Trop J Nat Prod Res, July 2021; 5(7):1185-1191 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In vivo Antioxidant Activity of Hydroethanol Extracts of *Terminalia avicennioides* (Combretaceae) in *Salmonella typhi*-Infected Wistar Rat's Model

Louis-Claire N. Famen[1], Benjamin T. Talom[2], Richard S. Tagne[2], Siméon P. C. Fodouop[2], Gabriel T. Kamsu[1], Norbert Kodjio[1], Alain B. Fowa[1], Donatien Gatsing[1]*

[1]*Research Unit of Microbiology and Antimicrobial Substances, Department of Biochemistry Faculty of Science, University of Dschang, P.O. Box 67 Dschang, Cameroon*
[2]*Department of Biomedicals Sciences, Faculty of Science, The University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon*

ARTICLE INFO

Article history:
Received 20 November 2020
Revised 18 March 2021
Accepted 17 July 2021
Published online 02 August 2021

ABSTRACT

Infectious diseases such as typhoid fever leads to the formation of free radicals which can have a damaging effect on the body. This study evaluated the *in vivo* antisalmonella activity of the hydroethanol extract of *Terminalia avicennioides* stem bark in rats infected with *Salmonella typh*i (ATCC 6539), and its antioxidant activity. The *S. typhi*-infected rats concurrently received daily doses of *T. avicennioides* extract (5, 23 and 46 mg/kg) or ciprofloxacin (14 mg/kg) as positive control, for 15 days. At the end of the treatment period, the animals were sacrificed and blood, liver, kidney, heart, lung and spleen were collected for evaluation of biochemical parameters, which included assays of superoxide dismutase, catalase, peroxidase, malondialdehyde and nitric oxide, as well as biological responses. The hydroethanol extract of *T. avicennioides* bark (5, 23 and 46 mg/kg) cured the infected rats between the 8th and 11th day of treatment. The extracts also significantly reduced ($p < 0.05$) blood malondialdehyde and nitric oxide levels, and significantly increased ($p<0.05$) the activity of superoxide dismutase, catalase and peroxidase in the infected rats. Phytochemical analysis revealed the presence of flavonoids, alkaloids, phenols, saponins, anthocyanins and anthraquinones in the hydroethanol bark extract of *T. avicennioides*. The results show that the hydroethanol bark extract of *T. avicennioides* possesses antisalmonella activity and reduces the state of oxidative stress caused by *S. typhi* during rat's infection.

Keywords: *Terminalia avicennioides*, Phytochemical, Oxidative stress, *Salmonella typhi*.

Introduction

Typhoid and paratyphoid fevers are systemic bacterial infections with a digestive origin, caused by bacteria of the *Salmonella* genus, respectively the serotypes Typhi and Paratyphi (A and B).[1] The World Health Organization (WHO) estimates 22 million cases of typhoid and 216,500 deaths related to typhoid fever occurring each year worldwide.[2] Although typhoid fever is primarily considered as an endemic disease, its epidemics frequently occur as a result of failures in water supply, and it remains a major public health problem in developing countries with an incidence of 77.4 cases/100,000 inhabitants in Asia, Africa and Latin America.[3] In Cameroon typhoid fever is a public health problem and the second disease after malaria commonly reported by health personnel.[4] Typhoid fever is an acute, fatal and febrile disease. Without treatment, its lethality rate is 10 to 30%, falling to 1 - 4% with appropriate therapy. Typhoid fever infection is characterized by the production of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and nitric oxide (NO) by macrophage cells of the immune system, to destroy these salmonellas in their phagosomes.[5]

*Corresponding author. E mail: gatsingd@yahoo.com
Tel: (237) 677516740

Citation: **Famen** LCN, Talom BT, Tagne RS, Fodouop SPC, Kamsu GT, Kodjio N, Fowa AB, Gatsing D. *In vivo* Antioxidant Activity of Hydroethanol Extracts of *Terminalia avicennioides* (Combretaceae) in *Salmonella typhi*-Infected Wistar rat's model. Trop J Nat Prod Res. 2021; 5(7):1185-1191. doi.org/10.26538/tjnpr/v5i7.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

But at high concentrations, these ROS lead to oxidative cellular stress and denature lipid membranes.[6] Antibiotics are the most commonly used means of control, with fluoroquinolones and third-generation cephalosporins being the most widely used.[7] However, the bacterial strain have been developing resistance to antibiotics (especially ampicillin and phenicolates) in recent years.[8] In many developing countries, access to conventional medicine remains limited to large urban areas,[9] where alternative medicine is commonly used to treat or prevent common chronic diseases, and to improve the quality of life. In Africa, more than 80% of the population uses traditional medicine and medicinal plants for primary health care.[10,11] *T. avicennioides* is traditionally used for the treatment of typhoid fever in the West region of Cameroon. Akanbi showed the *in vivo* efficacy of bark roots of *T. avicennioides* against *Plasmodium berghei*.[12] Studies have also shown that extracts of *T. avicennioides* exhibit antisalmonella activities *in vitro*.[13] But little is known on the *in vivo* antisalmonella activities of the extract of this plant, and on the biochemical parameters it may affect in a living organism. Therefore, this study evaluated the *in vivo* antisalmonella and antioxidant activities of the hydroethanol extract of *T. avicennioides* on *Salmonella typhi* ATCC6539.

Materials and Methods

Plant material

The fresh stem bark sample of *T. avicennioides* was harvested in July 2018 at Noun division, Western region of Cameroon. It was identified by Dr. Tchiengue Bathelemy (Botanist). The herbarium specimen with voucher number 7908/SRF-Cam was deposited at the National Herbarium of Cameroon.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Preparation of plant extract

The fresh stem bark was air-dried at room temperature (24 to 27°C) until constant weight. Ground and powdered dried stem bark (100 g) was macerated with 70% ethanol (1 L) at room temperature for 2 days. The resulting hydroethanol residue was evaporated at 40°C using rotary evaporator (BUCHI R-200) under reduced pressure and stored at -20°C until further use.

Phytochemical screening

The extracts were screened for different classes of compounds, including alkaloids, anthocyanins, anthraquinones, flavonoids, phenols, saponins, tannins, steroids and triterpenes, using standard methods.[14]

Bacteria strain

Stock cultures of *S.* Typhi (ATCC 6539) used in this study were obtained from Centre Pasteur of Cameroon. Their pure cultures were maintained in Muller-Hinton agar and stored at 4°C.

