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Antisamonellal Property and Modes of Action of Leaf Extracts of *Dracaena deisteliana* Engl. (Dracaenaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. Author DG designed the research, Author HBLF took part in all experiments as the principal investigator and performed the experiments, she was assisted by authors MN, GTK, DG and EYK carried out the statistical analysis. Authors HBLF, GKT, JBS and NK readied the manuscript. Authors LCNF and DG read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: In Cameroon, typhoid fever is after malaria, the disease with which health workers are most confronted because its incidence is constantly increasing. Salmonella has become over time multiresistant to antibiotics available on the market, for this reason, it becomes imperative to use drugs made from extracts of parts of medicinal plants consisting of several secondary metabolites at the origin of their good efficacity.
Objective: Determine whether the aqueous and hydroethanolics leaf extract of *Dracaena deisteliana* have *in vitro* antisalmonellal activities, and to determine the site of action of the most active extract on *Salmonella* Typhi strain (ATCC6539).
Methods: To do this, the *in vitro* antisalmonellal activity of aqueous and hydroethanolics leaf extracts of *Dracaena deisteliana* was evaluated by the liquid microdilution method. The modes of action of the 55% hydroethanolic leaf extract of *Dracaena deisteliana* was also done in order to verify the sites of action of this extract.
Results: The *in vitro* antisalmonellal activity revealed that the decocted and 55% hydroethanolic leaf extract of *Dracaena deisteliana* presented MICs of 256 and 128 µg/ml respectively on the *Salmonella* Typhi (ATCC6539) strain on which they were tested. Phytochemical analysis revealed the presence of steroids, terpenoids, tannins, phenols, and saponins in aqueous and hydroethanolics leaf extracts of *Dracaena deisteliana.* The modes by which the 55% hydroethanolic leaf extract of *Dracaena deisteliana* inhibits the growth of *Salmonella,* were studied by following bacterial decay, bacteriolysis, inhibition of biofilms, inhibition of ATPASES^{-}H+ proton pumps, protein synthesis and biofilm formation. The study of growth kinetics on the *Salmonella* Typhi strain (ATCC6539) showed that at all concentrations (2MIC, MIC and 1/2MIC), the 55% hydroethanolic leaf extract of *Dracaena deisteliana* induced the inhibition of bacterial ATPASES^{-}H+ proton pumps. This extract also inhibits the formation of biofilms from 28 to 72% and prevents the synthesis of *Salmonella* Typhi strain (ATCC6539) proteins.
Conclusion: Aqueous and Hydroethanolics leaf extracts of *Dracaena deisteliana* possess *in vitro* antisalmonellal activities.

Keywords: Alternative medicine; antisalmonellal; Dracaena deisteliana; extract; bacterial decay; Modes.

1. INTRODUCTION

"Typhoid fever is an acute generalized infection of the mononuclear phagocyte system, intestinal lymphoid tissue, and gallbladder caused by *Salmonella enterica* serovar Typhi (*Salmonella* Typhi) and associated with poor sanitation and untreated water supply" [1]. "It is endemic in countries, especially throughout Asia and Africa" [2]. The most recent global data show 20 million cases and more than 200.000 deaths per year [3,4]. In Cameroon, over the same period, 145.281 cases were recorded in 2015 compared to 173.603 cases in 2016 [4], indicating a clear increase in the disease [3]. "Typhoid and paratyphoid fevers manifest one or two weeks following infection and the most clinical signs are fever and malaise, abdominal pain and are associated or not with headache, myalgias, nausea, anorexia, constipations and occasionally diarrhea" [5]. "In the absence of adequate treatment, these manifestations can be life-threatening for the patient. Appropriate antibiotic therapy against these salmonellosis lowers the risk of mortality to less than 1%, except that the adaptability of microorganisms and the misuse of these antibiotics induce a proliferation of germs resistant to available antibiotics. Reasons why increasing strains resistant to fluoroquinolones, 3rd generation cephalosporins, and ciprofloxacin are being isolated" [6,3]. "This disease continues to be a major public health problem in developing countries where it remains endemic. Thus, it becomes essential to look for new sources of treatment to fight *Salmonella* infection. The immense property of medicinal plants in the prevention, diagnosis, and treatment of various diseases is summarized by numerous studies" [7-10]. Traditionally, leaves of *Dracaena deisteliana* mixed with stems of *Cenecio biafrae* fight against infertility in women [11]. The stems of *Dracaena deisteliana* taken alone fight toothache [12]. Scientifically, the ethanolic extract from leaves of this plant harvested from the Bamboutos had no effect on *paratyphi* A and B and on *Salmonella Typhi* strain ATCC 6539 *in vitro* [13]. Methanolic leaf extract of *Dracaena deisteliana* is constituted of phenolic compound,

saponins, alkaloids, terpenoids, anthraquinones, and flavonoids [14]. However, plant activities may vary depending on the harvest location, extraction method, and solvent system used for extraction [15]. Where from the importance of this work, which consisted of determining the most active extract of the leaves of *Dracaena deisteliana* harvested in Dschang, and prepared using various methods and various solvent systems of extraction (aqueous and hydroethanolique 95%, 85%, 75%, 65%, 55% and 45%), as well as the different sites of action of the latter on the strain of *Salmonella* Typhi ATTCC6539.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant material

The leaves of *Dracaena deisteliana* were harvested in Dschang (Menoua division, West Region of Cameroon) in January 2020. The plant was identified at the Cameroon National Herbarium (Yaoundé) by comparing our sample with the available deposited specimen having voucher number 53011HNC.

