	−	−	−	−	−	512	512
	CMB	−	−	−	−	−	−	−	−	1024	1024
	CMB/CMI	−	−	−	−	−	−	−	−	2	2
Ciprofloxacine	CMI	0,5	0,5	1	0,25	2	0,5	0,5	1	0,25	2
	CMB	2	2	4	1	4	2	2	4	1	4
	CMB/CMI	4	4	4	4	2	4	4	4	4	2

STs: Salmonella Typhi ATCC6539, ST: Salmonella Typhi, STM: Salmonella Typhimurium, SPB: Salmonella Paratyphi B and SPA: Salmonella Paratyphi A). MIC= Minimum Inhibitory Concentration. MBC= Minimum Bactericidal Concentration, the line (-) = No concentration.

Table 4. Percentage of DPPH$^{\bullet}$ free radical inhibition for hydroethanolic and aqueous extracts of *B. rufescens* leaves and stem bark

Extracts	Concentrations (µg / ml)	Percentage of inhibition ± Standard deviation						
		EtOH 95%	EtOH 75%	EtOH 50%	Decocted	Macerated	Infused	Vit C
Leaves	12.5	82.007±3.8249^{cd}	63.620±4.920^{b}	39.425±11.792^{a}	79.554±3.899^{c}	82.788±11.063^{cd}	71.084±12.698^{bc}	95.734±0.223^{d}
	25	89.713±0.508^{ef}	86.701±6.356^{de}	37.551±5.121^{a}	88.517±0.365^{de}	86.689±1.106^{de}	82.271±4.846^{de}	96.666±0.654^{f}
	50	91.612±0.215^{ef}	87.639±1.706^{cde}	85.940±2.811^{bcde}	84.358±6.080^{bcd}	84.222±1.146^{bcd}	82.386±3.963^{bc}	96.738±0.062^{f}
	100	93.010±1.516^{fgh}	91.505±1.777^{efgh}	76.332±1.196^{a}	90.099±1.473^{efg}	87.378±0.358^{cde}	86.058±0.172^{cd}	96.845±0.124^{i}
	200	94.516±0.284^{cd}	95.957±0.878^{cd}	88.810±1.330^{ab}	84.534±6.696^{a}	86.861±1.599^{a}	94.664±1.204^{cd}	97.276±0.328^{d}
Stem bark	12.5	82.776±3.801^{cd}	39.127±7.831^{b}	72.461±1.641^{bc}	72.544±4.737^{bc}	78.888±7.862^{c}	46.559±4.190^{a}	95.734±0.223^{d}
	25	86.291±1.522^{de}	67.756±8.504^{c}	79.403±7.612^{d}	81.505±3.182^{de}	86.881±0.654^{de}	56.236±0.558^{b}	96.666±0.654^{f}
	50	85.3544±2.661^{bcd}	80.091±3.184^{ab}	85.197±5.844^{bcd}	89.569±0.107^{de}	89.856±0.407^{de}	75.304±1.894^{a}	96.738±0.062^{f}
	100	90.158±2.324^{ef}	84.509±1.984^{bc}	88.755±3.791^{def}	92.222±0.345^{f}	94.767±0.620^{hi}	81.254±2.740^{b}	96.845±0.124^{i}
	200	93.555±1.129^{cd}	91.623±1.037^{bc}	93.631±2.257^{cd}	97.096±0.492^{d}	96.523±0.124^{d}	84.623±1.182^{a}	97.276±0.328^{d}

The numbers bearing the letters a, b, c…. are significantly different at the 5% level (p <0.05). The underlying values are of the form of means ± Standard deviation

Table 5. Antiradical activity of *B. rufescens* extracts expressed in term of IC_{50}

Extracts	IC_{50} in µg / ml	
	Leaves	Stem bark
EtOH 95%	9.780±0.125^{ab}	10.469±0.051^{b}
EtOH 75%	10.754±0.331^{b}	14.940±0.754^{c}
EtOH 50%	19.531±2.502^{e}	11.347±0.866^{b}
Decocted	11.285±0.482^{b}	10.414±0.049^{b}
Macerated	11.255±0.383^{b}	9.845±0.220^{ab}
Infused	11.575±1.175^{b}	17.701±0.798^{d}
Vitamin C	8.570±0.016^{a}	