Experimental animals

Young, healthy Wistar rats (150 – 200 g) of each sex were bred at the University of Dschang animal house. Animal housing and *in vivo* experiments were carried out following the guidelines of the European Union on Animal Care (CEE Council 86/609)[15] that were adopted by the Institutional Committee of the Ministry of Scientific Research and Innovation of Cameroon. They were housed in the laboratory under a natural temperature and 12 hours dark/light cycle. The animals were fed with standard diet and received water *ad libitum*. All animal procedures were performed after approval by the University of Dschang-Cameroon Ethics Committee (Project N° BCH1202/FS/UDs/2018).

Typhoid fever induction and treatment

Throughout the experiment, in order to reduce the level of commensals anaerobic bacteria that normally colonize the rat intestine, animals were given azithromycin solution (5 mg/mL).[16] A *S. typhi* suspension was prepared to 0.5 on the McFarland turbidity scale. Saline solution (1 mL of 0.9% NaCl), containing about 1.5 x 10^8 colony-forming units was given orally to each animal.[17]

Sixty male and female albinos' rats of the Wistar strain aged between 08 to 10 weeks were divided into 12 groups of 5 animals each, including 6 male groups and 6 female groups. The selected animals were acclimatized for one week. With the exception of group 1 animals (uninfected and untreated), all the other groups (2-6) were infected. They received orally, a single dose (1 mL) of 1.5 × 10^8 CFU suspension of *S. typhi* (ATCC 6539). Infection monitoring in animals was done by colony-counting blood culture on Salmonella-Shigella agar and converted to CFU salmonella per milliliter of blood. The effectiveness of the infection was achieved when the bacteria concentration of the blood was more than 4 × 10^5 CFU /ml of blood, then by the excretion of watery stools, the presence of mucus in the stool, the reduced activity and exponential increase in systemic load in *S. typhi* in rats.[17] Each animal in each group was housed in its own cage and these animals were treated as follows: group 1 (negative control group) received distilled water; group 2 (typhoid control group, infected and untreated) received distilled water; group 3 (positive control group) received ciprofloxacin (14 mg/kg); groups 4, 5 and 6 (test groups) received hydroethanol stem bark extract of *T. avicennioides* (5, 23 and 46 mg/kg, respectively). These doses were obtained from traditional practitioner's dose (46 mg/kg of body weight). Then every 2 days during the experimental period, the blood was collected into heparinized tubes and assessed immediately for the bacterial load. At the end of the experiment, the animals were fasted for 12 h and then anesthetized with chloroform. Blood sample was collected by the cardiac puncture, centrifuged at 3000 g for 15 min, and the obtained serum was used for biochemical assays.

Animals were further dissected and the liver, kidney, heart, lung and spleen were removed. A sample (0.20 g) of each organ was ground in a mortar containing ice and then centrifuged. Homogenates of these organs were prepared in phosphate buffer pH 7.2 (20% organ in 80% phosphate buffer) and then centrifuged at 3000 g for 25 min. The supernatant (organ homogenate) was stored at -20°C for assays of oxidative stress parameters.

Determination of superoxide dismutase (SOD) activity

The activity of SOD in the sample was determined by the method of Fridovich and Misra with some modifications.[18] Homogenates (150 µL) were added to 500 µL of carbonate-bicarbonate buffer (pH 10.2, 0.3M, pKa 10.3), then 250 µL of an EDTA solution (0.6 mM), and 350 µL distilled water were further added. The mixture obtained was homogenized and 250 µL of adrenaline (4.5 µM) was added in order to initiate the reaction. Auto-oxidation of adrenaline was measured by reading the OD at 480 nm, 30 seconds and 180 seconds after adding epinephrine. The SOD was activity expressed as inhibition percentage and was calculated as follows:

$$\%\ \text{inhibition} = \left[100 - \left(\frac{\Delta\ OD\ sample}{\Delta\ OD\ blank}\right)\right] \times 100$$

A 50% inhibition corresponds to one unit of activity.

Determination of catalase activity

Catalase activity was determined by the method of Dimo *et al*.[19] Phosphate buffer pH 7.4 (375 µL) was added to 25 µL of tissue homogenate, followed by 100 µL of H_2O_2 (50 mM). One minute later, 1 mL of potassium dichromate (5%) prepared in 1% acetic acid was introduced into the reaction medium. The mixture obtained was incubated for 10 min in a boiling water bath and then cooled in an ice bath. The optical density was obtained at 570 nm against the blank (the extract was replaced by distilled water in the blank tubes). The enzymatic activity of catalase was deduced by the Beer-Lambert law.

Determination of Peroxidase activity (POD)

Peroxidase activity was determined in tissues and serum by Habbu *et al* method.[20] Test sample (0.5 mL) was added to 1 mL of 10 mM KI solution and 1 mL of sodium acetate (40 mM). The absorbance of potassium iodide was read at 353 nm, which indicates the amount of peroxidase. Then 20 µL of H_2O_2 (15 mM) was added, and the change in the absorbance in 5 min was recorded. Units of Peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity was expressed in terms of units per mg of proteins.

Determination of malondialdehyde (MDA)

Lipid peroxidation was evaluated by the determination of malondialdehyde (MDA) according to the method by Oyedemi *et al*[21] with certain modifications. Malondialdehyde is one of the final products of the decomposition of polyunsaturated fatty acids (PUFAs) under the effect of free radicals released during stress. In a hot acidic medium (pH 2 to 3; 100°C), an MDA molecule condenses with two thiobarbituric molecules (TBA) to form a pink colored complex (reading at 532 nm). Five hundred microliters of 1% orthophosphoric acid and 500 µL of precipitation mixture (1% thiobarbituric acid in 1% acetic acid) were added to 100 µL of homogenate. The resulting reaction mixture was homogenized and incubated for 15 minutes in a boiling water bath. After cooling in an ice bath, the mixture was centrifuged at 3500 rpm for 10 min. The absorbance of the supernatants was read at 532 nm against the blank. Lipid peroxidation was calculated on the basis of the MDA molar extinction coefficient and expressed in micromoles of MDA per gram of tissue using the Beer-Lambert law.