2.1.2 Microorganisms and culture media

The microorganisms used to determine the antibacterial activities of the extracts were Gram-negative bacteria: 2 resistant clinical isolates of *Salmonella enterica* serovar Typhi (ST and S566), a sensitive clinical isolate of Salmonella enterica serovar Enteridis (SE), a sensitive clinical isolate of *Salmonella enterica* serovar Typhimurium (STM) obtained from the "Centre Pasteur du Cameroun" and a sensitive strain of *Salmonella* Typhi ATCC6539 from the American Type Culture Collection. The microorganisms were stored at 4°C on *Salmonella* Shigella Agar (SSA) (Accumix, Belgium). This medium was used for the activation of bacteria. Mueller Hinton broth (MHB) (Liofilchem, Italy) was used as a basic enrichment medium for the determination of Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC). The various culture media were prepared as described by the manufacturers.

2.1.3 Standard antibiotic

Ciprofloxacin (Sigma Aldrich, Germany) was used as the reference antibiotic. Its choice is justified by the fact that it is used in the first-line treatment of typhoid fever (typhoid and paratyphoid fevers) in Cameroon [16,17].

2.2 Methods

2.2.1 Preparation of extracts

The harvested *Dracaena deisteliana* leaves were dried out from the sun and crushed using moulinex (SINGSUNG, SINGAPOREE BL-530). The powder obtained made it possible to prepare the aqueous extracts (infused and decocted) according to the protocol described by Duke (2000) and hydroethanolic extracts (95%, 85%, 75%, 65%, 55% and 45%) according to the protocol described by [11]. These different extracts were stored at -4°C and were used for the evaluation of *in vitro* antisalmonellal activity of leaf extracts of *Dracaena deisteliana*.

2.2.2 Extraction efficiency

The yield as a percentage of extract relative to the mass of the treated plant material was calculated according to the formula of [18]: R= (m ×100)/m°

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

2.2.3 Study of *in vitro* antisalmonellal activity of *Dracaena deisteliana* leaf extracts

2.2.3.1 Preparation of bacterial inocula

The bacterial suspensions were prepared by taking samples from 18 hours culture colonies that have been diluted in sterile physiological water to obtain a turbidity identical to that of point 0,5 on the Mc Farland scale, corresponding to a concentration of 1.5×10^8 CFU/ml. These suspensions were diluted with Mueller-Hinton broth until the concentration of bacteria desired for the test (1.5×10^6 CFU/ml).

2.2.3.2 Preparation of stock solutions of extracts of Dracaena deisteliana leaf

For the evaluation of antisalmonellal activity in liquid media (determination of MIC and MBC), the stock solutions of extracts were prepared at 4096 µg/ml in 2.5% DMSO combined with 2.5% distilled water till the complete dissolution of extract. The mixture was diluted so that the final concentration ranged from 1024 µg/ml to 8 µg/ml. The stock solution of ciprofloxacin (positive control) was prepared at 256 µg/ml in 2.5% DMSO combined with 2.5% distilled water

so that the final concentration ranged from 64 µg/ml to 0.5 µg/ml.

2.2.3.3 Determination of Minimum Inhibitory Concentrations (MIC)

The inhibitory property of bacterial growth of leaf extracts of *D. deisteliana* was determined by the microdilution method as described by [18]. In each well of a microplate of 96 wells, 100 µl of culture broth (MHB) was introduced, then, 100 µl of each extract was added, respectively, into the first 3 wells of the first line and serial dilutions following a geometric progression of reason 2 were carried out. A volume of 100 µl of bacterial inoculum was introduced into each well. Ciprofloxacin was used as the reference antibiotic and was the positive control. Negative controls were performed in wells containing culture medium, mixture culture medium and DMSO (2.5%), and the inoculum. Neutral controls were performed in wells containing 200 µl of culture medium. The plates were carefully covered with a sterile lid and then sealed with parafilm paper and incubated at 37°C. After 18 h of incubation, the MIC values of each extract were obtained by adding 51.84 µl of an aqueous solution of para-iodonitrotetrazolium chloride (INT 0.02%) to each well and incubating again for 30 min at 37 °C. All concentrations that prevented the appearance of the pink color were taken as inhibitory concentrations and the smallest was noted as MIC. For each extract three columns were made and the revelation was made in two columns. The third was used to determine the minimum bactericidal concentrations. This test was performed in three independent repetitions.

2.2.3.4 Determination of Minimum Bactericidal Concentrations (MBC)

For the determination of MBC values, 50 µl from each well where there was inhibition of bacterial growth (no pink staining) were plated on 150 µl MHB and incubated at 37°C for 48 hours. The lowest concentrations that yielded no color change 30 minutes after the addition of INT were taken as the MBC values.

2.2.3.5 Phytochemical screening of various extracts of the leaves of Dracaena deisteliana

In order to determine the different classes (alkaloids, phenols, flavonoids, tannins, anthraquinones, anthocyanins, terpenes, and Saponins) of property bioactive compounds present in *D. deisteliana* extracts, phytochemical tests were carried out according to the standard methods of [19].

2.2.4 Action mechanism of 55% hydroethanolic leaf extract of *Dracaena deisteliana*

2.2.4.1 Measurement of the lytic activity of the extract

The lytic activity of the extract on *Salmonella* Typhi (ATCC6539) was evaluated according to the method described by [20]. Briefly, 18 hours bacterial colonies were picked on agar and introduced into 0.9% NaCl solution. The resulting suspension was standardized to have a turbidity corresponding to Mc Farland 0.5 standard (1.5×10^8 CFU/ml). Then, 100 µl of plant extract was introduced into the wells of a microplate containing the suspension to have ½ MIC, MIC and 2 MIC as the final concentration in the wells. The resulting mixtures were shaken and the absorbances were immediately measured at 620 nm (T0). Then, the plates were incubated at 37°C with shaking and the absorbances were measured at 620 nm, respectively, every hour for 5 h (T1; T2; T3; T4 and T5). The absorbances measured at T0 were used to determine the relative absorbances as a function of time. The test was performed in triplicate.

