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In vitro antisalmonellal and antioxidant properties of leaves extracts of *Zehneria scabra* (L.F.) sond (Cucurbitaceae)

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Abstract
Conventional antibiotic are usually used in poultry farming to treat salmonellosis. Unfortunately, these antibiotics have negative side effects both on animal and human health, and also lead to anti bio-resistance; hence the need for an alternative means of treatment. Therefore, the aim of this study was to investigate the *in-vitro* antisalmonellal and antioxidant activities of aqueous, ethanolic, and hydro-ethanolic leaves extracts of *Zehneria scabra* against four *Salmonella* isolates. Antisalmonellal activity was evaluated using the broth microdilution method. To evaluate the antioxidants properties, the quantitative determination of total phenols, flavonoids and the phytochemical screening were performed using standard methods; four extract concentrations were evaluated (12.5, 25, 50, 100 and 200µg/mL). The ethanolic extract had the greatest antisalmonellal activities, with minimum inhibitory concentrations (MICs) value of 128µg/ml against *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi, and 256µg/ml against *Salmonella* Typhi ATCC6539. The ethanolic extract exhibited similar 1, 1-diphenyl-2picrylhydrazyl (DPPH) scavenging activity with control vitamin C, but had the strongest DPPH scavenging activity compared with aqueous and hydroethanolic extracts at 200 µg/mL. For the extract concentration of 200µg/ml and 100µg/ml, ethanolic and aqueous extracts showed comparable Nitric oxide scavenging activity with vitamin C. At 12.5, 100 and 200µg/ml, ethanolic extract showed a significantly (p<0.05) lower ferric reducing power as compared with all other extracts, as well as vitamin C. Phytochemical screening of extracts revealed the presence of flavonoids, phenol, tannins, and triterpenes in all the extracts while alkaloids, steroids and anthraquinone were absent. The results showed that *Zehneria scabra* leaves extracts are promising for a successful treatment of salmonellosis in poultry as well as in *Salmonella*-susceptible mammals but, *in vivo* studies are needed to confirm the efficacy and safety of this plant extract against *Salmonella.*

Keywords: salmonellosis, zoonosis, *zehneria scabra* activity, poultry

1. Introduction
Salmonellosis is an infectious disease caused by *Salmonella enterica*, a gram negative bacillus. *Salmonella enterica* is one of the pathogenic bacteria that thrive in the gastrointestinal tract of livestock. Avian salmonellosis is an important disease causing a serious impediment to the development of poultry industry in developing countries especially in Asia and Africa (Rajagopal and Mini, 2013). Ohl and Miller (2001) [25]. states that *Salmonella* Typhimurium and Enteritidis are the most infectious bacteria that attacks birds, rendering the products harmful for human consumption. In human theses serovars are the causative agents of gastroenteritis whereas the other serovars (*Salmonella* Typhi and Paratyphi) are the causative agents of typhoid and paratyphoid fever in humans (Ali and Sultana, 2012) [1]. Salmonella contamination is also one of the main routes of food poisoning in human.
Avian salmonellosis affects domestic and wild birds, resulting in high mortalities and morbidities, stunted growth, a decrease in oviposition in the poultry industry with significant economic losses (Ramachandranpillai *et al*., 2013). It causes a reduced ability to hatch infected eggs (Wigley *et al*., 2005) [5]. Salmonella contamination of eggs can lead to a very high level of embryonic mortality and rapid death of newly hatched chicks, before clinical signs are observed (Gast, 2003) [14]. In order to prevent gastrointestinal infection with *Salmonella*, farmers usually use antibiotics to suppress the growth of pathogenic bacteria. Unfortunately the use of conventional antibiotics often leads to negative side effects in animals, and to resistance of the target pathogenic microorganisms (Retnani *et al*., 2014). Thus, in developing countries the use of antibiotics as growth promoter for instance in animals is prohibited. For these reasons, there is an increasing need for alternative products with antimicrobial proprieties (Varmuzova *et al*., 2015) [34].
Salmonella infection causes the production of superoxide and nitric oxide radical which in turn react to form peroxynitrite, a strong biological oxidant (Rastaldo *et al*., 2007) [30]. Consequently, pathological conditions characterized by oxidative stress can result in typhoid fever or other bacterial infections (Cook and Samman, 1996 [10]; Sokoudjou *et al*., 2018). Thus there is a need to search for new substances with both antisalmonellal and antioxidant activities. Many plants used for health care by people in various traditional systems show immense medicinal potential (Atsafack *et al*., 2016) [3, 18]. This revival of interest in plant-derived drugs is mainly due to the fact that medicinal herbs are safer and more dependable than the costly synthetic drug, with adverse effects (Pamploma, 1999; Ayachi *et al*., 2009) [14]. Based on the information obtained from traditional practitioners, *Zehneria scabra*

