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In vitro Antisalmonellal and Antioxidant Activities of the Crude Extracts and Fractions from the Stem Bark of *Albizia gummifera* (J. F. Gmel.) C. A. Sm.

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Authors' contributions

This work was carried out in collaboration between all authors. Author SSA was the field investigator and drafted the manuscript. Author NK contributed to the antioxidant studies. Author SPCF contributed to the preparation of plant extract and phytochemical studies. Authors AN and JRK contributed to the manuscript writing and revision. Author DG designed the study and supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Typhoid fever is an infectious disease, which continues to be a serious health problem and remains a major cause of morbidity and mortality in the developing country. Considering the ethnopharmacological relevance of *Albizia gummifera* (Leguminosae/Fabaceae), this study was designed to investigate the *in vitro* antisalmonellal and antioxidant activities of various extracts and fractions of this plant against five *Salmonella* species.
Study Design: Extraction, fractionation, antibacterial and antioxidant evaluation, phytochemical

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screening.
Place and Duration of the Study: Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon, between October 2013 and November 2014.
Methods: Antimicrobial activity was evaluated by the broth microdilution method, whereas the evaluation of antioxidant properties, quantitative determination of total phenols and flavonoids, phytochemical screening were performed by other standard methods.
Results: The data obtained showed that minimal inhibitory concentration (MIC) values ranged from 64 to 512 µg/ml. Crude extract, ethyl acetate and hexane fractions showed significant antibacterial activity (MIC = 64 µg/ml). The extracts and fractions exhibited moderate antioxidant properties (IC_{50} values obtained ranged from 48.80 to 61.90 µg/mL for the aqueous extracts, and from 28.10 to 34.10 µg/mL for the $MeOH/CH_2Cl_2$ extract and fractions). Ethyl acetate fraction at 200 µg/ml showed the highest hydroxyl radical scavenging and ferric reducing power activity. Phytochemical screening showed the presence of alkaloids, flavonoids, saponins and phenols in all the extracts and fractions.
Conclusion: The results suggest that stem extract of *A. gummifera* contains antisalmonellal and antioxidant substances, which could be developed for the treatment of typhoid fever and other salmonellosis.

Keywords: Albizia gummifera; antisalmonellal activity; antioxidant activity.

1. INTRODUCTION

*Salmonella typhi, Salmonella paratyph*i A and *Salmonella paratyphi* B are the Salmonellae of medical importance which cause typhoid fever, paratyphoid A and B fevers respectively [1-3]. Typhoid fever continues to be a serious health problem and remains a major cause of morbidity and mortality in the developing country [3]. Estimate for the year 2008 suggested that there are approximately 33 million infections and 500,000 deaths from typhoid fever [4]. Several multi-drug resistance (MDR) such as chloramphenicol, amoxicillin and fluoroquinolones (e.g. ciprofloxacin) increase throughout the world including the developing countries like Cameroon [5,6]. Consequently, efforts have to be made to evaluate and develop new agents to be used as anti-typhoid therapy.

Plants are possible source of antimicrobial agents [3] and rich source of free radical scavenging molecules, such as vitamins (E, C, A), terpenoids, phenolic acids, lignins, stilbenes, tanni ns, flavonoids, quinones, coumarins, and other metabolites with antioxidant activity [7]. The genus *Albizia* comprises approximately 150 species, mostly wide-spread in tropical and subtropical regions of Asia and Africa. Phytochemical investigation of different species belonging to genus *Albizia* afforded different classes of secondary metabolites such as saponins, terpenes, alkaloids and flavonoids [8]. Some bioactive compounds (triterpenoid saponins, novel macrocyclic alkaloids and flavonol glycosides) isolated and identified from this genus showed different biological activities such as antitumor, antiplatelets aggregation and bactericidal activities [8]. *Albizia gummifera* is a species that is used in folk medicine for the treatment of various ailments such as bacterial infections, skin diseases, malaria and stomach pains [8,9]. Previous studies have reported the presence of spermine alkaloids, oleanane saponins and triterpenes in the stem bark of this plant and shown their relative biological activities against *Neisseria gonorrhoeae*, trypanosome and *Plasmodium* [10-12]. To the best of our knowledge, no scientific investigation has so far been reported in literature regarding its action against typhoid fever and oxidative stress. Therefore the present investigation has been designed to study the *in vitro* antisalmonellal and antioxidant effect of various extracts and fractions of *A. gummifera* stem bark.