Determination of nitric oxide (NO)

Nitric oxide content in serum and tissue homogenates was measured by Griess reagent.[22] Absorption of the chromophore during ionization of nitrite with sulfanilamide coupled with naphthylethylenediamine (NED) was read at 520 nm. Three hundred and forty microliters of 1% sulfanilamide (prepared in 5% orthophosphoric acid) was introduced into 340 µL of serum and homogenates. The resulting mixture was homogenized and left in the dark for 5 minutes at room temperature. Next, 340 µL of 0.1% NED was added to the reaction medium and the whole was left once more in the dark for 5 minutes. The optical densities were then read at 520 nm against the blank. The NO content was determined from a calibration curve derived from the different Na_2NO concentrations.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Statistical analysis

Results were expressed as mean ± standard error of mean. Statistical significance was determined by the one-way analysis of variance followed by Waller-Duncan test using the software SPSS 22 version. Differences were considered significant at $P < 0.05$.

Results and Discussion

The *in vivo* antisalmonella activity of the 70% hydroethanol extract was evaluated in rats infected with *Salmonella typhi* ATCC 6539 strain. According to Naughton *et al*, the pathogenicity and virulence of *salmonella* are host-specific.[23] Therefore, *S. typhi* induces systemic infection in humans and in some animals (rabbits, rats), while *S. typhimurium* induces only localized gastroenteritis in humans.[24-26] Thus, the *S. typhi* strain was used to infect rats to evaluate *in vivo* activity of hydroethanol extract of *T. avicennioides*. In addition, several important factors of *salmonella* infection, such as intestinal transport, electrolyte transport and colonization site (ileum) are similar in rats and humans.[23] For this reason, Wistar rats were used in the study.

Figures 1 and 2 represent the bacterial load in the blood of rats as a function of time, in males and females, respectively. After infection, during the 24 h of latency period, there was no significant increase of bacteria load in the animal's blood. This period is related to the time of acclimatization of bacteria in the rat's body. During this adaptation period, many metabolic reactions occur, like the synthesis of particular enzymes necessary to metabolize the substrate.[27] The exponential increase in number of *S. typhi* in the rat's blood after latency phase indicates that infection is established and could be explained by the excretion of watery stools and the reduction of rat physical activities. The slight decrease in bacterial load observed after 8 days and 10, 12 and 14 days in male and 10 and 16 days in untreated infected female would be due to the action of the immune system, while the increase observed in animals treated with *T. avicennioides* bark extract would be due to the combined action of the immune system and the plant extract.[28] The blood of animals treated with different doses of extract was practically clear of *salmonella* between the 8th and 12th day of treatment in both males' and females'. Therefore, animals treated with the dose from the MIC and its multiples found a dose-dependent cure: the 8th day (46 mg /kg), the 10th day (23 mg /kg) and the 11th day (5 mg /kg) of treatment. The results show that the extract possesses inhibitory activity against *S. typhi* in rats. Other reports have shown that plant extracts can treat induced salmonellosis in rats.[27,29]

The stem bark extract of *T. avicennioides* revealed the presence of flavonoids, alkaloids, phenols, saponins, anthocyanins, and anthraquinones (Table 1). This is similar to the report of Musa *et al* and Famen *et al*, who had already demonstrated the presence of some of these chemical constituents in the hydroethanol extract of stem barks of *T. avicennioides*.[13,30] Several studies have demonstrated the antibacterial activities of some bioactive compounds.[25] Reactive oxygen species (ROS) are oxidants formed in the body due to exogenous and endogenous factors and are considered responsible for many diseases such as cancer, cardiovascular disease, neurodegenerative diseases, inflammatory processes and aging.[31] Antioxidant systems are normally set up in living aerobic organisms to fight the effect of oxidative stress. The enzymatic antioxidants of superoxide dismutase (Table 2), catalase (Table 3) and peroxidase (Table 4) were measured in the organs and serum of rats infected with *S. typhi* ATCC 6539 and treated with the 70% hydroethanol extract of *T. avicennioides*. The redox status of the cells is maintained by a combination of enzymatic and non-enzymatic antioxidants. The antioxidant enzymes superoxide dismutase, catalase, and peroxidase are involved in a coordinated process that prevents oxidative damage by ROS.[32] Many studies have shown that the excessive production of highly oxidized and oxidizing free radicals in cells reduces the activities of these enzymes.[32,33] Superoxide dismutase (SOD), peroxidase and catalase are antioxidant enzyme proteins that constitute the first barrier to this antioxidant defense.[32] Their activity and location in the cell are complementary and ensure the elimination of superoxide anions and hydrogen peroxide in all intracellular compartments.[34] In this study, the levels of SOD, catalase and peroxidase in the serum and tissue for treated animals (with both ciprofloxacin and hydroethanol extract of *T. avicennioides*) were significantly higher compared to negative control animals.

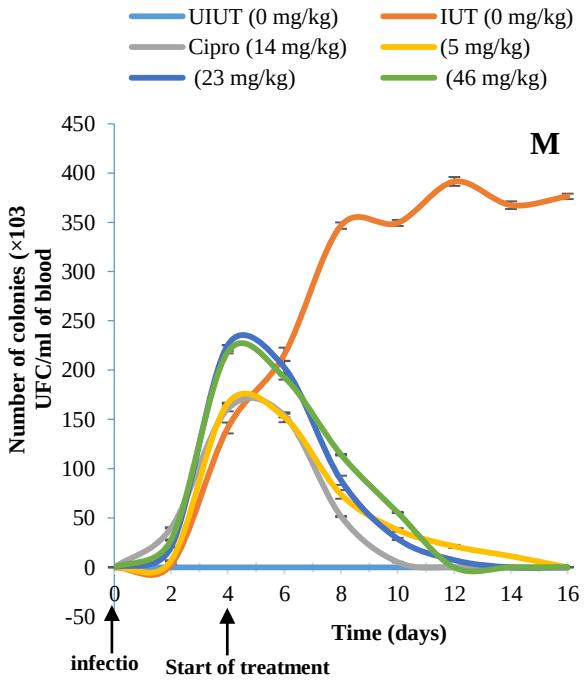

Figure 1: Effects of *Terminalia avicennioides* extract on the number of *Salmonella typhi* colonies (ATCC6539) as a function of the time in male rats. Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and Untreated; M: male; F: female.