(L.F) is one of the plants frequently used for the treatment of typhoid fever, diarrhea and stomach pain in humans in the West Region of Cameroon. Since Salmonella infection was recently reported as one of the main cause of health problem and decreased productivity in laying hens in Cameroon (Kouam *et al*., 2018, 2019) [19, 20], the aim of this study was therefore to assess the *in vitro* effect of *Zehneria scabra* (L.F) leaf extracts on *Salmonella* responsible for ill-health in poultry in Cameroon.

2. Materials and Methods

2.1 Collection and identification of plant material

Total aerial part of *Zehneria scabra* was collected in the dry season (February 2019) in Foumbot, a village of the Noun Division in the West Region of Cameroon. The plant was identified at the National Herbarium in Yaounde-Cameroon using a voucher specimen registered under the reference HNC N°66689 by Mr. Tadjouteu Fulbert.

2.2 Preparation of different extracts of *Zehneria scabra*

The leaves of *Z. scabra* were air-dried at room temperature and powdered to coarse particles using electronic blender. The extract was prepared using the method described by Duke (2000) [13]. For aqueous extraction, plant powder was macerated by pouring100g of the powder in 1000ml of distilled water and staring it two times daily. For ethanolic and hydroethanolic extraction, 100g of plant powder was dissolved in 95°C ethanol, and in a mixture of solvent 50/50 (distilled water/ 95°C ethanol) respectively; after dissolution the mixture was constantly starred for 48 hours and then were filtered using Whatman paper N°1. The filtrates were concentrated at 45°C in a rotating evaporator for 24hours and the obtained volume was later dried in an oven at 40°C for 5 days to allow the water to evaporate and obtain the extract. The plant extract of each solvent was stored in sterilized bottles at room temperature until usage. The extraction yield was calculated using the following formula: Yield= (Quantity of extract obtained/ Quantity of plant used) ^{x} 100. The extracts were prepared at a concentration of 4096µg/ml by dissolving 8,19mg of the extract in 200µl of Dimethyl-sulfoxide (DMSO) and completing the volume to 2ml with MBH broth. For antioxidant activity, extract were prepared at 2000µg/ml by dissolving 4 mg of extract in 2ml of distilled water.

2.3 *In vitro* antisalmonellal tests

2.3.1 Reference antibiotics and microbial growth indicator

Ciprofloxacin and oxytetracyclin were used as reference antibiotic. P-iodonitrotetrazolium chloride (INT) was used as microbial growth indicator. The presence of viable bacteria changes the yellow dye (INT) to pink color. Wells with no appearance of the pink colour (characteristic of bacterial growth) are noted as inhibitory concentrations.

2.3.2 Bacterial isolates tested and culture media

The tested bacteria were made up of three isolates *Salmonella* Typhi (ST), *Salmonella* Typhimurium (STM) and *Salmonella* Enteritidis (SE)] obtained from the Medical bacteriology Laboratory of "Centre Pasteur" of Yaounde, Cameroon and one strain of *Salmonella* Typhi (ATCC 6539), obtained from "the Research Unit in Microbiology and Anti-microbial Substances", University of Dschang. Salmonella-Shigella agar (SSA) 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) were used as culture media.

2.3.3 Preparation of bacteria Inocula

The activation of these isolates was performed with the streak technique on *Salmonella/Shigella* agar (SSA). This latter was then incubated at 37 °C for 18 hours for activation. The bacterial suspensions were therefore prepared by taking from this culture, colonies which were diluted in sterile physiological water until a turbidity identical to that of the point 0.5 on the scale of Mc Farland, corresponding to a concentration of 1.5×10^8 CFU/ml. These suspensions were diluted100 times with MHB until the desired bacteria concentration for the *in vitro* antibacterial test (1.5×10^6 CFU/ml) was obtained.