2. MATERIALS AND METHODS

2.1 Plant Material

The stem bark of *A. gummifera* was collected in Foto, Menoua Division, West Region of Cameroon, in March 2013. Identification of the plant was done at the National Herbarium, in Yaounde-Cameroon, using a voucher specimen registered under the reference HNC N°20859/SRF-Cam by Ngansop Eric.

2.2 Preparation of Extracts and Fractions

A. gummifera stem bark were air-dried at room temperature (23±2°C) away from sunlight and milled to coarse particles.

Aqueous extracts (infusion, maceration and decoction) were prepared according to the methods described by Duke [13]. The maceration (macerated extract) was prepared by dissolving 100 g of plant powder into 1000 mL of water. Extraction was allowed to process for 48 h with constant stirring (three times per day). The decoction (decocted extract) was prepared by dissolving 100 g of plant powder in 1000 mL of water and then boiling at 100°C for 15 min. The infusion (Infused extract) was prepared by boiling water and then immediately putting the plant powder in this boiled water for 15 min. These mixtures were then filtered using Whatman paper N°1 and the filtrates were concentrated by evaporating the solvent at 40°C in an oven for 48 hours.

For the organic extract, 100 g of the powdered stem bark were macerated three times at room temperature in 1000 ml of a mixture of methylene chloride/methanol (CH_2Cl_2/MeOH) (1:1) for 48 hours, and then filtered with Whatman paper N°1. The filtrate was concentrated at 45°C using a rotary evaporator (Büchi R200) and the obtained volume was later dried at 40°C. The plant extracts were stored in sterilized bottles at room temperature until usage.

The *A. gummifera* CH_2Cl_2/MeOH (1:1) stem bark extract (CrE) was fractionated by solvent–solvent partitioning to obtain three fractions containing compounds soluble in methanol/water (*ReF*), ethyl acetate (AeF) and n-hexane (HeF). The recoveries of ReF, AeF, and HeF were about 48.08%, 20.17% and 31.15% respectively.

2.3 In vitro Antimicrobial Tests

2.3.1 Test bacteria and culture media

The test microorganisms including four isolates: *Salmonella typhi* (ST), *Salmonella paratyphi* A (SPA), *Salmonella paratyphi* B (SPB), *Salmonella typhimurium* (STM), 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 in this study were Salmonella-Shigella Agar (Italy Liofilchem) for activation and maintenance of *Salmonella* strain/isolates, and Mueller Hinton Broth (MHB) for the determination of the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs).

2.3.2 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. For this purpose, 18 hours old bacterial cultures were prepared in Salmonella-Shigella Agar (SSA). A few colonies of bacteria were collected aseptically with a sterile wire loop and introduced into 10 mL of sterile 0.9% saline water. These suspensions were diluted 100 times with MHB to yield about 1.5×10^6 CFU/mL before use.

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

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-micro well sterile plates as described by Newton et al. [14]. For this, the crude extracts and fractions were dissolved in 2.5% 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. Hundred microliters (100 µL) of each of 1.5×10^6 CFU/mL bacterial suspensions were added to respective wells containing the test samples (except the extract and media sterility control wells) and mixed thoroughly to give the final concentrations ranging from 8 to 1024 µg/mL (for extracts and fractions). Oxytetracycline and ciprofloxacin were used as standards antibiotics at concentrations ranging from 1 to 128 µg/mL and 0.5 to 68 µg/mL respectively. These preparations were further incubated at 37°C during 18 hours. The inhibitory concentration of the extracts and fractions was detected after addition of 40 µL of 0.2 mg/mL of p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, South Africa) and incubation at 37°C for 30 min. Viable bacteria change the yellow dye (INT) to pink colour. The lowest concentrations at which there were no visible colour changes were considered as MIC. The MBC values 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 MBCs were revealed by the addition of INT as above. All extract concentrations at which no colour changes were considered as bactericidal concentrations, and the smallest of these concentrations was considered as the MBC. These tests were

carried out in triplicates at three different occasions.

2.4 Phytochemical Screening

The phytochemical screening was performed using standard methods described by Harbone [15]. The extracts and fractions of *A. gummifera* stem bark were screened for the following classes of phytochemical compounds: Alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes.