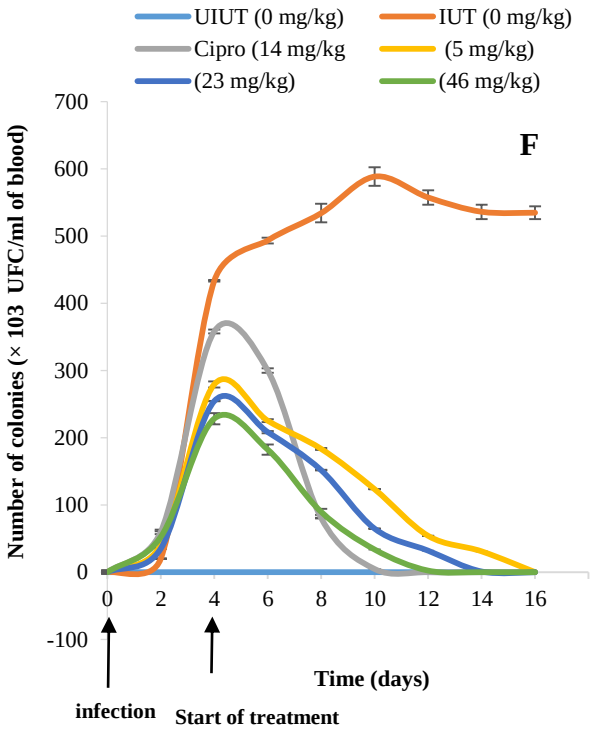

Figure 2: Effects of *Terminalia avicennioides* extract on the number of *Salmonella typh*i colonies (ATCC6539) as a function of the time in female rats. Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and Untreated; M: male; F: female.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The decrease in catalase, peroxidase and Superoxide dismutase levels in the negative control indicates that there was an oxidative environment that led to oxidative stress in the negative control, which could result to a high bacterial load. The increase in catalase, peroxidase and SOD levels in the positive control animals and the groups treated with the different doses of extracts shows that there was no oxidizing environment and no stress in the treated groups. This may be due to the reduction in bacterial load in the treatment groups compared to the negative control. This suggests that hydroethanol extract contains antioxidant substances that neutralize reactive oxygen species, hence, preventing the inhibition or destruction of catalase, peroxidase and SOD and, promoting the protection of these organs against any tissue damage induced by these reactive compounds.

The results are consistent with the report of Akanbi, that showed a significant increase in serum catalase and SOD levels in mice infected with *Plasmodium berghei* and treated with methanol extract of the stem bark of *T. avicennioides*.[12] The current results are also similar to those of Fowa *et al* who showed an increase in serum and tissue levels of catalase and SOD in rats infected with *S. typhi* ATCC 6539 and treated with 70% of hydroethanol extract of *Adenia lobata leaves*.[30]

Table 1: Phytochemical composition of the hydroethanol extract from the stem bark of *T. avicennioides*

Class of compound	Detection in stem bark extract of *Terminalia avicennioides* (Ethanol 70%)
Alkaloids	+
Anthocyanin	+
Anthraquinone	+
Flavonoids	+
Phenols	+
Tannins	-
Triterpenes	-
Saponins	+
Steroids	-

+ : Present ; - : Absent.

Table 2: Effects of hydroethanol extracts of *T. avicennioides* on superoxide dismutase (SOD) activity of in serum and tissues of rats

Sex	Doses	Organs and Quantity of SOD (activity/mg of tissues and activity/mL of blood)					
		Serum	Liver	Kidneys	Lungs	Spleen	Heart
Female	UIUT (0 mg/Kg)	0.21 ± 0.02^{b}	0.33 ± 0.02^{b}	0.22 ± 0.01^{b}	4.96 ± 1.03^{b}	1.83 ± 0.30^{bc}	3.34 ± 0.26^{b}
	IUT (0 mg/Kg)	0.17 ± 0.01^{a}	0.23 ± 0.01^{a}	0.16 ± 0.01^{a}	1.97 ± 0.26^{a}	1.05 ± 0.25^{a}	2.55 ± 0.05^{a}
	Cipro (14 mg/kg)	0.24 ± 0.03^{b}	0.31 ± 0.02^{b}	0.20 ± 0.01^{b}	4.25 ± 0.71^{b}	1.43 ± 0.29^{ab}	3.08 ± 0.07^{b}
	5 mg/Kg	0.20 ± 0.01^{b}	0.34 ± 0.02^{b}	0.22 ± 0.02^{b}	3.64 ± 0.55^{b}	1.71 ± 0.14^{b}	2.95 ± 0.19^{b}
	23 mg/Kg	0.22 ± 0.02^{b}	0.32 ± 0.01^{b}	0.20 ± 0.01^{b}	4.43 ± 1.26^{b}	1.86 ± 0.06^{b}	2.95 ± 0.12^{b}
	46 mg/Kg	0.23 ± 0.03^{b}	0.32 ± 0.00^{b}	0.23 ± 0.02^{b}	3.86 ± 0.55^{b}	2.22 ± 0.16^{c}	3.16 ± 0.15^{b}
Male	UIUT (0 mg/Kg)	0.21 ± 0.01^{c}	0.29 ± 0.01^{b}	1.77 ± 0.06^{c}	1.61 ± 0.10^{c}	1.47 ± 0.02^{b}	2.54 ± 0.13^{bc}
	IUT (0 mg/Kg)	0.14 ± 0.01^{a}	0.23 ± 0.01^{a}	1.06 ± 0.05^{a}	0.86 ± 0.02^{a}	1.33 ± 0.01^{a}	1.97 ± 0.06^{a}
	Cipro (14 mg/kg)	0.20 ± 0.00^{c}	0.29 ± 0.02^{b}	1.19 ± 0.01^{b}	2.11 ± 0.09^{d}	1.64 ± 0.005^{c}	2.56 ± 0.05^{b}
	5 mg/Kg	0.15 ± 0.01^{ab}	0.28 ± 0.02^{b}	1.19 ± 0.01^{b}	1.09 ± 0.05^{b}	1.46 ± 0.02^{b}	2.45 ± 0.02^{b}
	23 mg/Kg	0.17 ± 0.01^{b}	0.31 ± 0.01^{b}	1.95 ± 0.06^{d}	1.70 ± 0.14^{c}	1.48 ± 0.04^{b}	2.50 ± 0.06^{b}
	46 mg/Kg	0.20 ± 0.01^{c}	0.37 ± 0.02^{c}	2.19 ± 0.15^{e}	1.96 ± 0.08^{d}	1.60 ± 0.08^{c}	2.67 ± 0.04^{c}

Data are mean ± standard error of mean (n = 5). In the same column and for the same sex, values with different letters are significantly different ($p < 0.05$). Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and untreated.