2.3.4 Determination of minimum inhibitory and minimum bactericidal concentrations

The susceptibility of *Salmonella* species was tested by broth micro-dilution method. MICs values of *Z. scabra* extracts, and antibiotic on *salmonella* isolates were determined using rapid INT colorimetric assay (Mativandlela *et al*., 2006). These tests were performed in triplicates. The ratio MBC/MIC was calculated to determine the bactericidal (MBC/MIC $\leq$ 4) or bacteriostatic (MBC/MIC > 4) activities of the extracts (Gatsing *et al*., 2006) [15].

2.4 Phytochemical screening

After obtaining the extract from plant material, phytochemical screening was performed according to the standard methods described by Harbone (1973) to determine the presence of alkoloids, tannins, saponins, steroids, triterpenes, phenols, flavonoids, anthocyanins and anthraquinons.

2.5 Antioxidant assay

2.5.1 DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2picrylhydrazyl) assay method was used to study the free radical scavenging activity of leaf extracts of *Zehneria scabra* as previously described (Menson *et al*., 2001).

2.5.2 Nitric oxide (NO) radical scavenging assay

Nitric oxide (NO) generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitric ions, which are measured using the Griess reaction (Green *et al*., 1982) [17].

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 according to the method described by Padmaja *et al*., (2O11) [26]. Increasing absorbance of the reaction mixture indicated higher reduction capacity of the tested extracts (Mohammed *et al*., 2013) [23].

2.5.4 Total phenolic compounds determination

The folin- ciocateu method described by Namekong *et al* (2017) was used.

2.5.5 Total flavonoids content determination

The colorimetric aluminum chloride method was used to

have the total flavonoids content of the *Zehneria scabra* extract. In fact 100µl of extracts (2000µg/ml) was mixed with 1.49ml of distilled water and 0.03 ml of 5% NANO solution. After 5min, 0.03 ml of 1O%ALCL$_3$H$_2$O solution was added. After 6min, 0.2 ml of 0.1 m sodium hydroxide and 0.24 ml of distilled water were added. The solution was well mixed and increase in absorbance was measured at 510 nm using a UV- visible spectrophotometer. The total flavonoids content was calculated using standard method catechin calibration curve. The results were expressed as milligrams of equivalent catechin (mgECT) per gram of extract.

2.6 Statistical Analysis
Data obtained were expressed as mean value ± standard deviation (SD). Significant difference between test and control groups was carried out using one-way analysis of variance (ANOVA) and means were separated using Waller- Duncan test at the significant of 5% through the statistical package for the social science (SPSS) computer software version 20.0

3. Results
3.1 Yield and Physical features of *Zehneria scabra*
Following the extraction of *Zehneria scabra* (L.F) leaf, marked differences were observed at the level of their output as well as their physical appearance. The yield and physical appearance of the various extracts are shown in Table 1. Yield varied from 10.8 to 11.68%, while color varied from dark to dark brown and physical appearance were tender and crystal

Table 1: Yield and physical appearance of *Zehneria scabra* leaves extract

		Physical characteristics	
Extract	**Yield (%)**	**Color**	**Physical appearance**
Aqueous extract	10.8	Dark	Crystal
EE 95°	11.68	Dark brown	Tender
HEE 50/50	11.60	Dark	Tender

EE: 95° Ethanolic extract; HEE: Hydro-ethanolic extract

3.2 Anti-salmonellal assay
The different extracts of *Zehneria scabra* leaf showed antibacterial activities on the tested microorganisms with the MICs values ranging from 128 to 1024 µg/ml. The ethanolic (95%) extract had the lowest activity (128 to 256µg/ml), indicating the greatest antisalmonellal activities, with MIC value of 128µg/ml against *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi and 256µg/ml against *Salmonella* Typhi ATCC6539 respectively. Aqueous extract and hydro-ethanolic extracts had the less active extracts with MICs ranging from 512 to 1024µg/ml.The ratio MBC/MIC was ≤ 4 for ethanolic extract for all the tested bacteria indicating that this extract is the most bactericidal on all the tested bacteria. Hydroethanolic extract showed bactericidal effect only on *Salmonella* Enteritidis. Oxytetracyclin is less active than Ciprofloxacin with MICs of 4µg/ml; the controls were more active than ethanolic extracts. Ciprofloxacin had a bacteriostatic effect only on Serotype typhi ATCC6539. Ethanolic extract are more bactericidal than Ciprofloxacin for all isolates, and is as bactericidal as Oxytetracyclin for STM and ST (Table 2).