2.5 Antioxidant Assay

2.5.1 DPPH radical scavenging assay

The antioxidants can react with DPPH, a violet coloured stable free radical, converting it into a yellow coloured α,α-diphenil-β-picrylhydrazine. The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of the antioxidant.

The free radical scavenging activities of the crude extracts and fractions of *A. gummifera* were evaluated using the DPPH assay method as described by Mensor et al. [16]. Briefly, the extract/fraction (2000 µg/mL) was two-fold serially diluted with methanol. Hundred microliters (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 density was measured at 517 nm using spectrophotometer "Jenway, model 1605". Ascorbic acid (Vitamin C) was used as control. Each assay was done in triplicate and the results, recorded as the mean ± SD of the three findings, were illustrated in tabular form. The radical scavenging activity (RSA, in %) was calculated as follows:

$$RSA\ (\%) = [(A_{DPPH} - A_{sample}) / A_{DPPH}] \times 100;$$

where A = Absorbance.

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

2.5.2 Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [17]. Reagent mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µl of 1 mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.5 mL of extract at various concentrations. H_2O_2 was added to the mixture to start the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture was measured at 560 nm with spectrophotometer "Jenway, model 1605". Butylated hydroxytoluene (BHT) was used as standard antioxidant and the hydroxyl radicals scavenging (HRS) activity was calculated.

$$HRS\ (\%) = [(A_{control} - A_{sample}) / A_{control}] \times 100;$$

where *A* = *Absorbance*

2.5.3 Ferric Reducing/Antioxidant Power (FRAP) assay

The ferric reducing power was determined by the Fe^{3+} to 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. Briefly, the extract/fraction (2090 µg/mL) was two-fold serially diluted with methanol. Four hundred microliters (400 µL) of the diluted extract/fraction were mixed with 500 µL of phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide and incubated at 50℃ for 20 min. Then 0.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant (0.5 mL) was diluted with 0.5 mL of water and mixed with 0.1 mL of freshly prepared 0.1% ferric chloride, to give a final extract/fraction concentration range of 12.5 - 200 µg/mL (12.5, 25, 50, 100 and 200 µg/mL). 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 the positive control. Increased absorbance of the reaction mixture indicated higher reduction capacity of the sample (extracts/fractions) [11].

2.5.4 The quantitative determination of total phenolic compounds

The total phenolic compounds were determined by the Folin-Ciocateu Reagent method. The reaction mixture consisted of 0.02 ml of extract or fraction (2000 µg/ml), 1.38 mL of distilled water,

0.02 mL of 2N Folin ciocalteu reagent and 0.4 mL of a 20% sodium carbonate solution. 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 and the results were expressed as milligrams of Equivalents Gallic Acid (mgEGA) per gram of extract/fraction.

2.5.5 Determination of total flavonoids content

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

2.6 Statistical Analysis

The experimental data were expressed as mean value ± standard deviation (SD) of three replications. The comparison was 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 16.0.

3. RESULTS

3.1 Antimicrobial Activity

The *A. gummifera* extracts and fractions showed antibacterial activity against all the four clinical bacterial isolates and one strain tested. MIC values obtained ranged from 64 - 512 µg/ml (Table 1). For ciprofloxacin and oxytetracycline, these values were about 100 times lower than those of the extracts and fractions. In general, *Salmonella typhi* and *Salmonella typhimurium* were found to be more sensitive than the other bacteria. CrE, AeF and HeF presented the same MIC (64 µg/ml). Except the activity of EaF and HeF on *Salmonella paratyhi B* and *Salmonella paratyphi A* respectively, other activities of extracts and fractions on all microorganisms showed MIC values less than 256 µg/ml (Table 1).

3.2 Qualitative Phytochemical Composition

Table 2 presents the phytochemical composition of *A. gummifera* stem bark. The phytochemical screening of the extracts and fractions revealed the presence of different groups of secondary metabolites, including alkaloids, tannins, flavonoids, saponins, triterpenes, phenols and anthocyanins. Tannins were absent in the hexane and residual fractions, whereas anthocyanins were absent in the hexane, ethyl acetate and residual fractions. Anthraquinones were absent in the extracts and fractions while triterpenes were only absent in different aqueous extracts and residual fraction.