The numbers bearing the letters a, b, c….. are significantly different at the 5% level (p <0.05). The underlying values are of the form of means ± Standard deviation

Table 6. Iron reducing power (FRAP) of hydroethanolic and aqueous extracts of leaves and stem bark of *B. rufescens*

Extracts	Concentration (µg/ml)	Optical density ± Standard deviation						
		EtOH 95%	EtOH 75%	EtOH 50%	Decocted	Macerated	Infused	VitC
Leaves	12.5	0.180±0.002 ^{abc}	0.142±0.019 ^{a}	0.197±0.009 ^{bc}	0.178±0.020 ^{ab}	0.132±0.041 ^{a}	0.176±0.001 ^{bc}	0.204±0.006^{bc}
	25	0.467±0.050^{e}	0.223±0.023 ^{a}	0.226±0.002 ^{a}	0.228±0.013 ^{a}	0.229±0.008 ^{a}	0.240±0.021 ^{a}	0.452±0.025 ^{de}
	50	0.686±0.028 ^{gh}	0.353±0.007 ^{cd}	0.299±0.013^{ab}	0.279±0.033 ^{a}	0.315±0.018 ^{abc}	0.410±0.002 ^{ef}	0.992±0.026 ^{i}
	100	0.763±0.016 ^{d}	0.472±0.014 ^{ab}	0.745±0.000 ^{c}	0.424±0.019 ^{a}	0.508±0.066 ^{ab}	0.444±0.004 ^{ab}	1.813±0.005^{e}
	200	0.938±0.002 ^{cd}	0.810±0.026 ^{b}	0.857±0.025 ^{bc}	0.802±0.052 ^{b}	0.781±0.009 ^{b}	0.663±0.016 ^{a}	1.829±0.003^{e}
Stem bark	12.5	0.230±0.007^{cde}	0.271±0.014 ^{de}	0.280±0.021 ^{e}	0.265±0.045 ^{de}	0.224±0.010 ^{bcd}	0.193±0.008 ^{bc}	0.204±0.006^{bc}
	25	0.286±0.003 ^{ab}	0.345±0.070 ^{bc}	0.453±0.018^{de}	0.392±0.009 ^{cd}	0.371±0.022 ^{c}	0.221±0.029 ^{a}	0.452±0.025^{bc}
	50	0.395±0.017 ^{de}	0.642±0.009 ^{fg}	0.784±0.014 ^{h}	0.446±0.003 ^{fg}	0.652±0.037 ^{g}	0.333±0.015 ^{bc}	0.992±0.026 ^{i}
	100	0.855±0.072 ^{cd}	0.762±0.126 ^{c}	0.920±0.067 ^{d}	0.859±0.043 ^{cd}	0.970±0.012 ^{d}	0.554±0.001 ^{b}	1.813±0.005 ^{e}
	200	1.004±0.022 ^{d}	0.939±0.013 ^{cd}	0.967±0.017 ^{d}	0.926±0.092^{b}	0.965±0.026 ^{d}	0.804±0.067^{b}	1.829±0.003 ^{e}

VitC = Vitamin C; The numbers bearing the letters a, b, c… are significantly different at the 5% level (p <0.05). The underlying values are of the form of means ± Standard deviation

Table 7. Total phenol and flavonoid content of hydroethanolic and aqueous extracts of *B. rufescens* leaves and stem bark