Table 2: Inhibition parameters (MIC, MBC) of *Zehneria scabra* leaves extracts against different test microorganisms

		Strain/ isolates			
Tested samples	**Studied parameter**	**STM**	**SE**	**ST**	**STs**
Aqueous	MIC (µg/ml)	1024	1024	512	1024
	MBC (µg/ml)	>1024	>1024	>1024	>1024
	MBC/MIC	/	/	/	/
EE (95%)	MIC (µg/ml)	128	128	128	256
	MBC (µg/ml)	256	512	256	1024
	MBC/MIC	2	4	2	4
HEE 50/50	MIC (µg/ml)	512	512	1024	1024
	MBC (µg/ml)	>1024	1024	>1024	>1024
	MBC/MIC	/	2	/	/
Oxytetracyclin	MIC (µg/ml)	4	4	4	4
	MBC (µg/ml)	8	8	8	16
	MBC/MIC	2	2	2	4
Ciprofloxacin	MIC (µg/ml)	0.5	1	0.5	1
	MBC (µg/ml)	2	4	2	8
	MBC/MIC	4	4	4	8

ST: Salmonella Typhi, STs: Salmonella Typhi ATCC6539, SE: Salmonella Enteritidis, STM: Salmonella Typhimurium. EE: 95% ethanolic extract, HE: hydro-ethanolic extract, MIC= Minimum inhibitory concentration, MBC= Minimum bactericidal concentration.

3.3 Phytochemical composition of *Zehneria scabra* extracts
The phytochemical screening of the extract revealed the presence of different groups of secondary metabolites including alkaloids, anthocyanin, anthraquinone, phenols, flavonoids saponins, steroids, tannins and triterpènes. Alkaloids, anthraquinone and steroids were absent in all the extracts. Saponins and anthocyanin were present only in EE 95° (Table 3)

Table 3: Phytochemical composition of *Zehneria scabra* leaves extracts.

Phytochemical group	Aqueous	EE95°	HE50/50
Alkaloids	-	-	-
Anthocyanin	-	+	-
Anthraquinone	-	-	-
Phenols	+	+	+
Flavonoids	+	+	+
Saponins	-	+	-
Steroids	-	-	-
Tannins	+	+	+
Triterpenes	+	+	+

EE: 95% ethanolic extract, HE: hydro-ethanolic extract; +: present; -: absent.

3.4 Antioxidant activities
3.4.1 DPPH radical scavenging activity
From Table 4, it appears that from all the tested concentrations of the extracts without vitamin c, ethanolic extract showed the best antioxidant activity (lower IC50) than aqueous and hydroethanolic extracts.

Table 4: DPPH radical scavenging activity of leaves extracts of *Zehneria scabra.*

Extract	Extract concentration (µg/mL)					IC50 (µg/ ml)
	12.5	25	50	100	200	
Aqueous	16.33±0.74^{c}	17.40±0.78^{c}	23.68±1.25^{c}	24.28±0.42^{c}	33.33±3.83^{b}	4852
EE 95%	26.02±0.73^{b}	30.77±1.26^{b}	42.28±2.69^{b}	61.31±4.18^{b}	90.80±0.25^{a}	49
HE 50/50	11.48±3.06^{d}	16.73±1.53^{c}	24.18±0.25^{c}	23.66±0.25^{c}	31.39±1.90^{b}	3411
Vitamin C	80.06±0.62^{a}	86.18±0.62^{a}	87.26±0.75^{a}	90.15±1.03^{a}	93.46±0.37^{a}	10

EE: 95% ethanolic extract; HE: hydro-ethanolic extract; ^{a,b,c}: in each column, values with a different letter are significantly different ($p < 0.05$).