Table 3: Effects of hydroethanol extracts of *T. avicennioides* on catalase activity in serum and tissues of rats

Sex	Doses	Organs and Quantity of catalase (µmol/min/mg of tissues and µmol/min/mL of blood)					
		Serum	Liver	Kidneys	Lungs	Spleen	Heart
Females	UIUT (0 mg/Kg)	0.16 ± 0.06^{b}	8.01 ± 0.95^{d}	0.22 ± 0.01^{b}	3.53 ± 0.28^{d}	3.41 ± 0.29^{c}	2.41 ± 0.22^{bc}
	IUT (0 mg/Kg)	0.07 ± 0.00^{a}	2.71 ± 0.18^{a}	0.16 ± 0.00^{a}	1.48 ± 0.10^{a}	2.16 ± 0.10^{a}	1.92 ± 0.10^{a}
	Cipro (14 mg/kg)	0.08 ± 0.01^{a}	2.87 ± 0.06^{a}	0.36 ± 0.02^{c}	2.54 ± 0.43^{c}	2.32 ± 0.19^{a}	3.33 ± 0.33^{d}
	5 mg/Kg	0.08 ± 0.00^{a}	3.89 ± 0.74^{b}	0.17 ± 0.01^{a}	1.83 ± 0.10^{b}	2.74 ± 0.07^{b}	2.23 ± 0.08^{b}
	23 mg/Kg	0.10 ± 0.01^{b}	4.11 ± 0.65^{b}	0.17 ± 0.01^{a}	2.57 ± 0.18^{c}	3.25 ± 0.24^{c}	2.64 ± 0.26^{c}
	46 mg/Kg	0.11 ± 0.01^{b}	5.54 ± 0.25^{c}	0.21 ± 0.02^{b}	2.97 ± 0.33^{cd}	3.11 ± 0.10^{c}	2.56 ± 0.07^{c}
Males	UIUT (0 mg/Kg)	0.09 ± 0.01^{b}	6.28 ± 0.22^{c}	12.70 ± 1.58^{b}	4.67 ± 0.60^{c}	6.20 ± 0.93^{c}	3.65 ± 0.23^{c}
	IUT (0 mg/Kg)	0.05 ± 0.02^{a}	4.18 ± 0.16^{a}	7.07 ± 1.15^{a}	3.10 ± 0.23^{a}	3.14 ± 0.25^{a}	2.37 ± 0.56^{a}
	Cipro (14 mg/kg)	0.06 ± 0.01^{a}	4.80 ± 0.27^{b}	8.78 ± 0.83^{a}	4.67 ± 0.80^{bc}	5.23 ± 0.58^{c}	2.98 ± 0.03^{a}
	5 mg/Kg	0.07 ± 0.00^{a}	4.56 ± 0.24^{b}	12.07 ± 1.65^{b}	3.83 ± 0.27^{b}	3.86 ± 0.11^{b}	2.95 ± 0.04^{a}
	23 mg/Kg	0.12 ± 0.01^{c}	5.96 ± 0.43^{c}	11.63 ± 0.77^{b}	4.80 ± 0.53^{c}	4.39 ± 0.78^{bc}	3.09 ± 0.02^{b}
	46 mg/Kg	0.15 ± 0.03^{c}	8.16 ± 0.53^{d}	11.92 ± 1.17^{b}	4.64 ± 0.35^{c}	4.48 ± 0.85^{bc}	4.64 ± 0.42^{d}

Data are mean ± standard error of mean (n = 5). In the same column and for the same sex, values with different letters are significantly different ($p < 0.05$). Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and untreated.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 4: Effects of hydroethanol extracts of *T. avicennioides* on peroxydase activity in serum and tissues of rats

Sex	Doses	Organs and Quantity of peroxydase (µmol/min/g of tissues et µmol/min/mL of blood)					
		Serum	Liver	Kidneys	Lungs	Spleen	Heart
Females	UIUT (0 mg/Kg)	2.60 ± 0.31^{b}	5.53 ± 0.18^{bc}	4.54 ± 0.26^{d}	2.77 ± 0.33^{b}	3.48 ± 0.51^{b}	2.53 ± 0.52^{d}
	IUT (0 mg/Kg)	1.54 ± 0.13^{a}	3.16 ± 0.46^{a}	2.90 ± 0.44^{a}	2.20 ± 0.41^{a}	2.24 ± 0.33^{a}	1.19 ± 0.05^{a}
	Cipro (14 mg/Kg)	2.69 ± 0.46^{b}	6.19 ± 0.62^{c}	3.29 ± 0.24^{abc}	3.15 ± 0.35^{b}	3.85 ± 0.24^{b}	1.87 ± 0.08^{bc}
	5 mg/Kg	3.47 ± 0.02^{c}	4.69 ± 0.35^{b}	4.20 ± 0.56^{cd}	3.03 ± 0.15^{b}	3.29 ± 0.31^{b}	2.56 ± 0.36^{d}
	23 mg/Kg	3.07 ± 0.19^{bc}	5.50 ± 0.38^{bc}	3.03 ± 0.15^{ab}	3.09 ± 0.06^{b}	3.89 ± 0.69^{b}	1.56 ± 0.25^{ab}
	46 mg/Kg	3.57 ± 0.18^{c}	5.93 ± 0.48^{c}	3.91 ± 0.79^{bcd}	3.21 ± 0.05^{b}	6.73 ± 0.16^{c}	2.18 ± 0.30^{cd}
Males	UIUT (0 mg/Kg)	1.76 ± 0.41^{b}	5.48 ± 0.29^{ab}	4.02 ± 0.72^{b}	3.43 ± 0.25^{b}	3.99 ± 0.36^{b}	2.45 ± 0.37^{b}
	IUT (0 mg/Kg)	1.00 ± 0.10^{a}	4.91 ± 0.68^{a}	1.58 ± 0.20^{a}	2.48 ± 0.31^{a}	3.17 ± 0.06^{a}	1.20 ± 0.06^{a}
	Cipro (14 mg/Kg)	2.28 ± 0.04^{c}	6.50 ± 1.18^{abc}	4.30 ± 1.46^{b}	4.64 ± 0.16^{c}	4.88 ± 0.76^{c}	1.92 ± 0.21^{b}
	5 mg/Kg	1.54 ± 0.01^{b}	7.30 ± 1.31^{c}	3.25 ± 0.29^{ab}	3.75 ± 0.05^{b}	4.89 ± 0.34^{c}	2.01 ± 0.31^{b}
	23 mg/Kg	3.72 ± 0.14^{d}	6.62 ± 0.33^{bc}	4.55 ± 0.80^{b}	3.90 ± 0.21^{b}	4.41 ± 0.18^{bc}	3.61 ± 0.30^{c}
	46 mg/Kg	2.39 ± 0.23^{c}	6.48 ± 0.44^{abc}	4.61 ± 0.90^{b}	3.65 ± 0.30^{b}	4.35 ± 0.23^{bc}	3.49 ± 0.42^{c}