Extracts		Concentrations of total phenols (mgGAE/g) ± Standard deviation	Concentrations of total flavonoids (mgCE/g) ± Standard deviation
Leaves	EtOH 95%	2.895±0.081 ^{d}	2.335±0.265 ^{ab}
	EtOH 75%	1.215±0.098 ^{a}	2.505±0.605 ^{ab}
	EtOH 50%	2.081±0.488 ^{c}	2.590±0.469 ^{ab}
	Decocted	1.203±0.168b ^{a}	2.717±0.447 ^{ab}
	Macerated	1.377±0.005 ^{ab}	2.335±0.383 ^{ab}
	Infused	0.994±0.145 ^{a}	1.337±0.220 ^{a}
Stem bark	EtOH 95%	4.000±0.011 ^{f}	2.165±0.168 ^{a}
	EtOH 75%	3.500±0.058 ^{ef}	2.462±0.715 ^{ab}
	EtOH 50%	3.075±0.180 ^{de}	4.225±0.574 ^{b}
	Decocted	1.779±0.453 ^{bc}	1.231±0.447 ^{a}
	Macerated	0.877±0.122 ^{a}	2.760±0.669 ^{ab}
	Infused	1.232±0.533^{a}	2.144±0.715 ^{a}

The numbers bearing the letters a, b, c….. are significantly different the 5% level (p <0.05). The underlying values are of the form of means ± Standard deviation

4. DISCUSSION

The hydroethanolic extracts (95%, 75% and 50%) showed the highest yields compared to the aqueous extracts. In addition, the addition of water to ethanol results in alcohol dilution and reduces the yield instead. These results are in agreement with those of Mohammedi and Atik [19] who revealed that mixed solvents are very efficient to extract. In addition, the yield of the decoction was higher than that of the other aqueous extracts. The superiority of this yield over the maceration and infusion suggests that temperature would play a positive effect, improving extraction [20]. The results of antimicrobial tests (MIC and MBC) on the leaves and stem barks of *B. rufescens* show that they contain substances with antisalmonella activity. Indeed, many plant extracts and isolated compounds from the Fabaceae family have been presented as possessing antibacterial activity [21]. Hydroethanolic and aqueous extracts of *B. rufescens* leaves and stem bark exhibited significant to low Minimum Inhibitory Concentrations (MICs) (256 to 1024 µg/ml).

According to Kuete [22], the antibacterial activity of plant extracts is considered significant when MIC < 100 µg/ml, moderate when 100 µg/ml ≤ MIC ≤ 625 µg/ml and low when MIC > 625 µg/ml. The 95% hydroethanolic extract of the leaves exhibited the moderate activity on three isolates and strain of *Salmonella* (*Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Typhimurium and *Salmonella* Typhi ATCC6539 with concentrations of 512 µg/ml. The hydroethanolic extracts and the aqueous extract of the stem bark exhibited moderate activity on isolates (*Salmonella* Typhi, *Salmonella* Typhi B and *Salmonella* Paratyphi A) with concentrations ranging from 256 to 512 µg/ml. These results corroborate those of Muhammad and Sirat [21] who showed that the methanolic extract of this plant inhibited the growth of some pathogenic bacteria like *P*. *aeroginosa*. The differences in inhibitory activities observed with the same extract towards the different isolates and the *Salmonella* strain could be due to the difference in molecular structure between the tested pathogens [23, 5].

The null activity observed with the aqueous extracts (macerated and decocted) of the leaves could be related to the absence of anthocyanins and saponins in these extracts. The inhibition of *Salmonella* growth by the different extracts of *B. rufescens* may be due to the presence of phenolic compounds. This result corroborates the work of Sokoudjou et al. [24] who showed that phenolic compounds such as gallic acid and scopoletin were active (MIC ranging from 16 to 128 µg/ml) against ST, STM and *S*. Typhi 6539. Antibacterial substances can be classified as bactericidal, when the BMC/MIC ratio ≤ 4 or bacteriostatic, when the MBC/MIC ratio > 4 [25]. Based on these criteria, the majority of the hydroethanolic extracts were bactericidal. The solvent system and the extraction methods used influenced the different results obtained. Indeed, several works have already shown that the solvent system used for an extraction can strongly influence its secondary metabolites content [26] and that the method of extraction of plant extracts can influence their activities [27, 28].

The difference in the observed activity between the different extracts on the one hand and the isolates on the other hand may be due either to the constitutional or structural variability of the germs tested, or to the difference in solubility of the active substances present in each extract. It could also be due to the difference in the chemical composition of the genetic elements of transferable resistance between strains [25], or to the difference in the composition of secondary metabolites found in each extract.