2.2.4.2 Effect on protein content

This method allows the evaluation of the effect of the extract on bacterial protein synthesis. It was implemented following the protocol described by [21]. In 15 tubes each containing 9 ml of MHB, 0.5 ml of bacterial suspension (standardized to Mc Farland 0.5 scale) was added. Then, 0.5 ml of extract from the leaf of *Dracaena deisteliana* was added to each tube to obtain the following concentrations: 2 MIC, MIC, and ½ MIC (each treatment was done in triplicate). The control tube was treated under the same conditions and received 0.5 ml of MHB instead of the plant extract. The tubes were incubated at 37°C with shaking. After 24 hours of incubation and centrifugation at 12,000 rpm for 3 minutes, there was recovery of the pellet that represented the bacterial cells. These cells were weighed and mixed with lysis buffer (10 mM Tris/HCl, pH 7.4; 100 mM EDTA, pH 7.4; 20 mM NaCl, 1% (w/v) SDS) at a rate of 40 mg bacteria per 500 µl buffer. Once the bacteria were lysed, the samples were centrifuged at 12,000 rpm for 3

minutes and the protein concentration was assessed in the supernatant by the Bradford method using a microplate reader (SoftMax Pro. USA). The blank was the lysis buffer. Protein synthesis will be inhibited if the protein content decreases compared to the control group not treated with the extract.

2.2.4.3 *External membrane permeability test of the bacteria*

The potentiating effect of the plant extract on the membrane permeability to erythromycin was performed according to the method described by [22]. With some modifications. In sterile microplates of 96 wells, 50 µl of bacterial inoculum of *Salmonella Typhi* (ATCC 6539) at the concentration of 1.5×10^6 CFU/ml and prepared from a bacterial culture of 18 h was inoculated in Mueller Hilton broth containing 50 µl of the extract prepared to have 128 µl as the final concentration of the extract combined with 50 µl of erythromycin at concentrations ranging from 0.0625 to 8 µg/ml. The whole was incubated at 37°C for 18 h. Then the growth of the bacteria was measured in a microplate reader (SoftMax Pro. USA) at 450 nm and the results were expressed as optical density. The test was performed in triplicate.

2.2.4.4 *Evaluation of the effect of the extract on bacterial ATP-H+ proton pump*

This was done by acidifying the external environment of the bacteria using a pH electrode according to the method described by [23]. Briefly, an 18 h bacterial colony was taken and introduced into 20 ml of Muller Hinton broth contained in an Erlen-Meyer to have a concentration of 1.5×10^6 CFU/ml. The preculture was prepared by maintaining the whole at 37°C for 18 hours. Then, aliquots of this bacterial preculture were taken and diluted 1:100 (v/v in MHB contained in Erlen-Meyer) and 100 ml of bacterial culture was then taken and centrifuged at 4249 rpm for 30 minutes at 4°C. The pellet was washed with distilled water, then with KCl (50 mM) and re-suspended in 50 ml of KCl (50 mM). The suspension was then stored at 4°C for 18 hours (for glucose starvation). To 4 ml of these solutions, 0.5 ml of extract dissolved in DMSO was added to obtain final concentrations corresponding to 64 µg/ml. The pH was adjusted to 6.4 by adding HCl and/or NaOH. After 10 minutes of incubation at 37°C, the acidification of the medium was triggered by adding 0.5 ml of a 20% glucose solution whose rapid catabolism will be accompanied by the release of protons into the medium. Thereafter, the pH of the medium was measured every 10 minutes for 1 hour. For the negative control, the extract was replaced by 2.5% DMSO. The recorded pH values were used to plot pH versus time curves. Any inhibition of the acidification of the medium in the presence of a plant extract was attributed to an inhibitory effect of the operation of ATPase-H+ pumps by this extract. This test was performed in three replicates.

2.2.4.5 *Evaluation of the antibiofilm activity of the extracts*

The anti-biofilm property of the extracts was determined according to the method described by [24], using the MTT colorimetric assay to quantify the biofilms formed. Briefly, 100 µl of MHB supplemented with 2% glucose was introduced into a 96-well microplate. Extracts at 1024 µg/ml were introduced into the upper wells and microdiluted in a geometric progression of reason 2. Subsequently, 100 µl of a Mc Farland 0.5 scale bacterial suspension was introduced into the wells. Negative control wells consisted of Muller Hinton broth and inoculum, while positive control wells consisted of Muller Hinton broth, inoculum, and ciprofloxacin. After being carefully covered and sealed with kerosene paper, the plates were incubated for 48 hours at 37°C. After incubation, the microplates were washed in phosphate buffer (0.2 M; pH 7.4). The plates were subsequently dried. The biofilm formed by the adherent cells was quantified after 2 hours of incubation at 37°C in the microplate wells where 100 µl of MTT solution (0.5 mg/ml) was previously introduced. The plate wells were then emptied and 200 µl of DMSO was added to solubilize the reduced formazan. Optical densities were read at 570 nm with a microplate reader (SoftMax Pro). Biofilm inhibition occurred if the number of biofilms formed decreased compared to the control group not treated with the extract.

% inhibition of biofilms= ((Do control cells – Do treated cells)/(Do cells – Do treated cells)) x 100

3. RESULTS

3.1 Yield of Various Hydroethanolic and Aqueous Extracts

The extraction of the leaves of *Dracaena deisteliana* with different solvent gave various yields. The best yields were obtained with the

65% (43, 27%) hydroethanolic system. While the smallest yield was obtained with 85% (1, 293%) hydroethanolic leaf extract of Dracaena deisteliana system (Fig. 1).