Data are mean ± standard error of mean (n = 5). In the same column and for the same sex, values with different letters are significantly different ($p < 0.05$). Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and untreated.

Table 5: Effects of hydroethanol extracts of *T. avicennioides* on nitric oxide (NO) levels in the blood and tissues of rats

Sex	Doses	Organs and Quantity of Oxide Nitric (µmol/g of tissues and µmol/mL of blood)					
		Serum	Liver	Kidneys	Lungs	Spleen	Heart
Females	UIUT (0 mg/Kg)	1.26 ± 0.00^{a}	0.08 ± 0.02^{a}	0.12 ± 0.00^{a}	0.07 ± 0.00^{e}	0.08 ± 0.02^{b}	0.12 ± 0.00^{a}
	IUT (0 mg/Kg)	14.75 ± 0.36^{d}	0.36 ± 0.05^{d}	0.45 ± 0.10^{c}	0.09 ± 0.00^{f}	0.34 ± 0.03^{c}	0.17 ± 0.00^{c}
	Cipro (14 mg/Kg)	1.05 ± 0.02^{a}	0.15 ± 0.00^{b}	0.24 ± 0.00^{b}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.12 ± 0.01^{a}
	5 mg/Kg	8.55 ± 0.39^{c}	0.12 ± 0.00^{ab}	0.22 ± 0.00^{b}	0.03 ± 0.00^{ab}	0.05 ± 0.00^{ab}	0.15 ± 0.00^{b}
	23 mg/Kg	3.63 ± 0.14^{b}	0.23 ± 0.04^{c}	0.20 ± 0.00^{ab}	0.04 ± 0.00^{bc}	0.07 ± 0.01^{b}	0.12 ± 0.00^{a}
	46 mg/Kg	1.44 ± 0.15^{a}	0.14 ± 0.02^{b}	0.19 ± 0.00^{ab}	0.04 ± 0.01^{c}	0.05 ± 0.00^{ab}	0.12 ± 0.00^{a}
Males	UIUT (0 mg/Kg)	1.72 ± 0.04^{b}	0.38 ± 0.10^{b}	0.18 ± 0.00^{a}	0.35 ± 0.04^{c}	0.36 ± 0.04^{b}	0.21 ± 0.01^{c}
	IUT (0 mg/Kg)	6.35 ± 0.29^{f}	0.75 ± 0.04^{c}	0.41 ± 0.01^{e}	0.51 ± 0.07^{d}	0.89 ± 0.10^{c}	0.26 ± 0.00^{d}
	Cipro (14 mg/Kg)	3.07 ± 0.01^{d}	0.15 ± 0.03^{a}	0.35 ± 0.00^{d}	0.11 ± 0.00^{a}	0.31 ± 0.00^{ab}	0.13 ± 0.00^{a}
	5 mg/Kg	4.00 ± 0.00^{e}	0.23 ± 0.02^{a}	0.27 ± 0.01^{c}	0.09 ± 0.01^{a}	0.36 ± 0.03^{b}	0.20 ± 0.00^{c}
	23 mg/Kg	1.32 ± 0.02^{a}	0.22 ± 0.01^{a}	0.21 ± 0.00^{b}	0.23 ± 0.03^{a}	0.34 ± 0.00^{b}	0.17 ± 0.00^{b}
	46 mg/Kg	2.05 ± 0.02^{c}	0.24 ± 0.02^{a}	0.23 ± 0.01^{b}	0.24 ± 0.00^{a}	0.25 ± 0.01^{a}	0.18 ± 0.01^{b}

Data are mean ± standard error of mean (n = 5). In the same column and for the same sex, values with different letters are significantly different ($p<0.05$). Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and untreated.

The results of two markers of oxidative stress NO (Table 5) and MDA (Table 6) were measured from the organs (liver, kidneys, lungs, spleen and heart) and serum of rats infected with *S. typhi* (ATCC 6539). Lipid degradation leads to the formation of malondialdehyde (MDA), which is used as a marker of damage caused by free radicals.[27] The amplification of damage caused by salmonella or stress is related to the increase in neutrophil infiltration into tissues. Serum and tissue MDA levels were significantly higher in the negative control group than in the other animals. This shows that there was an oxidative environment and stress in the negative control animals, which could result from a high bacterial load. The significant reduction in serum and tissue MDA levels in the positive control and in the groups treated with the extract suggests that there was no oxidizing environment and no stress in the treated groups. This may be due to the reduction in bacterial load in the treated animals compared to the negative control. The extract would therefore prevent the destruction of the bilayer membrane of cells by neutralizing free radicals. These results are consistent with the work of Akanbi that showed a significant reduction in MDA levels in mice infected with *P. berghei* and treated with methanol extract of the stem bark of *T. avicennioides*.[12] The results are also similar to those of Kodjio *et al* and Fowa *et al* which showed that extracts of *Albizia gummifera*, *Curcuma longa* and *A. lobata* have protective effects on membrane lipids.[25,30] The significant decrease in nitric oxide levels in the tissues of infected rats treated with the extract compared to the negative control indicates that the extract regulated nitric oxide production compared to the uninfected and untreated animals. Due to its ability to regulate nitric oxide production, the extract has an antioxidant potential and an activity that can give it potential to control salmonellosis. This may be due to the antioxidant phytochemicals it contains. The hydroethanol extract of *T. avicennioides* contained some secondary metabolites (flavonoids and phenolic compounds) which could be responsible for the antioxidants activities. These secondary metabolites act through the stabilization of free radicals by giving them electrons. They also chelate metal ions such as iron and copper, inhibit enzymes that produce free radicals, act as a lipid peroxidation chain destroyer and regulate antioxidant enzymes.[35]

