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Reduction of the flux of bacteriopolluants in the aquatic microcosm by extracts of *Sida rhombifolia* (Malvaceae) in response to storage in containers of a different nature

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Abstract

The present study aims at evaluating the influence of the temperature of conservation, time of contact, nature of conditioning and *in-vitro* antibacterial activity of *Sida rhombifolia* extracts on some bacteria strains.

Methodology: The extracts of *Sida rhombifolia* were screened for different classes of compounds. Water samples from which bacteria strains were isolated, were collected from wells in Nsam- Yaoundé Cameroon. Standard guidelines were used for the antimicrobial activity with variation of nature of conditioning, temperature of conservation and the time of contact on the different bacteria strains.

Results: The classes of secondary metabolites identified from the extracts were phenols, flavonoids, triterpenes, tannins, coumarins and saponins. The ethanolic extract showed remarkable activity (max = 100% inhibition) after 6 and 12 hours of contact period with *Salmonella typhi* conditioned in glass; which differs slightly from those of *E. coli* (max = 99.9%). A maximum of 76.7 to 99.7% were observed for *Salmonella typhi* and *E. coli*, in the polyethelyne conditioning for the ethanolic extract. The correlations were different but not very significant on the tested strains ($p \leq 0.05$). The ethanolic extract showed, best results at 37°C (99.9%) on *E. coli* and at all temperatures for *S. typhi (*100%*).* For the aqueous extract, it was at 7°C and 37°C (99.6%) on *E. co*li; and 37°C (99.8%) for *S. typhi.* At concentrations 2 mg/mL (99.9%) and 0.5-2 mg/mL (100%), the ethanolic extract demonstrated significant activities against *E. coli* and *S. typhi*; and 2 mg/mL (99.8%) and 0.1-2 mg/mL (99%), for the aqueous extract. As concerns the contact period, at 12 hours, the ethanolic extract showed good results on *E. coli (*99.9%*),* while100% activity was observed for *S. typhi.* The aqueous extract at 12 hours showed significant results on *E. coli (*99.8%*)* and *S. typhi* (99.8%). The decrease in bacterial abundances was significant and negatively correlated ($p < 0.001$) to the increase in concentrations of *E. coli* and *S. typhi* strains. The ethanolic and aqueous extracts had MIC values of 25 and 12.5 mg/mL on *E. coli*; and 3.125 and 6.25 mg/mL on *S. typhi.* The extracts showed great bactericidal effects (MBC/MIC ratio between 1 and 4); though, the aqueous extracts showed bacteriostatic effects on *E. coli* (MBC/MIC ratio = 8).

Conclusion: *Salmonella typhi* was more sensitive to the plant extracts than *Escherichia coli*. This study supports the traditional use of *Sida rhombifolia* in the treatment of infectious diseases. The data obtained from this exploratory work make it possible to consider the use of extracts of *Sida rhombifolia* as an alternative process in the disinfection of water.

Keywords

Sida rhombifolia stems, *Salmonella typhi, Escherichia coli,* nature of the container and temperature of conservation.

Introduction

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites, fungi, protozoa, ectoparasites, and prions. Some are transmitted through bites from insects while others are caused by ingesting contaminated food or water [1, 2].These diseases are a leading cause of death, accounting for a quarter to a third of the estimated 54 million deaths worldwide [3]. In Cameroon in particular, Communicable Diseases (CDs) accounted for 40.7% of the burden of diseases. Lower respiratory tract infections: 10.10%; HIV/ AIDS: 11.5%; malaria: 10.80%; diarrheal diseases: 5.60%; tuberculosis: 1.40% and Sexually transmitted infections: 1.30%. These CDs account for 41.1% of deaths (Global Burden of Disease, 2013) [4].

Most of the pathogens involved in these infections are found in the soil and water as well. Water represents an integral part of man's daily activities (cooking and cleaning) as well as a means of transportation of many microorganisms which constitutes the main means/bridge of contamination with living organisms [5]. The global picture of water and health has a strong local dimension with some 1.1 billion people lacking access to improved drinking water sources and some 2.4 billion to adequate sanitation. WHO estimates that up to 80% of ill health in developing countries is water and sanitation related (2015). The population often at times sort for alternative means (from wells, springs, streams, rivers and bore holes) of acquiring portable water of whose quality is very doubtful [6–8].