3.2 *In vitro* antisalmonellal Activity of the Leaf Extracts of *D. deisteliana.*

3.2.1 Determination of Minimum Inhibitory Concentrations (MIC)

The antibacterial activity of the different extracts of this plant is presented in Table 1. Analysis of this table reveals that the minimum inhibitory concentrations (MIC) range from 64 to 1024 µg/ml for the strain and isolates tested. The 55% hydroethanolic extract had the best MIC (128 µg/ml) on STs while the decocted extract had the best MIC 256 µg/ml I on STs among the tested extracts. The MBC of 95% hydroethanolic extract had the good MBC 128 µg/ml on STs, and the decocted extract had an MBC on STs of 1024 µg/ml in. The 75% hydroethanolic extract had a little ratio MBC/MIC 2.

3.2.2 Phytochemical study of leaf extracts of *Dracaena deisteliana*

Qualitative phytochemical screening of extracts from *Dracaena deisteliana* leaves has led to the identification of several classes of secondary metabolites. Table 2 groups the different compounds detected and reveals the presence of phenols, flavonoids, saponins, anthocyanins, and terpenes in all extracts tested. However, tannins are present in 65%, 75%, 85% extracts and in all aqueous extracts. Sterols are present only in hydroethanolic extracts (65, 75, 85, and 95%). Anthraquinones are present in all extracts except in the extract 95%.

3.3 Probable Modes of the Antisalmonellal Activity of the 55% Hydroethanolic Leaf extract of *Dracaena deisteliana*

3.3.1 Bacteriolytic activity of the extract

Fig. 2 shows the lytic activity of the 55% hydroethanolic leaf extract of *Dracaena deisteliana* at different concentrations (1/2 MIC, MIC and 2 MIC) on *Salmonella* Typhi (ATCC6539) as a function of time. Analysis of this figure reveals a decrease in absorbance in bacterial cells treated with different concentrations of extract compared to untreated cells. This decrease is significant in cells treated with 2 MIC during the whole test and for the first hour in bacterial cells treated with extract at MIC and 1/2 MIC compared to untreated cells.

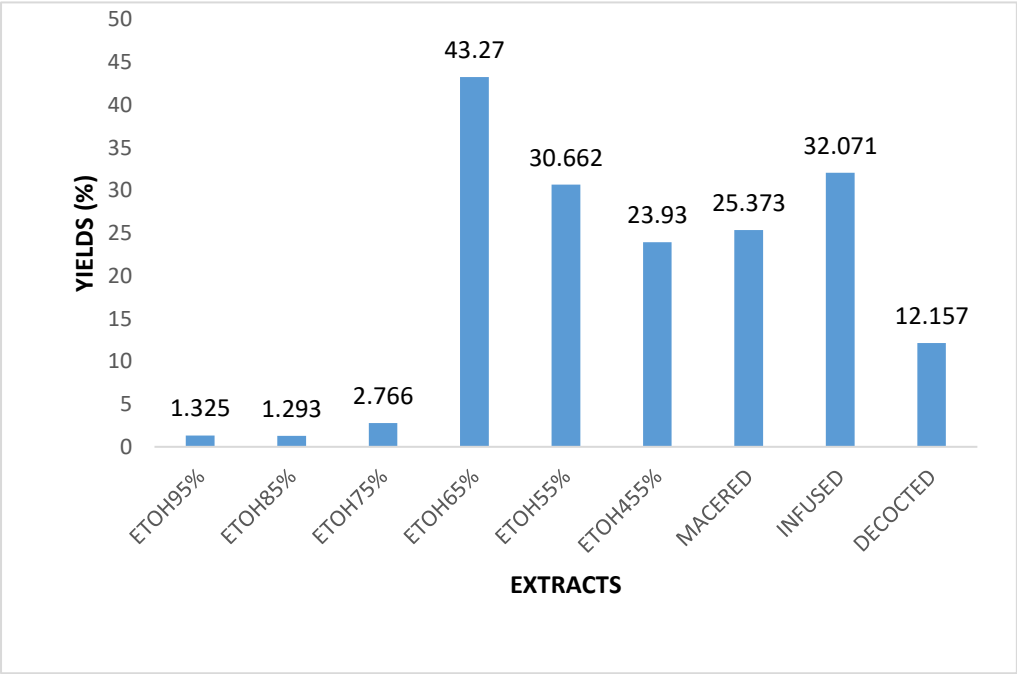

Fig. 1. Yields of hydroethanolics and aqueous leaf extracts of *Dracaena deisteliana*. EtOH: hydroethanolic; MA: Aqueous Macerate, AI: Aqueous Infused, DA: Aqueous Decocted

Table 1. MIC, MBC, and MBC/MIC values of the different leaf extracts of *Dracaena deisteliana* against different isolates and bacterial strains

Extract		Bacteria Isolated and Strain				
		STM	**STs**	**SE**	**ST**	**S566**
ETOH 95% (Macerated)	**MIC (µg/ml)**	1024	128	256	128	512
	MBC (µg/ml)	1024	128	1024	512	1024
	MBC/MIC	1	1	4	4	2
ETOH 85% (Macerated)	**MIC (µg/ml)**	512	128	128	128	1024
	MBC (µg/ml)	512	512	128	512	1024
	MBC/MIC	1	4	1	4	1
ETOH 75% (Macerated)	**MIC (µg/ml)**	512	128	128	128	512
	MBC (µg/ml)	_	512	1024	512	1024
	MBC/MIC	_	2	8	4	2
ETOH (Macerated)	**MIC (µg/ml)**	128	128	128	128	512
	MBC (µg/ml)	512	512	256	512	_
	MBC/MIC	4	4	2	4	_
ETOH 55%(Macerated)	**MIC (µg/ml)**	128	128	128	64	512
	MBC (µg/ml)	512	512	512	256	1024
	MBC/MIC	4	4	4	4	2
ETOH 45%(Macerated)	**MIC (µg/ml)**	128	254	128	64	512
	MBC (µg/ml)	512	512	256	128	1024
	MBC/MIC	4	2	2	2	2
AD	**MIC (µg/ml)**	128	256	512	256	512
	MBC (µg/ml)	512	1024	1024	512	512
	MBC/MIC	4	4	2	2	1
IA	**MIC (µg/ml)**	1024	1024	1024	1024	1024
	MBC (µg/ml)	_	_	_	_	_
	MBC/MIC	_	_	_	_	_
AM	**MIC (µg/ml)**	256	512	512	256	512
	MBC (µg/ml)	256	1024	1024	512	_
	MBC/MIC	1	2	2	2	_
Ciprofloxacin	**MIC (µg/ml)**	0,5	0,5	0,5	0,5	64
	MBC (µg/ml)	16	16	16	16	1024
	MBC/MIC	32	32	32	32	16