Table 6: Effects of hydroethanol extracts of *T. avicennioides* on malondialdehyde (MDA) levels in the blood and tissues of rats

Sex	Doses	Organs and Quantity of Malondialdehyde (µM/g of tissues and µM/mLof blood)					
		Serum	Liver	Kidneys	Lungs	Spleen	Heart
Females	UIUT (0 mg/Kg)	0,01 ± 0,00^{a}	0,22 ± 0,00^{a}	0,05 ± 0,01^{c}	0,06 ± 0,00^{a}	0,13 ± 0,00^{a}	0,11 ± 0,01^{a}
	IUT (0 mg/Kg)	0,04 ± 0,00^{d}	0,41 ± 0,00^{c}	0,12 ± 0,00^{e}	0,14 ± 0,00^{c}	0,21 ± 0,00^{b}	0,25 ± 0,04^{c}
	Cipro (14 mg/Kg)	0,02 ± 0,00^{b}	0,27 ± 0,02^{b}	0,04 ± 0,00^{b}	0,08 ± 0,00^{b}	0,17 ± 0,00^{d}	0,12 ± 0,01^{a}
	5 mg/Kg	0,03 ± 0,00^{c}	0,19 ± 0,00^{a}	0,02 ± 0,01^{a}	0,09 ± 0,00^{b}	0,19 ± 0,00^{c}	0,19 ± 0,02^{b}
	23 mg/Kg	0,03 ± 0,00^{c}	0,26 ± 0,01^{b}	0,07 ± 0,00^{d}	0,08 ± 0,00^{b}	0,18 ± 0,00^{c}	0,18 ± 0,01^{b}
	46 mg/Kg	0,01 ± 0,00^{a}	0,21 ± 0,01^{a}	0,07 ± 0,00^{d}	0,09 ± 0,00^{b}	0,12 ± 0,00^{a}	0,16 ± 0,03^{ab}
Males	UIUT (0 mg/Kg)	0,006 ± 0,00^{a}	0,23 ± 0,01^{ab}	0,06 ± 0,00^{a}	0,08 ± 0,00^{ab}	0,16 ± 0,00^{b}	0,22 ± 0,00^{a}
	IUT (0 mg/Kg)	0,028 ± 0,00^{d}	0,47 ± 0,05^{c}	0,19 ± 0,01^{d}	0,17 ± 0,00^{d}	0,27 ± 0,01^{d}	0,33 ± 0,01^{d}
	Cipro (14 mg/Kg)	0,011 ± 0,00^{c}	0,19 ± 0,03^{a}	0,06 ± 0,00^{a}	0,11 ± 0,00^{c}	0,15 ± 0,00^{b}	0,30 ± 0,04^{cd}
	5 mg/Kg	0,008 ± 0,00^{b}	0,24 ± 0,03^{ab}	0,13 ± 0,00^{c}	0,11 ± 0,00^{c}	0,19 ± 0,00^{c}	0,22 ± 0,00^{a}
	23 mg/Kg	0,006 ± 0,00^{a}	0,28 ± 0,00^{b}	0,09 ± 0,00^{b}	0,09 ± 0,00^{b}	0,15 ± 0,00^{b}	0,27 ± 0,00^{bc}
	46 mg/Kg	0,005 ± 0,00^{a}	0,21 ± 0,00^{a}	0,09 ± 0,01^{b}	0,06 ± 0,01^{a}	0,11 ± 0,02^{a}	0,26 ± 0,00^{b}

Data are mean ± standard error of mean (n = 5). In the same column and for the same sex, values with different letters are significantly different ($p < 0.05$). Cipro: ciproflaxacin; IUT: Infected and untreated; UIUT: Uninfected and untreated.

Conclusion

This work shows that the hydroethanol extract of *Terminalia avicennioides* can reduce *S. typhi* load and reduce the state of oxidative stress caused by infection of *S. typhi* in rats. The plant extract also enhanced the activity of antioxidant enzymes, and reduced the level of oxidative stress markers, showing a promising potential treatment against typhoid fever. However, further studies should be conducted to determine the side effects and define the therapeutic dose that would allow safety use.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors acknowledge the Laboratory of Bacteriology of Centre Pasteur du Cameroun for providing Salmonella strain and the Cameroon National Herbarium (Yaounde) for plant identification. The authors also acknowledge Mrs. Yacouba Patriciale for her contribution.

References

1. Crump JA. Progress in Typhoid Fever Epidemiology. Clin. Infect. Dis. 2019; 68(1):S4-S9.
2. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Org. 2004; 82(5):346-353.
3. Buckle GC, Walker CLF, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. J Glob Health**.** 2012; 2(1):1-9.
4. Achonduh-Atijegbe OA, Mfuh KO, Mbange HEA, Chedjou JP, Taylor DW, Nerurkar VR, Mbacham FW, Leke R. Prevalence of malaria, typhoid, toxoplasmosis and rubella among febrile children in Cameroon. BMC Infect Dis. 2016; 16(658):1-9.
5. Raffatellu MRP, Wilson SE, Winter AJ, Baumler. Clinical pathogenesis of typhoid fever. J Infect Dev Ctries. 2008; 2(4):260-266.
6. Erdemoglu N, Turan NN, Cakõcõ I, Sener B, Aydõn A. Antioxidant activities of some Lamiaceae plant extracts. Phytother Res. 2006; 20:9-13.
7. Ugboko H and De N. Mechanisms of Antibiotic resistance in *Salmonella typhi*. Int J Curr Microbiol Appl Sci. 2014; 3(12):461-476.
8. Vikesland P, Garner E, Gupta S. Kang S, Maile-Moskowitz A, Zhu N. Differential Drivers of Antimicrobial Resistance across the World. Acc Chem Res. 2019; 52(4):916-924.
9. Usha PTA, Jose S, Nisha AR. Antimicrobial Drug Resistance – a global concern. Vet World. 2010; 3(3):138-139.
10. Cowan MM. Plant Products as Antimicrobial Agents. Clin Microbiol Rev. 1999; 12(4):564-582.
11. Jiofack T, Fokunang C, Guedje N, Kemeuze V, Fongnzossie E, Nkongmeneck BA, Mapongmetsem PM, Tsabang N. Ethnobotanical uses of medicinal plants of two ethnoecological regions of Cameroon. Int Med Med Sci. 2010; 2(3):60-79.
12. Akanbi OM. *In vivo* Study of Antiplasmodial Activity of *Terminalia avicennioides* and Its Effect on Lipid Profile and Oxidative Stress in Mice Infected with *Plasmodium berghei*. Br Microbiol Res J. 2013; 3(4):501-513.
13. Famen LCN, Talom TB, Tagne SR, Kamsu TG, Kodjio N, Lacmata TS, Gatsing D. In vitro Antioxidant Activities and Effect of Hydroethanolic and Aqueous Extracts of *Terminalia avicennioides* (Combretaceae) on Salmonella. Microbiol Res J Int. 2020; 30(1):1-14.
14. Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. (3rd éd) Springer Netherlands; 1998. 302 p.
15. Smith JA, van den Broek FAR, Martorell JC, Hackbarth H, Ruksenas O, Zeller W. Principles and practice in ethical review of animal experiments across Europe: summary of the report of a FELASA working group on ethical evaluation of animal experiments. Lab Anim. 2007; 41:143-160.
16. Choi JG, Kang OH, Lee YS, Chae HS, Oh YC, Brice OO, Kim MS, Sohn DH, Kim HS, Park H, Shin DW, Rho JR, Kwon DY. *In Vitro* and *In Vivo* Antibacterial Activity of Punica granatum Peel Ethanol Extract against Salmonella. Evid-Based Compl Altern Med. 2011; 2011(690518):1-8.