3.3 Antioxidant Activity

3.3.1 DPPH free-radical scavenging activity

The ability of various stem bark extracts and fractions of *A. gummifera* to quench reactive species by hydrogen donation was measured through the DPPH radical scavenging activity test. All the extracts exhibit significant DPPH radical-scavenging activity (Table 3). The DPPH scavenging activity of the various samples and fractions increased in a dose dependent manner, as presented in Table 3. However, the scavenging activity of ascorbic acid, a well-known antioxidant, was relatively more pronounced than that of the samples. The IC_{50} (concentration of the extract required to scavenge 50% of DPPH radical) value express the antioxidant activity. By comparing the IC_{50} value of the various extracts and those of active fractions with that of the standard antioxidant, ascorbic acid (AsA) (Table 3), it was found that the antioxidant activity of AeF (IC_{50}: 28.10 µg/mL) was lower than that of AsA (IC_{50}: 19.30 µg/mL), but not significantly different from that of CrF (IC_{50}: 30.40 µg/mL), ReF (IC_{50}: 33.50 µg/mL) and HeF (IC_{50}: 34.10 µg/mL). By comparing various extracts and active fractions, the free radical-scavenging activities followed the order: AeF > CrF > ReF > HeF > DE > ME > IE. The free radical-scavenging activity of the aqueous extracts was lower than that of the organic solvent extract and fractions.

Table 1. MICs, MBCs, MBCs/MICs of different extracts and fractions of *A. gummifera* stem bark on isolates and strain of *Salmonella*

Substances tests			Strain/isolates				
			ST	**STS**	**STM**	**SPA**	**SPB**
		MIC (µg/mL)	**128**	256	256	**128**	**128**
	DE	MBC (µg/mL)	512	512	1024	1024	512
		MBC/MIC	4	2	4	8	4
		MIC (µg/mL)	256	256	**128**	256	256
	IE	MBC (µg/mL)	1024	512	512	512	512
Extracts		MBC/MIC	4	2	4	2	2
		MIC (µg/mL)	**128**	256	**128**	**128**	256
	ME	MBC (µg/mL)	512	1024	1024	512	1024
		MBC/MIC	4	4	8	4	4
		MIC (µg/mL)	**128**	**128**	**64**	**128**	**128**
	CrE	MBC (µg/mL)	1024	256	256	512	512
		MBC/MIC	8	2	4	4	4
		MIC (µg/mL)	256	**128**	256	512	512
	ReF	MBC (µg/mL)	1024	512	1024	1024	1024
		MBC/MIC	4	4	4	2	2
		MIC (µg/mL)	256	256	**64**	256	512
Fractions	**EaF**	MBC (µg/mL)	>1024	512	512	1024	>1024
		MBC/MIC	-	2	8	4	-
		MIC (µg/mL)	**64**	**128**	512	**128**	256
	HeF	MBC (µg/mL)	512	512	1024	512	1024
		MBC/MIC	8	4	2	4	4
		MIC (µg/mL)	16	8	16	8	16
	Oxy	MBC (µg/mL)	>128	64	128	32	32
		MBC/MIC	-	8	8	4	2
Antibiotics		MIC (µg/mL)	0.5	0.5	1	2	4
	Cip	MBC (µg/mL)	2	1	4	4	8
		MBC/MIC	4	2	4	2	2

CrE= Methylene chloride/methanol extract, EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract, Oxy= Oxytetracyclin Cip = ciprofloxacin. ST=Salmonella typhi, STS=Salmonella typhi ATCC1369, STM=Salmonella typhimurium, SPA=Salmonella paratyphi A, SPB=Salmonella paratyphi B.

Table 2. Qualitative phytochemical composition of the extracts and fractions of stem bark of *A. gummifera*

Phytochemical compounds	Extracts and fractions						
	CrE	**EaF**	**HeF**	**ReF**	**IE**	**DE**	**ME**
Alkaloids	+	+	+	+	+	+	+
Anthocyanins	+	–	–	–	+	+	–
Anthraquinons	–	–	–	–	–	–	–
Tannins	+	+	–	–	+	+	+
Triterpenes	+	+	+	–	–	–	–
Saponins	+	+	+	+	+	+	+
Steroids	-	–	–	–	–	–	–
Phenols	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+

CrE= Methylene chloride/methanol extract, EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract; + = Presence; - = absence.