ETOH: Hydroethanolic extract, AD: Aqueous decoctate, AI: Aqueous infused, AM: Aqueous Macerate; STs: Salmonella Typhi strain ATCC6539; S566: multidrug-resistant (Salmonella Typhi); ST: Salmonella Typhi; STM: Salmonella Typhimurium; SE: Salmonella Enteritidis; MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration

Table 2. Phytochemical composition of extracts from the leaves of *Dracaena deisteliana*

EXTRACTS	ETOH 95%	ETOH 85%	ETOH 75%	ETOH 65%	ETOH 55%	ETOH 45%	AD	AM	IA
Alkaloids	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Tannins	-	+	+	+	-	-	+	+	+
Anthraquinones	-	+	+	+	+	+	+	+	+
Anthocyanines	+	+	+	+	+	+	+	+	+
Terpenes	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	-	-	-	-	-
Saponins	+	+	+	+	+	+	+	+	+

ETOH: hydroethanolic extract, (-) Absent, (+) present, AM: Aqueous Macerated, AD: Aqueous Decoctate, IA: Aqueous Infused

3.3.2 Effect of different concentrations of 55% hydroethanolic leaf extract of *Dracaena deisteliana* on the protein synthesis of *Salmonella Typhi* (ATCC6539)

Fig. 3 illustrates the effect of different concentrations of 55% hydroethanolic leaf extract of *Dracaena deisteliana* on the protein synthesis of *Salmonella* Typhi (ATCC6539). This figure shows a significant decrease (0.0125; 0.0226, 0.0328; 0.0435) in the amount of protein in treated bacteria inversely proportional to the different concentrations of the extract, compared to untreated bacteria (Control).

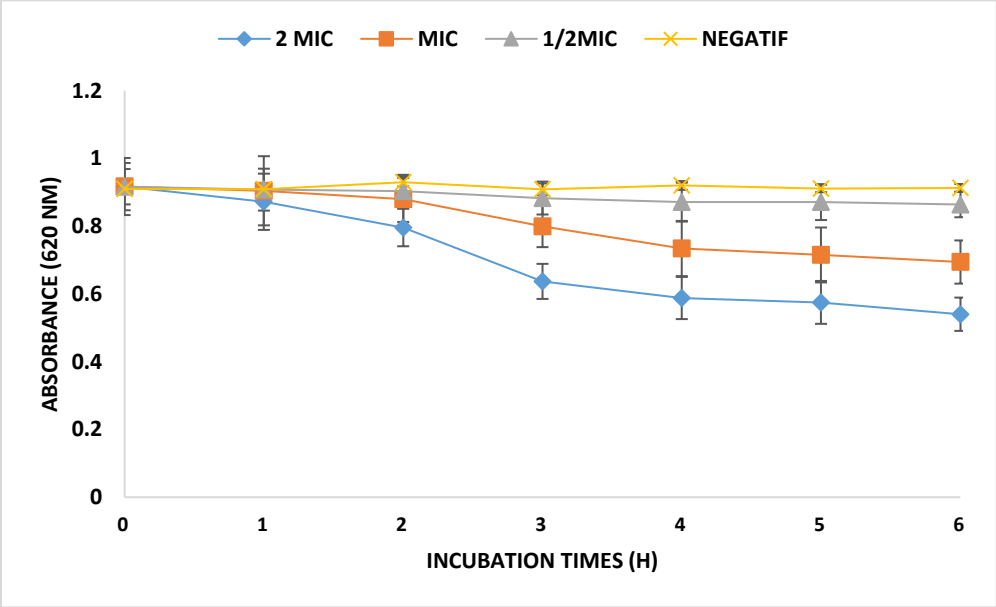

Fig. 2. Effect of 55% hydroethanolic leaf extract of *Dracaena deisteliana* at different concentrations and the lytic activity of *Salmonella* Typhi