17. Tala DS, Gatsing D, Fodouop SPC, Fokunang C, Kengni F, Djimeli MN. In vivo anti-salmonella activity of aqueous extract of *Euphorbia prostrata Aiton* (Euphorbiaceae) and its toxicological evaluation. Asian Pac J Trop Biomed. 2015; 5(4):310-318.
18. Misra PH and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247(10):3170-3175.
19. Théophile D, Emery TD, Djomeni D, Véronique PB, Njikam N. Effects of *Alafia multiflora* Stapf on lipid peroxidation and antioxidant enzyme status in carbon tetrachloride-treated rats. Pharmacol online. 2006; 2:76-89.
20. Habbu P, Shastry R, Mahadevan KM, Joshi H, Das S. Hepatoprotective and Antioxidant Effects of Argyreia Speciosa in Rats. Afr J Trad Compl Altern Med. 2008; 5(2):158-164.21.
21. Oyedemi SO, Bradley G, Afolayan AJ. *In-vitro* and *in-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol. 2010; 4(2):70-78.
22. Griess P. Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt, Ueber einige Azoverbindungen". Ber. dtsch. chem. Ges. 1879; 12(1):426-428.
23. Napolitano DR, Mineo JR, de Souza MA, de Paula JE, Espindola LS, Espindola FS. Down-modulation of nitric oxide production in murine macrophages treated with crude plant extracts from the Brazilian Cerrado. J Ethnopharmacol. 2005; 99(1):37-41.
24. Adeyi AO, Jinadu AM, Arojojoye OA, Alao OO, Ighodaro OM, Adeyi OE. *In vivo* and *in vitro* antibacterial activities of *Momordica charantia* on *Salmonella typhi* and its effect on liver function in typhoid-infected rats. J Pharmacogn Phytother. 2013; 5(11):183-188.
25. Kodjio N, Atsafack SS, Njateng GSS, Sokoudjou JB, Kuiate JR, Gatsing D. Antioxidant Effect of Aqueous Extract of *Curcuma longa* Rhizomes (Zingiberaceae) in the Typhoid Fever Induced in Wistar Rats Model. J Adv Med Pharm Sci. 2016; 7(3):1-13.
26. Naughton PJ, Grant G, Spencer RJ, Bardocz S, Pusztai A. A rat model of infection by *Salmonella typhimurium* or Salm. enteritidis. J Appl Bacteriol. 1996; 81(6):651-656.
27. Wilkins EGL and Roberts C. Extraintestinal salmonellosis. Epidemiol. Infect. 1988; 100(3):361-368.
28. Sato G. Infection of *Salmonella pullorum*, *Salmonella newington* or *Salmonella enteritidis* in laboratory rats by oral inoculation. Jpn J vet Res. 1965; 13(2):19-32.
29. Djimeli MN, Fodouop CSP, Njateng GSS, Fokunang C, Tala SD, Kengni F, Gatsing D. Antibacterial activities and toxicological study of the aqueous extract from leaves of *Alchornea cordifolia* (Euphorbiaceae). BMC Compl Altern Med. 2017; 17(1):1-10.
30. Fowa AB, Fodouop CSP, Fokunang NC, Djoueudom GF, Famen NLC, Ongbayokolak NS, Gatsing D. Antityphoid and antioxidant activities of hydroethanolic leaf extract of *Adenia lobata* Jacq. (*Passifloraceae*) on *Salmonella typhi* infected wistar rats. J Med Plants Stud. 2019; 7(1):13-22.
31. Musa FM, Ameh JB, Ado SA, Olonitola OS. Evaluation of phytochemical and antibacterial properties of *Terminalia avicennioides* crude extract against selected bacteria from diarrhoeic patients.Bayero J Pure Appl Sci. 2016; 9(1):129-137.
32. Ajboye TO, Yakubu MT, Salau AK, Oladiji AT, Akanji MA, Okogun JI. Antioxidant and drug detoxification potential of aqueous extract of *Annona senegalensis* leaves in carbon tetrachloride-induced hepatocellular damage. Pharm Biol. 2010; 48(12):1361-1370.
33. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westeroh N, Losano G. Nitric oxide and cardiac function. Life Sci. 2007; 81(10):779-793.
34. Chen B, Ning M, Yang G. Effect of Paeonol on Antioxidant and Immune Regulatory Activity in Hepatocellular Carcinoma Rats. Molecules. 2012; 17(4):4672-4683.
35. Soulère L, Viodé C, Périé J, Hoffmann P. Selective Inhibition of Fe- *versus* Cu/Zn-Superoxide Dismutases by 2,3-Dihydroxybenzoic Acid Derivatives. Chem Pharm Bull. 2002; 50(5):578-582.