3.4.2 Nitric oxide radical scavenging capacity assay.

The leaf extracts of *Zehneria scabra* showed potential antioxidant proprieties against nitric oxide at different concentrations (Table 5). Nitric oxide scavenging activity ranged from 3.91 to 59.58. For the extract concentration of 200µg/ml and 100µg/ml, ethanolic and aqueous extracts showed comparable activity with vitamin C while hydro-ethanolic extract exhibited the lower NO radical scavenging activity than that of vitamin C.

Table 5: Nitric oxide (NO) radical scavenging effect of *leaf* extracts of *Zehneria scabra.*

Extract	Concentration of extracts (µg/mL)				
	12.5	25	50	100	200
Aqueous	56.91±0.14^{a}	57.16±1.50^{a}	58.66±0.87^{a}	55.00±1.08^{a}	49.41±0.38^{a}
EE 95%	53.25±0.25^{b}	56.41±2.37^{a}	59.58±3.05^{a}	58.50±1.75^{a}	51.41±0.87^{a}
HE 50/50	46.75±1.00^{d}	49.00±0.75^{b}	34.91±0.62^{c}	25.58±14.44^{b}	3.91±3.22^{b}
Vitamin C	50.00±1.29^{c}	50.75±0.25^{b}	49.16±0.62^{b}	49.25±0.25^{a}	49.08±0.62^{a}

EE: 95% ethanolic extract, HE: hydro-ethanolic extract; ^{a,b,c}: in each column, values with a different letter are significantly different ($p < 0.05$).

3.4.3 Ferric reducing antioxidant power

Vitamin C (standard) exhibited the greatest ferric reducing activity. At 12.5, 100 and 200µg/ml, ethanolic extract showed a significantly ($p<0.05$) lower reducing power as compared with all other extracts (Table 6).

Table 6: Ferric reducing power of the leaf extracts of *Zehneria scabra*

Extract	Concentration of extract (µg/mL) and absorbance of plant extract				
	12.5	25	50	100	200
Aqueous	0.34±0.001^{b}	0.42±0.002^{b}	0.62±0.004^{b}	0.94±0.003^{b}	1.90±0.003^{b}
EE 95%	0.15±0.011^{d}	0.24±0.009^{c}	0.53±0.003^{c}	0.73±0.004^{d}	0.89±0.004^{d}
HE 50/50	0.20±0.001^{c}	0.26±0.008^{c}	0.53±0.010^{c}	0.77±0.013^{c}	1.46±0.005^{c}
Vitamin C	0.39±0.008^{a}	1.18±0.022^{a}	1.84±0.003^{a}	1.978±0.014^{a}	2.03±0.003^{a}

EE: 95% ethanolic extract; HE: hydro-ethanolic extract; ^{a,b,c}: in each column, values with the different letter are significantly different ($p < 0.05$).

3.4.4 Total phenolic and flavonoids contents of *leaf* extracts of *Zehneria scabra*

The concentration of phenolic compounds in ethanolic extract (mgGAE/g) was significantly ($p<0.05$) higher than in all other extracts. The lowest concentration was observed in aqueous (mgCATE/g) extract. The concentration of flavonoid compounds in aqueous extract was significantly ($p<0.05$) higher than in ethanolic and hydro-ethanolic extract (Table 7).

Table 7: Total phenolic and flavonoids contents of leaf extracts of *Zehneria scabra*

Extract	Total phenolic content (mg EGA/g of extract)	Total flavonoids content (mg ECAT/g of extract)
Aqueous	87.52±2.27^{c}	16.98±2.77^{a}
EE 95%	144.94±2.47^{a}	11.70±0.70^{b}
HE 50/50	127.50±2.90^{b}	3.86±0.75^{c}

EE: 95% ethanolic extract; HE: hydro-ethanolic extract; ^{a,b,c}: in each column, values with the different letter are significantly different ($p < 0.05$; Waller-Duncan test); mgEGA/g: milligrams of Gallic Acid Equivalents per gram of extract; mgECat/g: milligrams of Catechin Equivalents per gram of extract