Antibiotics have been used to treat many infectious diseases, but today, we are faced with their abusive and uncontrolled use. We are therefore witnessing the emergence of bacterial strains which are multi-resistant to treat and the cost of which is very expensive to manage and sometimes even inaccessible for some low class families [9].

For over 100 years medicinal plants have been exploited for their abundance in active secondary metabolites and their accessibility to many can justify their use in traditional medicine to about 80% of the African population in particular [10].The quality of traditional medicine, which plays a very important role in the health system, is determined by its active substances produced by the plants. Some factors such as, temperature, illumination and humidity, could contribute to the differences in the activities of the active ingredients. Other studies have been carried out on the factors that influence the activities of conventional drugs and plants extract using different techniques [11,12]. However, very limited information is available on the influence of the temperature of conservation, time of contact and nature of conditioning on the activities of a plant extracts. Based on these observations, the present experimental study was design to evaluate: the influence of the temperature of conservation, time of contact, nature of conditioning and *in-vitro* antibacterial activity of the extracts of *Sida rhombifolia* on some isolated strains of microcosm of water origin.

Materials and Methods

Plant material used

The plant material used were the stems of *Sida rhombifolia.* The whole plant was harvested in the town of Bazou, Western Region of Cameroon on 20th of October 2018, in the early hours. The choice was made on the basis of previous studies carried out in Cameroon and in many other countries. The plant was identified at the Cameroon National Herbarium under the code N° SA/2004/HNC.

Preparation of extracts

The plant was air dried and ground, to obtain 1 kg of the powder which was divided into two portions of 500 g each. In two separate 5 L vessels, 4 L each of ethanol and distilled water were added separately to the powder in the different containers. Stirring was done regularly to ensure full circulation of the solvent in the powder. This setup was left for 72 hours. The different filtrates were obtained by filtration on hydrophilic cotton to remove the solid particles. The different filtered fractions were concentrated (ethanolic extract at 80°C and 60°C for the aqueous extract) using a rotatory vaporization machine. Extraction yields were calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{mass of extract obtained}}{\text{mass of initial powder}} \times 100$$

Phytochemical screening

This is a set of techniques for qualitatively characterizing the chemical substances of a plant. The main classes of secondary metabolites are flavonoids, triterpenes, glycosides, saponins, tannins, coumarins, reducing sugars and cardiotonic glycosides. The phytochemical tests

were carried out according to the methods described by Harbone 1973, Alilou et al (2014), Maraei et al (2015) and N'Guessan et al [13–15].

Antibacterial activity Study

Bacterial isolation and storage

Bacteriological analysis was qualitative and quantitative. The variables considered in this study were the isolation and numbering of bacteria of the genus *Salmonella* and *Escherichia*, present in the different water samples. These bacterial species were selected because of their importance in hygiene and public health in indicating the microbiological quality of water intended for household consumption (Rodier, 2009; Holt et al., 2000) [16,17].

The water samples provided for this bacteriological analysis were collected from wells in the Nsam neighbourhood of Yaounde-Cameroon in sterile 500 mL glass vials. Two techniques were used to isolate the strains: the technique by spreading on Petri dishes and filter membrane method.

Preparation of bacterial suspensions

For activation, some bacterial colonies stored in glycerol at 5°C were defrosted at room temperature (23 ± 2 °C), and 100 µL was collected and spread on regular agar (PCA) on petri dishes. The colonies isolated after 24 hours were collected using a sterile Platinum loop and then spread again on ordinary agar slanted in slopes in different tubes. After 18 hours of incubation at 37°C, the bacterial suspensions were prepared by removing from this culture, colonies that were diluted in sterile distilled water until turbidity was obtained corresponding to 0.5 of the McFarland (H_2SO_4, $BaCl_2$) scale equivalent to the concentration of $1.5x10^8$ CFU/mL [18]. The main bacterial suspension was then diluted to various titers for antibacterial activity evaluation.