3.3.2 Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was determined using Fenton reaction [18] and the results obtained are presented in Table 4. From the study results, the infused extract had the highest, non-significant, activity among all aqueous extracts, while the ethyl acetate fraction

showed the best activity among all extracts and fractions. The residual fraction showed the lowest hydroxyl radical-scavenging activity, while the standard (BHT) exhibited the highest hydroxyl radical-scavenging activity at the concentration of 200 µg/ml. The ranking of the activities of various standards, extracts and fractions at this concentration was as follows: BHT > AeF > HeF > CrE > DE ≈ ME ≈ IE > AsA > ReF. All the remaining extract/fraction exhibited varied activities from one extract/fraction to another at each concentration.

3.3.3 Reducing power activity

The reducing power was determined by the Fe^{3+} to Fe^{2+} transformation in the presence of the extracts and fractions, and the results obtained are presented in Table 5. The residual fraction showed the lowest reducing power, while the standard (Vitamin C) exhibited the highest reducing power at the concentration of 200 µg/ml. The ranking of the activities of various extracts and fractions at this concentration was as follows: AsA > AeF > DE > HeF > CrE > ME > IE > ReF. All the remaining extract/fraction exhibited varied activities from one extract/fraction to another at each concentration.

3.3.4 Total phenolics and flavonoids content

It has been recognized that the total phenolic content of plant extracts is associated with their antioxidant activities due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. As presented in Table 6, the total phenolic content (TPC) ranged from 100.05 mgEGA/g to 126.01 mgEGA/g of dry extract. The lowest TPC was found in ReF (100.05 mgEGA/g of dry extract). The same results show that the flavonoids content (FC) ranged from

Table 3. DPPH radical scavenging activity (%) and IC_{50} values (µg/ml) of various extracts and fractions of stem bark of *A. gummifera* at different concentrations

Extracts	Extracts/fractions concentration (µg /ml)					
	12,5	25	50	100	200	IC_{50}
DE	29.53±0.96^{b}	37.29±0.49^{b}	40.12±1.08^{b}	44.92±1.14^{b}	61.94±3.39^{b}	48.80
IE	12.12±1.92^{a}	22.20±1.36^{a}	23.91±0.87^{a}	37.82±1.22^{a}	38.91±1.38^{a}	61.90
ME	30.64±2.89^{b}	44.82±0.98^{c}	47.47±3.09^{c}	56.96±2.64^{c}	77.05±0.65^{c}	49.40
CrE	56.20±1.18^{d}	57.55±2.74^{e}	64.73±1.08^{e}	68.25±0.91^{d}	86.45±2.35^{f}	30.40
ReF	46.56±0.69^{c}	49.45±1.57^{d}	63.32±7.63^{e}	75.30±1.67^{f}	78.50±0.49d^{e}	33.50
AeF	54.79±0.12^{d}	59.23±4.11^{e}	75.07±.0.31^{f}	82.14±2.78^{g}	88.52±3.51^{f}	28.10
HeF	44.44±1.05^{c}	46.55±1.49^{cd}	54.02±0.59^{d}	72.17±0.84^{e}	81.72±1.98^{e}	34.10
AsA	79.51±1.08^{e}	86.01±0.88^{f}	87.26±0.75^{g}	89.91±1.03^{h}	93.46±0.37^{g}	19.30

Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05). CrE= Methylene chloride/methanol extract, EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract, AsA = Ascorbic Acid

Table 4. Hydroxyl radical scavenging activity (%) of various extracts and fractions of stem bark of *A. gummifera* at different concentrations

Extracts	Extracts/fractions concentration (µg /ml)				
	12,5	25	50	100	200
DE	1.06±0.05^{b}	1.85±0.11^{b}	3.88±0.09^{ab}	5.74±0.05^{bc}	9.18±0.15^{c}
IE	1.76±0.05^{c}	2.62±0.05^{c}	3.02±0.05^{a}	6.33±0.32^{c}	9.65±0.17^{c}
ME	1.16±0.05^{b}	2.05±0.32^{b}	3.61±0.25^{ab}	5.30±0.60^{b}	9.05±0.69^{c}
CrE	2.78±0.09^{d}	3.11±0.05^{d}	3.94±0.05^{ab}	6.26±0.09^{c}	11.01±0.25^{d}
ReF	1.32±0.11^{bc}	1.82±0.15^{b}	2.22±0.06^{a}	2.65±0.05^{a}	3.84±0.05^{a}
AeF	6.80±0.30^{e}	7.66±0.17^{e}	23.38±5.61^{c}	40.03±0.20^{e}	62.92±0.64^{f}
HeF	1.19±0.09^{b}	2.69±0.09^{cd}	6.86±0.09^{b}	13.50±0.32^{d}	25.30±0.25^{e}
AsA	0.43±0.05^{a}	0.63±0.05^{a}	1.19±0.09^{a}	2.09±0.17^{a}	7.53±0.28^{b}
BHT	8.09±0.54^{f}	20.10±0.52^{f}	43.55±0.60^{d}	81.56±0.73^{f}	94.53±0.99^{g}

Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05). CrE= Methylene chloride/methanol extract. EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract, AsA = Ascorbic Acid. BHT=Butylated hydroxytoluene.

Table 5. Reducing power activity of various extracts and fractions of stem bark of *A. gummifera* at different concentrations

Extracts	Extracts/fractions concentration (µg /ml)				
	12,5	25	50	100	200
DE	0.068±0.004^{f}	0.083±0.003^{e}	0.119±0.002^{d}	0.150±0.001^{c}	0.256±0.001^{f}
IE	0.017±0.001^{b}	0.037±0.001^{b}	0.055±0.001^{b}	0.103±0.003^{b}	0.150±0.000^{b}
ME	0.022±0.001^{c}	0.053±0.001^{c}	0.094±0.001^{c}	0.150±0.001^{c}	0.167±0.001^{c}
CrE	0.051±0.001^{d}	0.085±0.001^{e}	0.124±0.003^{e}	0.167±0.001^{d}	0.214±0.001^{d}
ReF	0.011±0.001^{a}	0.014±0.000^{a}	0.033±0.004^{a}	0.058±0.002^{a}	0.116±0.020^{a}
AeF	0.077±0.003^{g}	0.126±0.004^{f}	0.180±0.000^{f}	0.224±0.001^{f}	0.267±0.002^{g}
HeF	0.049±0.002^{d}	0.079±0.000^{d}	0.095±0.002^{c}	0.173±0.001^{e}	0.242±0.001^{e}
AsA	0.064±0.001^{e}	0.131±0.001^{g}	0.195±0.001^{g}	0.247±0.002^{g}	0.298±0.002^{h}

Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05). CrE= Methylene chloride/methanol; extract, EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract, AsA = Ascorbic Acid.

5.31 mgECat/g to 13.52 mgECat/g of dry extract. The lowest FC was found in ReF (5.31 mgECat/g of dry extract) and the highest in HeF (13.52 mgECat/g of dry extract). The ranking of the amount of phenolics in plant samples was as follows: HeF > AeF > IE > CrE > ME > DE > ReF. These results demonstrate that flavonoids do not represent the main group of phenolic compounds in *A. gummifera* extracts and fractions.

4. DISCUSSION

4.1 Antisalmonellal Activity

The extracts and fractions from *A. gummifera* showed antibacterial activity on the five tested bacteria. According to Kuete [19], the activity of plant extracts are classified as significant (MIC < 100 µg/ml), moderate (100 < MIC ≤ 625 µg/ml) or weak (MIC > 625 µg/ml). Base on this scale, it can be conclude that these extracts and fractions have significant or moderate activity on the studied bacteria depending on the extract or fractions. This activity could be related to the phytochemical composition of these extracts/fractions [15]. Indeed, they were found to contain secondary metabolites such as alkaloids, phenols, flavonoids, saponins, tannins, triterpenes and anthocyanins which constitute a source of bioactive substances and are known for their antibacterial properties [20,21,22]. Similar results were noted by the previous studies on *A. gummifera* stem bark which reported biological activities against *Neisseria gonorrhoeae*, trypanosome and plasmodium [10-12]. Some triterpenes have presented a protective function by participating in the fight against microbial attacks [23]. *Salmonella typhi* and *Salmonella typhimurium* were more sensitive to tested extracts/fractions of *A. gummifera* stem bark than others. The differences in susceptibility may be explained either by the constitutional or structural variability of the tested germs or by the differences in genetic content of plasmids that can be easily transferred among microbial strains [24,25]. According to Marmonier [26] a plant extract exerts two types of activities: a bacteriostatic activity (MBC/MIC > 4) and a bactericidal activity (MBC/MIC ≤ 4). At the tested concentrations in this work, the MBC/MIC ratios were generally less than or equal to 4 for most extracts and fractions, indicating the bactericidal nature of the tested samples on the salmonellae species.