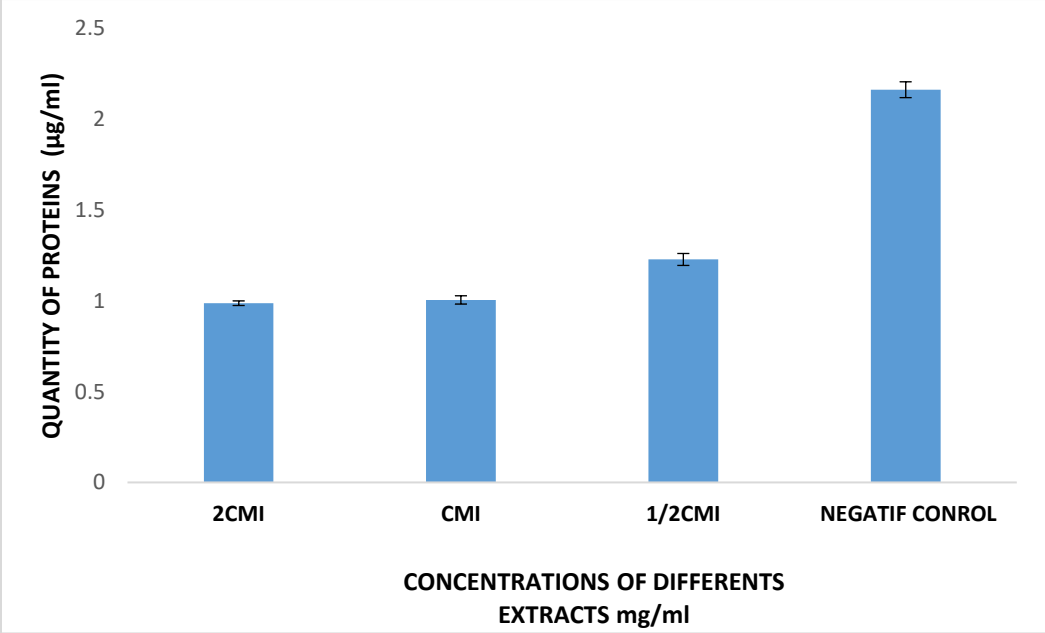

Fig. 3. Effect of 55% hydroethanolic leaf extract of *D. deisteliana* on protein synthesis of *Salmonella* Typhi

3.3.3 Effect of the combination of 55% hydroethanolic leaf extract of *D. deisteliana* with erythromycin on the growth of *Salmonella* Typhi (ATCC6539)

Fig. 4 shows the effect of the combination of 55% hydroethanolic leaf extract of *D. deisteliana* with erythromycin on the growth of *Salmonella* Typhi (ATCC6539). It was found that the extract and erythromycin combination induced growth inhibition in treated bacterial cells compared to untreated bacterial cells. This decrease was observed at all concentrations of erythromycin (0.0625 to 8 µg/ml) in combination compared to the same concentrations (0.0625 to 8 µg/ml) of erythromycin alone.

3.3.4 Effect of different concentrations of 55% hydroethanolic leaf extract of *D. deisteliana* on biofilm formation

The percentages of inhibition of *Salmonella* Typhi (ATCC6539) biofilms by the 55% hydroethanolic leaf extract of *D. deisteliana* are presented in Fig. 5. It is shown that this extract inhibits the formation of biofilms in a concentration-dependent manner. Compared to the effect of ciprofloxacin, the percentage inhibition of the different concentrations of the extract remained significantly low.

3.3.5 The pH changes of the reaction medium containing *Salmonella* Typhi cultures treated with different concentrations of the 55% hydroethanolic leaf extract of *Dracaena deisteliana*

Fig. 6 shows the pH changes of the reaction medium containing *Salmonella* Typhi cultures treated with different concentrations of the 55% hydroethanolic leaf extract of *Dracaena deisteliana.* It can be seen that the pH values of the medium decreased more during the whole test in bacterial cells treated with different concentrations of extracts in 60 minutes at 2 MIC (6.4 to 5.84); at MIC (6.4 to 5.44); at 1/2 MIC (6.4 to 5.08)) compared to the untreated bacterial cells (6.4 to 4.35).

4. DISCUSSION

4.1 *In vitro* Antisalmonellal Activity of the Leaf Extracts of *D. deisteliana.*

The search for alternative treatments is one of the priorities of health policies and is part of effective strategies to combat typhoid fever [25]. The results obtained following *in vitro* antimicrobial tests (MIC and MBC) showed that the extracts of *Dracaena deisteliana* possess interesting antisalmonellal activity. The

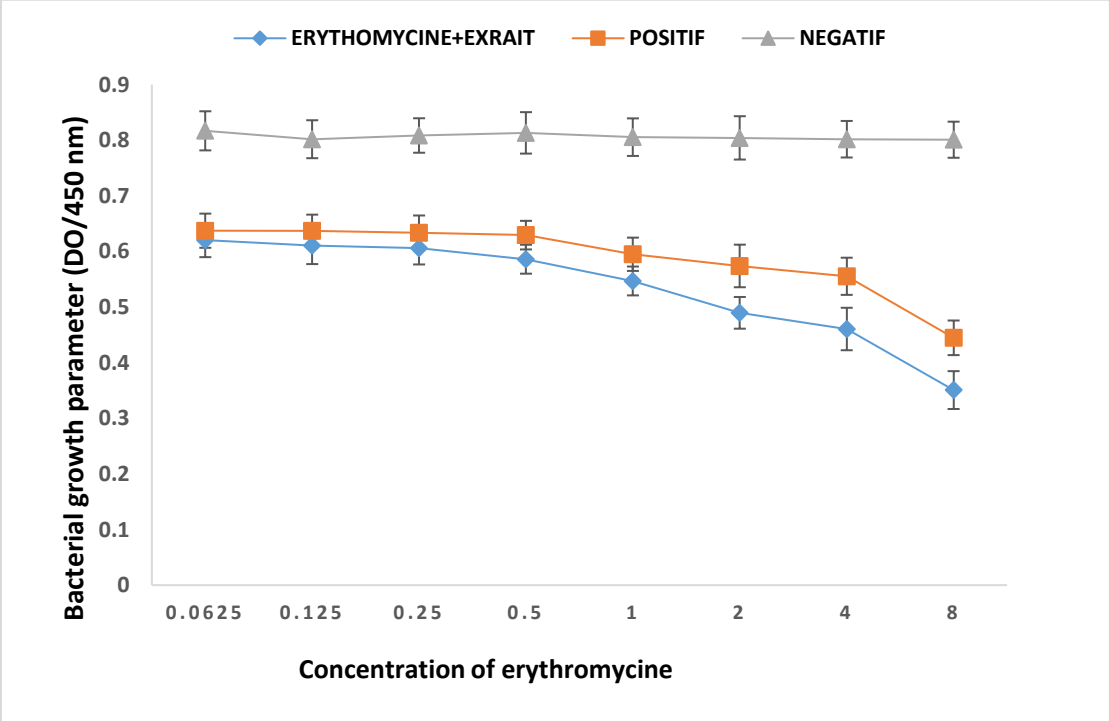

Fig. 4. Effect of 55% hydroethanolic leaf extract of *Dracaena deisteliana* on membrane permeability of *Salmonella* Typhi