4. Dicussion

In this study, the evaluation of *Z. scabra* antisalmonellal activity on *Salmonella* Typhi, *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Typhi ATCC6539 was carried out using aqueous, ethanolic and hydroethanolic extracts. The results showed that these extracts are active on all the *Salmonella* isolates studied. However, the MICs obtained with the ethanolic extract (128 - 256) were lower than those obtained with the aqueous and hydroethanolic extracts (512-1024).The ethanolic extract is therefore the solvent which better concentrate the antisalmonellal active ingredients contained in the plant than the other solvents. These results corroborate those of Namekong *et al*. (2017), Sokoudjou *et al*. (2018) who showed that the ethanolic extract of *Enantia chlorantha* and Canarium *schweinfurthii* were more active than the aqueous extract on *Salmonella*

Paratyphi A, *Salmonella* Typhi *Salmonella* Typhimurium and Paratyphi B respectively. However, they are different from those of Bolou *et al*. (2011) [6]. and Noghogne *et al*. (2015) [24]. who showed that the hydroalcoholic and aqueous extract of *Terminalia glaucescens* and *Mangifera indica* were more active than the ethanolic extract on *Salmonella* Typhimurium and *Salmonella* Typhi respectively. The difference in the activity of each extract may be due to the effect of each solvent on solubility, diffusion kinetics and mass transfer of bioactive compounds (Cacace and Mazza, 2003) [8]. Absolutely, it is well known that the affinity between solvent polarity and the compound of interest plays an important role in the extraction processes (Złotek *et al*., 2016) [36]; moreover, the antibacteriocidal effect of this plant was demonstrated against *Escherichia coli* and *Staphylococcus aureus*. The results showed that ethanolic extracts (70%) was more sensitive than other extracts (benzen, chloroform, acetone and distilled water) with a MIC of 1.95mg/ml and 31.25mg/ml for *Staphylococcus aureus* and *Escherichia coli* respectively (Bereket *et al.*, 2014) [5].

The results obtained shows that the ethanolic extract had a lower MIC than the other extracts. This means that its antisalmonellal activity is the most active in ethanolic extract studied at this stage. The difference in activity between these extracts could be explained by the nature of the molecule contained in each of these extracts. Indeed, there are differences in solubilization capacity and extraction of solvents with respect to phytomolecules. According to Cowan (1999) [11], during liquid-liquid extraction, phytomolecules are distributed among solvents according to their polarity and solubility. It could be inferred that the antisalmonellal substances contained in *Z. scabra* are more soluble in ethanol (95%) than in aqueous and hydroethanolic extracts used.

However, the phytochemical test carried out on these extracts revealed the presence of the same compounds (flavonoids, phenols and triterpenes) in all the extracts and the presence of saponins and anthocyanins only in the ethanolic extract. The observed results could be explained by the fact that these compounds are present in these extracts but not in equal quantity to induce the same activity, and that these active compounds are more concentrated in the ethanolic extract (95%). Moreover, the flavonoid assay in these different extracts shows that the ethanolic extract (95%) significantly contains fewer flavonoids compared to the aqueous extract. Thus, the antimicrobial activity of *Z. scabra* extracts is not due to the presence of flavonoids but probably due to the presence of Saponins and anthocyanins which may have a synergistic action. Saponins are known to be antibacterial and to inhibit mold (George *et al.*, 2002) [16]. Anthocyanins are reported to exert antimicrobial activity via inhibiting pathogenic bacterial growth (Cardona *et al.*, 2013).

In addition, the extracts of *Z. scabra* showed variable antibacterial activities against the tested microorganisms. Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC ≤ 4 and bacteriostatic when the ratio MBC/MIC > 4. For the *Z. scabra* extracts used, the ratio MBC/MIC was less than or equal to 4 for ethanolic extracts and more than 4 for Ciprofloxacin on Sérotype Typhi ATCC6539 suggesting that some may be classified as bactericidal agents, whereas others are bacteriostatic against the bacteria strain/isolates. Differences in observed antibacterial activities with respect to the different isolates and strain could be due either to the constitutional or structural variability of the treated bacteria or to the difference in the chemical composition of the genetic resistance elements transferable between strains such as plasmids (Gatsing and Adoga, 2007) [12]. Hence the most active extract (ethanolic 95%) has a greatest against STM and SE (MIC ≤128µg /ml). Oxytetracyclin and Ciprofloxacin were more active than extracts. This can be related to the extract composition which contains inactive compounds while antibiotics are a pure product. In contract the MBC/MIC ratio of ethanolic extract was bactericidal than Ciprofloxacin, and bactericidal effects of ethanolic extract are equals to Oxytetracyclin. Suggesting that ethanolic extract of this plant can be used in the treatment of *Salmonella*.