Preparation of extract solutions

For each extract, the solutions were prepared in a volume of 500 mL at concentrations of 2000 µg/mL, 1000 µg/mL, 500 µg/mL, and 100 µg/mL from sterile distilled water. The solutions were first filtered on hydrophilic cotton then on sterile Whatman paper and finally through a filter membrane of porosity 0.45 µm. All this was carried out around a Bunsen burner to limit any contamination [19].

Experimental section

Influence of different factors on the antibacterial activities of *Sida rhombifolia* extracts

For each bacterial species, 30 vials were used for the study. So, 15 vials for each conditioning .These vials were divided into 5 series A, B, C, D and E; 3 (representing the different temperatures: 7°C, room temperature and 37°C of conservation) of 350 mL according to the different concentration of each extract (maceration with distilled water and ethanol). Each of the 5 vials in series A contained 50 mL of saline water (NaCl: 8.5 g/L) used as control. The other vials of series B, C, D and E, each contained 50 mL of extract, at different concentrations: 100 µg/mL, 500 µg/mL, 1000 µg/mL, 2000 µg/mL, respectively.

0.5 mL of each bacterial suspension were introduced into each of the vials containing the plant extracts and this constituted the initial time of contact t_0. Same was done after 6 hours and 12 hours constituting different time of contact t_6 and t_{12} respectively. The bacterial cell concentration in each vial at time t_0 was 1.5×10^8 CFU/mL. 100 µL of sample were collected and seeded by the spreading method until exhaustion of the surface of the specific agar media (Endo for *E. coli* and Salmonella-Shigella for *Salmonella typhi*) on Petri dishes previously labelled according to the different concentrations of the extracts.

After 18-24 hours of incubation of the petri dishes (37°C for *S. typhi* and 44°C for *E. coli*), the bacterial colonies on the surface of the specific agar were counted using a colony counter. The bacterial cell abundance expressed in CFU/100 mL were obtained using the formula:

$$\text{Abundance (N)} = \frac{\text{Number of colonies counted on the petridishes}}{\text{volume of water aanalysed in mL}} \text{ x } 100$$

Then the percentages of inhibition (PI) after the bacterial action of the extracts on the different strains were calculated according to the following formula [19]:

$$PI = \frac{(No - Nn)}{No} \times 100$$

N_0 = Bacterial abundance in saline water (Positive control)
N_n= Bacterial abundance after antibacterial activities

Determination of minimum inhibitory concentrations (MICs)

The MIC of the various extracts was determined by the liquid micro dilution method using the M07-A9 protocol described by CLSI (2012) with some modifications [18]. It is the smallest concentration that can inhibit any visible growth of a microorganism after incubation at 37°C for 18 to 24 hours.

In each microplate well (96 wells), a volume of 100 µL of Mueller Hinton broth was added. Then, in the first wells of the series of three columns of which 1, 2.3, 5, 6, 7 and 9, 10, 11 were put in a volume of 100 µL of the main solution at a concentration of 500 mg/mL of the aqueous and ethanolic extract, respectively. Successive dilutions in series of a factor of 2 permitted us to obtain a range of concentrations ranging from 1.56 mg/mL to 100 mg/mL. A volume of 100 µL inoculum (2×10^6 CFU/mL) was later put into each well to get a final volume of 200 µL per well. The plates were sealed with cling film paper and incubated at 37°C for 18 to 24 hours.

Columns 5 and 10 served as a negative control for Mueller Hinton broth and contained exclusively MHB; and line F was used as a positive control for the bacterial strains and contained MHB + inoculum. The positive control "A" was made up of a reference antibiotic (Ampicillin).

After incubation, bacterial growth verification was made using Iodo-nitro-terazoliumchloride (INT). The principle of which is based on the uptake of protons emitted by dehydrogenases (enzymes present on the bacterial membrane) of living bacteria after glucose metabolism. It reduces and renders the medium pinkish after about 30 minutes of re-incubation [20]. 40 µL of the solution of INT were put into each of the wells of the microplate. The MICs were defined as the smallest concentrations at which there is total inhibition of bacterial growth.