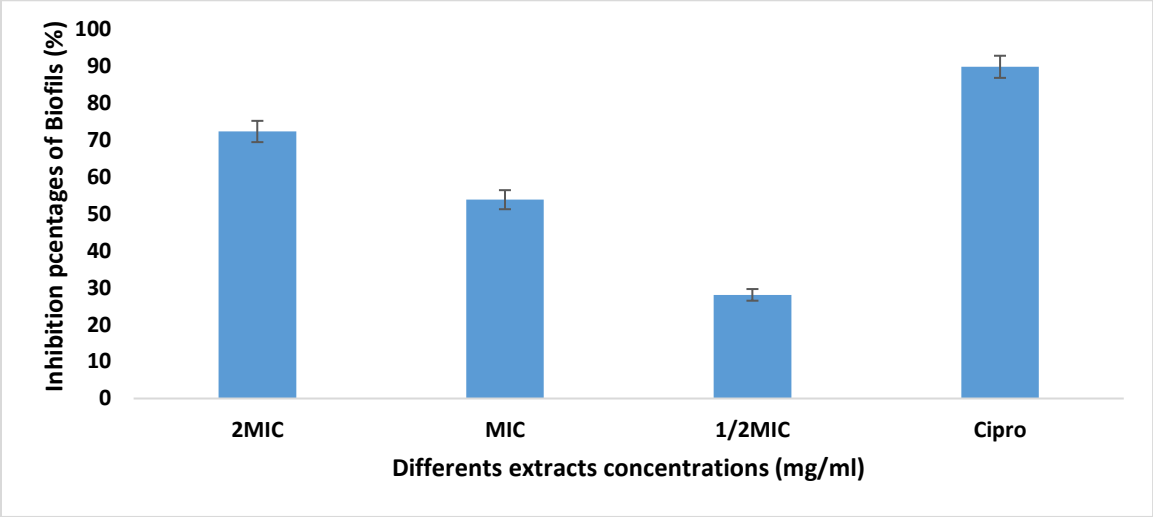

Fig. 5. Percentage of biofilm inhibition of the 55% hydroethanolic leaf extract of *Dracaena deisteliana*

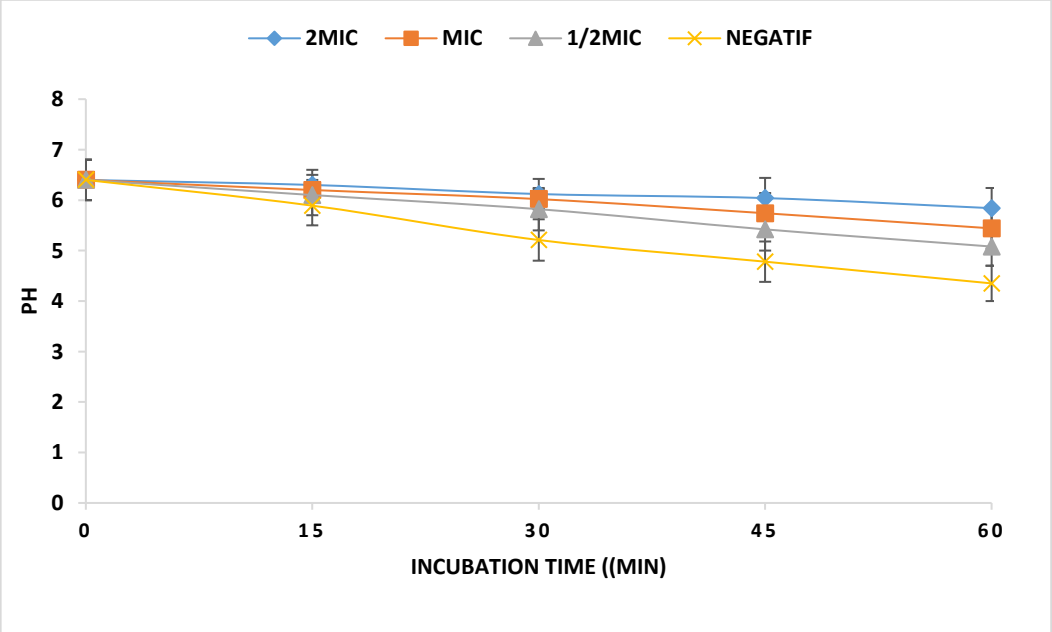

Fig. 6. Effect of 55% hydroethanolic leaf extract of *Dracaena dracaena* on ATPase-H+ pumps in the reaction medium inoculated with *Salmonella* Typhi

antibacterial activity of the extracts of this plant varied depending on the solvent system and the extraction method. Hydroethanolic extract 55% was found to be the most active (MIC = 128 µg/ml) on all germs against which they were tested. According to the activity classification scale of [24], 55% hydroethanolics leaf extract showed significant activity (MIC ≤ 512 µg/ml). According to this scale, this extract is significantly active because of the MIC ≤ 512 µg/ml obtained. These results obtained with the leaves of *Dracaena deisteliana* thus justify their traditional use in the treatment of typhoid fever and microbial infections.