4.2 Antioxidant Evaluation

The antioxidant profile of various extracts/fractions of *A. gummifera* stem bark as a prelude to finding agent(s) that could be used to reduce oxidative stress associated with typhoid and paratyphoid fevers were determined. Evaluation of antioxidant properties through different methods express the fact that actives constituents act generally according to two mechanisms: scavenging free radical and reducing prooxidant [27-29].

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) reacts with an antioxidant compound, which can donate hydrogen to stabilize it. The DPPH scavenging activity of the various extracts and fractions of *A. gummifera* stem bark was increased in a dose-dependent manner. It was suggested that the extracts and fractions have high proton-donating ability. However, different extracts and fractions of *A. gummifera* demonstrated a remarkable anti-radical activity with 20 < IC_{50} ≤ 75 µg/ml. In fact, according to Souris et al. [30],

Table 6. Total phenol and flavonoids content of extracts and fractions of *A. gummifera* stem bark

Extracts/ Fractions	Total phenol content (mgEGA/g of extract)	Flavonoids content (mgECat/g of extract)
DE	107.55±0.50[b]	6.71±0.02[b]
IE	111.30±4.76[b]	6.63±0.03[b]
ME	109.66±3.49[b]	5.60±0.02[a]
CrE	110.72±4.57[b]	8.92±0.01[c]
ReF	100.05±1.80[a]	5.31±0.02[a]
AeF	125.72±3.49[c]	8.60±0.01[c]
HeF	126.01±3.99[c]	13.52±0.05[d]

Along each column, values with the same superscripts are not significantly different, Waller Duncan (P > 0.05). CrE= Methylene chloride/methanol extract, EaF=Ethyl Acetate fraction, HeF =Hexane fraction, ReF=Residual fraction, IE=Infused extract, DE=Decocted extract, ME=Macerated extract.

the antioxidant activity of plant extracts are classified as significant (IC_{50} < 20 µg/ml), moderate (20 < IC_{50} ≤ 75 µg/ml) or weak (IC_{50} > 75 µg/ml). Base on this scale, it can be concluded that the extracts and fractions from *A. gummifera* have moderate antioxidant activity. According to Sujatha et al. [8], there are many reports on the antioxidant property of *Albizia* species such as *A. labbeck*, *A. amara* and *A. procera* which possess free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and reducing power activities. It is known that antioxidants could be reducing agents [28] with their compounds capable of donating a single electron or hydrogen atom for reduction.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [31]. The antioxidant activities of putative antioxidants have been attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction, and radical scavenging [32]. The reducing power of extracts and fractions from *A. gummifera* increased with increasing concentration of the extract/fraction. This result indicates that some compounds in extracts and fractions from *A. gummifera* are electron or hydrogen donors and can reduce the oxidized intermediates of lipid peroxidation processes by reacting with free radicals in other to convert them into more stable products and to terminate radical chain reactions.

Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and important biological damage reacting with polypeptides, saccharides, nucleotides, and organic acids [33,34]. In the present study, the ethyl acetate fraction exhibited a strong scavenging capacity towards the hydroxyl radicals generated by the Fenton reaction in the test concentration range and the scavenging effects increased with increasing concentration of the extract. Similar result was obtained with the methanolic extract from *Inula graveolens* [27].

Various phytochemical components, especially polyphenols (such as flavonoids, phenyl propanoids, phenolic acids, tannins, etc…) are known to be responsible for the free radical scavenging and antioxidant activities of plants [35]. They possess many biological effects which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals [35]. *A. gummifera* stem bark extract and fractions have both phenolic and flavonoid compounds. It has been recognized that flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants [36]. Mechanisms of action of flavonoids are through scavenging or chelating processes [37,38]. They act as quenchers of singlet oxygen or as proton donors to scavenge various oxidizing species, i.e. superoxide anion ($O_2^{\cdot -}$), hydroxyl radical, or peroxy radicals [16,27,39].

5. CONCLUSION

These results provide promising baseline information for the potential use of extracts of this plant, as well as some of the fractions, in the treatment of typhoid, paratyphoid fevers and oxidative stress. CrE, EaF and HeF by their high antibacterial activity could be potential candidates for the development of new antityphoid agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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