Determination of the minimum bactericidal concentration (MBC) of the various extracts

In order to determine the MBC, a volume of 100 µL of culture broth was introduced into new plates, then the volume was added to 200 µL by pipetting a volume of 100 µL of the contents of the concentration wells greater than or equal to the MIC. These plates were then incubated for 18-24 hours at 37°C followed by a verification using INT. All wells which did not have a pink coloration were considered as bactericidal and the smallest concentrations were noted as MBC. The tests were made in quadruplicates.

After determination of MBC, the MBC/MIC ratios were calculated. This report was used to characterize the activity of a given antibiotic;

- MBC/MIC ≤ 2: bactericidal effect,
- MBC/MIC 4 to 16: bacteriostatic effect,
- MBC/MIC > 16: the bacterium is said to be antibiotic tolerant [21].

Data analysis

The progression of the bacterial abundance was represented on graphs with histograms using Microsoft Excel 2016 software. The degree of

connection between bacterial abundances, periods of contact, type of conditioning and temperature for each concentration of the extracts were evaluated using Spearman's "r" correlation. Data comparisons of the mean bacterial abundance values were calculated using the Kruskal-Wallis H-test. All these analyses were carried out using the SPSS software version 16.0.

Results and Discussion

Results

Extraction yield and phytochemical screening of the extract

After the different extractions, the yields were evaluated according to the solvent used for extraction. These yields as well as the physical characteristics of the extracts are recorded in table 1. The results shown on the table indicate that, yields range from 2.56 to 2.8%. The highest extraction yield was obtained from distilled water and the lowest from ethanol. The extracts differ in their colour and texture (Table 1).

Table 1: Plant extract yields and characteristic of extracts

Solvents	Mass of the powder (g)	Mass of extract (g)	Yield (%)	Physical Characteristics
Ethanol (CH_3CH_2OH)	500	10.258	2.56	Dark green and sticky
Distilled water (H_2O)	500	11.258	2.8	Brown and very sticky

A qualitative test for the identification of different classes of secondary metabolites indicated that, the ethanolic extract (EE) contained flavonoids, saponins, tannins, phenols, coumarins and triterpenes; while the aqueous extract (AE) contained flavonoids, phenols, triterpenes and tannins. However, none of the extracts gave a positive test for alkaloids and sterols (Table 2).

Table 2: Secondary metabolites present in plant

Family of secondary metabolites	EE	AE
Alkaloids	-	-
Phenols	+	+
Flavonoids	+	+
Coumarins	+	-
Sterols	-	-
Triterpenes	+	+
Saponins	+	-
Tannins	+	+

–: absent; +: present.
EE: Ethanolic extract; AE: Aqueous extract.

Evaluation of the antibacterial activity of *Sida rhombifolia* extracts

Antibacterial activity of stem bark extracts of *Sida rhombifolia* on bacterial strains

The bacterial cell abundances of the different strains, as well as the percentages of inhibition of different extracts or ampicillin were calculated for each of the contact periods after direct counting of the different colonies obtained on the specific media sunk in Petri dish for the period 18 to 24 hours of incubation.

Effect of Aqueous and Ethanolic Extracts on the growth of *E. coli* at 7℃

Generally, there was a decrease in the bacterial abundances in the presence of extracts of *Sida rhombifolia* compared with those of the control that ranged between 5.0×10^9 and 6.05×10^{10} CFU/100mL. The ethanolic extract conditioned in glass greatly decreased the abundances of *E. coli* at 6hrs of contact compared to the aqueous extracts and those conditioned on polyethylene.