Phytochemical analysis of the different extracts tested revealed the presence of certain secondary metabolites (phenols, flavonoids, sterols, terpenoids, tannins, saponins, anthocyanins and anthraquinones). Indeed, anthraquinones are well known for their antisalmonellal activities [26]. A substance is bacteriostatic when its MBC/MIC ratio > 4 and bactericidal when it's MBC/MIC ratio ≤ 4 [27,9]

hence, all hydroethanolic leaf extract of *D. deisteliana* can be classified as bactericidal because all their MBC/MIC ratios are less than or equal to 4. Indeed, several studies have already shown that the solvent system and extraction method used can strongly influence the secondary metabolic content [28-30] and the activities of extracts [31-33]. This is confirmed in this work with the presence of many classes and subclasses of secondary metabolites present in each extract depending on the solvent used and the extraction method used. The difference in activity observed between the different extracts on the one hand and the isolates on the other hand may be due to the constitutional or structural variability of the germs tested. It could also be due to the difference in the chemical composition of the genetic elements between the germs [34], or due to the difference in the composition of secondary metabolites encountered in each extract. It is likely that the antimicrobial activity of the extracts is not attributable to a single compound, but to the synergistic action of the compounds present in the extracts at various sensitive locations of bacteria. According to [35,36], who had similar results in their work on plant extracts, some bacteriostatic or bactericidal substances act by interfering with cellular components and molecules essential for the survival of bacteria such as the cell wall, genome, proteins, and vitamins.

4.2 Modes of Action of the Antibacterial Activity

The study of the modes of action of a substance allows to determine the site(s) on the bacteria where this substance can react. For this reason, five modes of action of the 55% hydroethanolic leaf extract of *Dracaena deisteliana* have been realized. The substances are able to cause membrane damage by complete lysis of the bacterial cell. In this study, the decrease in absorbance observed at 620 nm in bacterial cells treated with the 55% hydroethanolic leaf extract of *Dracaena deisteliana* reflects the lytic action of the extract on *Salmonella* Typhi ATCC 6539. According to [37], lysis is marked by a decrease in the absorbance of the solution to 620. This activity is justified by the presence in this extract of anthraquinones and anthocyanins which are known for their lytic properties. Erythromycin is a macrolide antibiotic that acts by inhibiting protein synthesis by binding to the 50S ribosomal subunit. In small concentrations, this antibiotic is unable to penetrate the intact outer membrane of Gram-negative bacteria [38] because of their large size. In the presence of the extract, a greater activity of this molecule is observed at subinhibitory concentrations, this result could reflect the ability of the 55% hydroethanolic leaf extract of *Dracaena deisteliana* to facilitate the passage of erythromycin through the outer membrane of the bacterium, with consequent destabilization of the membrane causing the increase in membrane permeability. This result corroborates the work of [22] who showed that the hydroethanolic extract of *Leonotis nepetifolia* increased the membrane permeability of Gram-negative bacteria specifically that of *Escherichia coli*. According to, [39], this test is considered an indicator of irreversible bacterial cytoplasmic membrane damage. Because damage to the bacterial membrane can lead to the release of cytoplasmic constituents of the microbial cell [40]. Some biocidal substances exert their actions by inhibiting key enzymes of the synthetic voice of cellular macromolecules (nucleic acids, proteins) with the consequence of the decrease in the concentration of the molecule concerned in the bacterium [40,41]. In this work, the decrease in the amount of protein observed in bacterial cells treated with the 55% hydroethanolic leaf extract of *D. deisteliana* at different concentrations reflects the defect of protein synthesis. This means that this extract has compounds that interfere with one of the processes of protein synthesis in addition, to the presence of flavonoids that are known for their ability to inhibit topoisomerases (enzymes that regulate the binding of DNA molecules). They are able to cut one of the two paired DNA strands, pass one strand through the cut, and relegate the capture. This process adds or removes super strands to the DNA, an important mechanism for genome replication during cell multiplication. These small molecule inhibitors act as simple effective bactericides. The inhibition of eukaryotic topoisomerases induces DNA breaks that force cells to enter the cycle of programmed bacterial cell death (apoptosis) [42].

The decrease in the pH of a medium in the presence of an antibacterial substance compared to the negative control would result from the inhibitory effect of ATPase-H+ proton pumps by this substance [43,44]. Proton pump are a prime target for antimicrobial compounds [45]. The present results showed that the 55% hydroethanolic leaf extract of *D. deisteliana* inhibited the ATPase/H+ proton pumps of *Salmonella* Typhi ATCC(6539), indicating that ATPase/H+ proton pump were also one of the

property targets of this extract. Inhibition of these pumps by the extract will therefore be deleterious to the bacteria as it will prevent the excretion of protons in to the external environment, thus making the environment less acidic and compromising for the survival of the bacteria [46,47]. These results confirm the bacteriostatic and bactericidal activities observed above and corroborate the work of [48], who showed that the methanolic extract of *Enantia chlorantha* had an inhibitory effect on ATPASE-H+ proton pump on gram negative bacteria.

Biofilms are structured clusters of bacterial cells embedded in a polymeric matrix and attached to a surface that allow them to survive in hostile environmental conditions [48]. Some biocidal substances exert their antimicrobial actions by inhibiting biofilm formation [45]. In the present study, the 55% hydroethanolic leaf extract of *D. deisteliana* inhibited biofilm formation by *Salmonella* Typhi strain with the percentages of inhibition varying with the concentration of the extract 2MIC: 72.386; MIC: 53.897; 1/2MIC: 28.093 and ciprofloxacin 89.92. The ability of these extracts to inhibit biofilm formation would reside in their composition of phenolic compounds (notably flavonoids), which possess anti-adhesive activities that prevent the bacteria from adhering to a biotic surface and thus prevent biofilm formation [49]. These results corroborate those obtained by [50,51,52] who showed the ability of *Tristemma mauritianum* to inhibit biofilm formation of *Salmonella* Typhi ATCC 6539.

5. CONCLUSION

Aqueous and Hydroethanolics leaf extracts of *Dracaena deisteliana* possess *in vitro* antisalmonellal activities. The 55% hydroethanolic leaf extract of *Dracaena deisteliana* induced the inhibition of bacterial ATPASES-H+ proton pumps. This extract also inhibits the formation of biofilms from 28 to 72% and prevents the synthesis of *Salmonella* Typhi strain (ATCC6539) proteins.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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