The antioxidant activity of *Zehneria scabra* extracts was evaluated *in vitro* by the DPPH test, nitrite oxide (ON) and ferric reducing antioxidant power (FRAP). The results of the DPPH anti-radical test showed that the percentages of inhibition are between 11.48% and 90.80%. These percentages of inhibition indicate that the extracts contain an antiradical power. The antiradical activity of these extracts is explained by the presence of polyphenols and flavonoids (Bitchagno *et al.*, 2016) contained in extracts of *Z. scabra*. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron (Bruneton, 2009) [7]. This corroborates the work of Ćetković *et al*. (2007) who showed that the antioxidant activity of some plant extracts (*Solanum montana*) is related to their richness in phenolic compounds. Flavonoids protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, and reduce radicals of alpha-tocopherol or to inhibit oxidases (Ramde-Tiendrebeogo *et al*., 2012) [29].

The highest and lowest IC50 were respectively observed with aqueous (4852 µg/ml) and ethanolic 95% (49 µg/ml) extracts. The aqueous and hydroethanolic extracts with high IC50 value was showed less scavenging activity than ethanolic extracts and vitamin C which had a low IC50 value, suggesting that the latter are more potent scavengers of DPPH radical). Souri *et al*. (2008) [32]. scaled the antioxidant potential of a plant extract into tree ranges: significant when IC50 < 20 µg/ml, moderate when 20 µg/ml ≤ IC50 ≤ 75 µg/ml and weak when IC50 > 75 µg/ml; this suggest that ethanolic extract in this experiment have the best antiradical activity which is however moderate.

In this study, our plant had a reducing power against nitrite oxide, suggesting that this plant can be used to control oxidative stress induced by infection. The reducing power of nitrite oxide could be explained by the presence of both phenolic compounds and flavonoids as revealed by the titration of total phenolic compounds and total flavonoid content of this plant. These phytochemical groups (phenolic compounds and flavonoids) have already been showed to have antioxidant activity (Ara *et al.*, 2009).

The reducing power of iron was observed for all extracts of *Zehneria scabra*. This reducing power is probably due to the presence of hydroxyl groups in the phenolic compounds that can serve as electron donors. Indeed, the presence of reducing agents in the plant extracts would cause the reduction of Fe3 + / ferricyanide complex in ferrous form

(Fe2 +). This corroborates the work of Bougandoura and Bendimerad (2013) who previously showed that the reducing power of some medicinal plants is linked to the hydroxyl group in phenolic compounds.

The 95 % ethanolic extracts showed the best anti-oxidant properties, since its IC50 was the lowest compared to aqueous and hydroethanolic extracts and closer to vitamine C. Its capacity to reduce nitrite oxide and phenol content is very high as in vitamin C compared to other extracts. The effect of antioxidants on DPPH has been thought to be due to their hydrogen donating ability. Many studies showed that DPPH have been used as a substrate to evaluate the anti-oxidative or free radical scavenging activity of plant extracts (Kodjio *et al.*, 2016 [3, 18]; Namekong *et al.*, 2017, Sokoudjou *et al*., 2018).

In conclusion, this study showed that ethanolic extract of *Zehneria scabra* at a concentration of 128µg/ml and 200µg/ml has an antisalmonellal and antioxidant properties respectively. Therefore the extract of this plant are promising for a successful treatment of salmonellosis in poultry but, *in vivo* studies are needed to confirm the efficacy of this plant extract against *Salmonella*.

Ethical statement

This study did not involve animals

Funding statement

This study did not receive any funding from any funding organization

Conflict of interest

The authors declare that they have no competing interests.

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