It is likely that their antimicrobial activity is not attributable to a single mechanism, but their simultaneous action at different bacterial sites. This corroborates the work of Kobanski [29] who suggest that some bacteriostatic or bactericidal substances act by interfering with some essential structures of the bacteria such as the membrane wall, genome and proteins. The results of antimicrobial tests (MIC and MBC) of the leaves and stem bark of *B. rufescens* showed that this plant contains substances with antisalmonella activity that could be used in the treatment of typhoid and paratyphoid fevers.

Antioxidant compounds have diverse mechanisms of action and a single method would not be sufficient to assess the total capacity of an antioxidant [30, 5]. Therefore, it is best to use several methods for determining the antioxidant activity of a substance (in this case the DPPH and FRAP methods) for the same sample. The results of this study showed that the 95% hydroethanolic extract of leaves and the macerated stem bark exhibited higher antiradical activity than all extracts at the concentration of 100 µg/ml. The antioxidant activity of the extracts

could be explained by their richness in polyphenolic substances, tannins and more particularly in total flavonoids. These results are in agreement with those of several authors, who reported a positive correlation between all phenolic content and antioxidant activity [31, 5]. Hydroethanolic and aqueous extracts of *B. rufescens* leaves and stem barks showed IC_{50} values between 9.780 and 19.531 µg/ml. These results corroborate those of Promprom and Chatan [32] who showed that the ethanolic leaf extract of *Bauhinia nakhonphanomensis* presented an IC_{50} value of 17.07 ± 0.24 µg/ml. According to Souri et al*.* [33] the antioxidant potential of a plant is divided into three groups: high when IC_{50}< 20 µg/ml, moderate when 20 µg/ml ≤ IC_{50} ≤ 75 µg/ml and low when IC_{50}> 75 µg/ml. The IC_{50} values showed that the hydroethanolic and aqueous extracts of the leaves and stem barks of *B. rufescens* exhibited high antiradical activities because all these extracts have IC_{50}< 20 µg/ml. The high antiradical activity of the different extracts of the leaves and stem barks of *B. rufescens* could be explained by the high presence of polyphenolic compounds (total phenols, anthraquinones, and flavonoids). The antioxidant activity of other secondary metabolites is directly related to their hydroxyl group.

The iron reduction test is one of the methods for assessing antioxidant activity. The 95% hydroethanolic extract of the leaves showed the highest reducing power ($p<0.05$) compared to all extracts followed by the 50% hydroethanolic extract of the stem bark from the concentration 50 µg/ml. The antioxidant potential of *B. rufescens* leaf and bark extracts could be related to the presence of total phenol and flavonoids which were detected during the quantitative assay. Indeed, total phenols and flavonoids are powerful antioxidants [32]. These results suggest the reducing power of *B. rufescens* is likely due to the presence of hydroxyl group in phenolic compounds that can serve as protron donors. Therefore, antioxidants are considered to be reductants and inactivators of oxidants [34].

Phenolic compounds are important plant constituents, as they act against lipid peroxidation and inactivate free radicals [17]. The concentration of phenolic compounds is very high in the 95% hydroethanolic extract of leaves and stem bark to compare other extracts. In addition, flavonoids are powerful antioxidants, which possess redox properties that allow them to eliminate the effects of reactive oxygen species [35] as well as to chelate various transition metals [17]. These results corroborate those of the iron reduction test, where the 95% hydroethanolic extract of leaves and stem barks exhibited the greatest antioxidant activity. There was a positive correlation between the antioxidant activity and the phenol content in the extracts. Many authors have also shown this correlation [36, 37, 38]. These results suggest that the extracts of *B. rufescens* have an antioxidant activity, due to the phenolic compounds present in these extracts.

5. CONCLUSION

The results showed that the 95% hydroethanolic extract of the leaves of *B. rufescens* exhibited both antisalmonellal and antioxidant activity *in vitro*, due to the presence of free radical scavenging phytochemicals that could have the ability to inhibit a free radical and therefore could reduce oxidative stress. Additional studies will be conducted to determine *in vivo* antisalmonella and antioxidant activities, side effects and define the therapeutic dose that will allow safe use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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