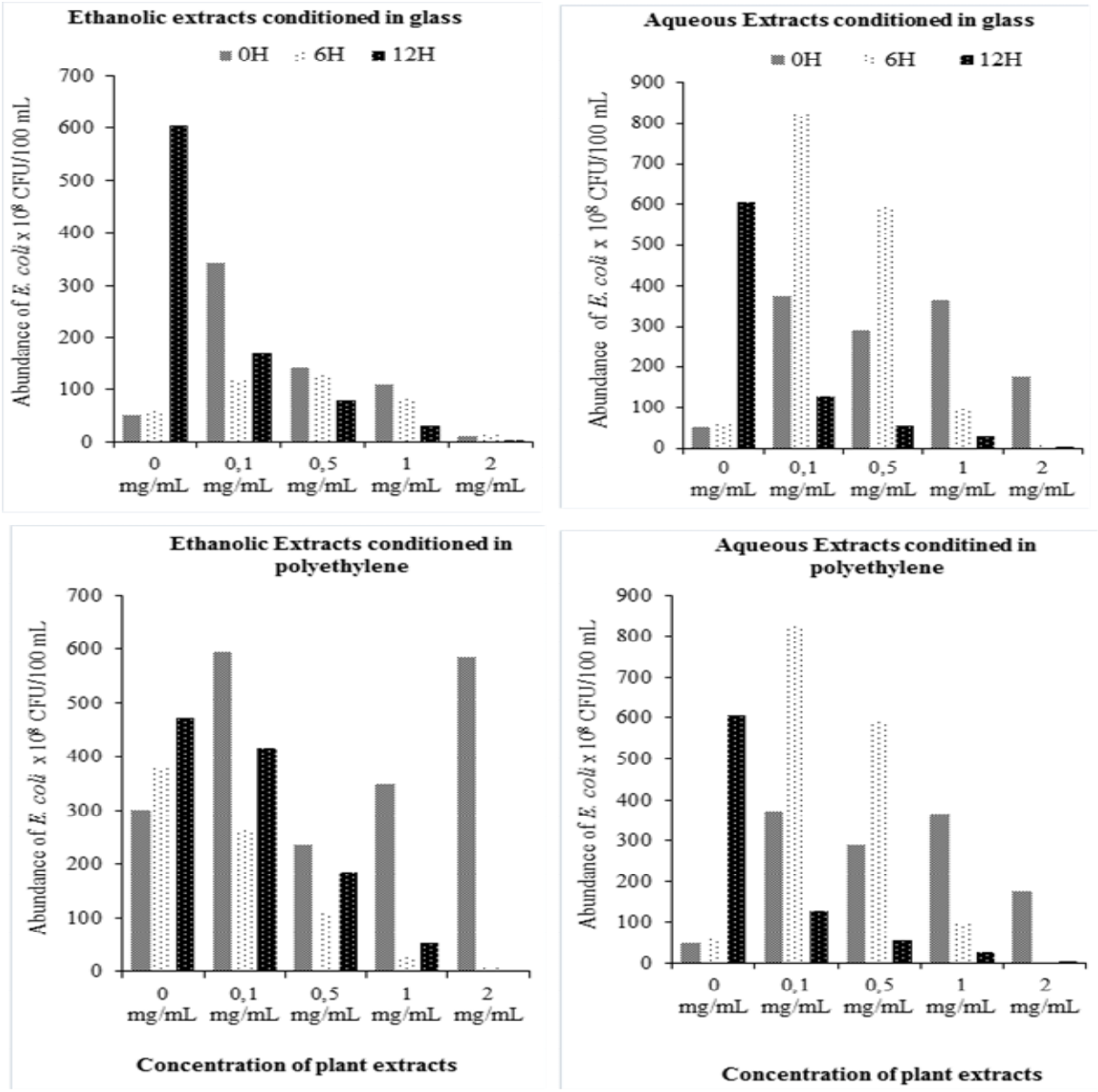

Figure 1: Effect of aqueous and ethanolic extracts on the growth of *E. coli* conditioned in glass and polyethylene at 7℃

Effect of Aqueous and Ethanolic Extracts on the growth of *E. coli* at Room temperature (23 ± 2 °C)

In the glass conditioning, the bacterial abundance of *E. coli* in the presence of ethanolic extract ranged from 4.0×10^{8} to 7.24×10^{10} CFU/100mL, while the aqueous extract ranged from $1x10^{8}$ to $5.08x10^{10}$ CFU/100mL.

In the polyethylene conditioning, the bacterial abundance of the *E. coli* in the presence of ethanolic extract ranged from 5.0×10^{8} to 7.25×10^{10} CFU/100mL. And that of the aqueous extract ranged from $1x10^{8}$ to $8.64x10^{10}$ CFU/100mL.

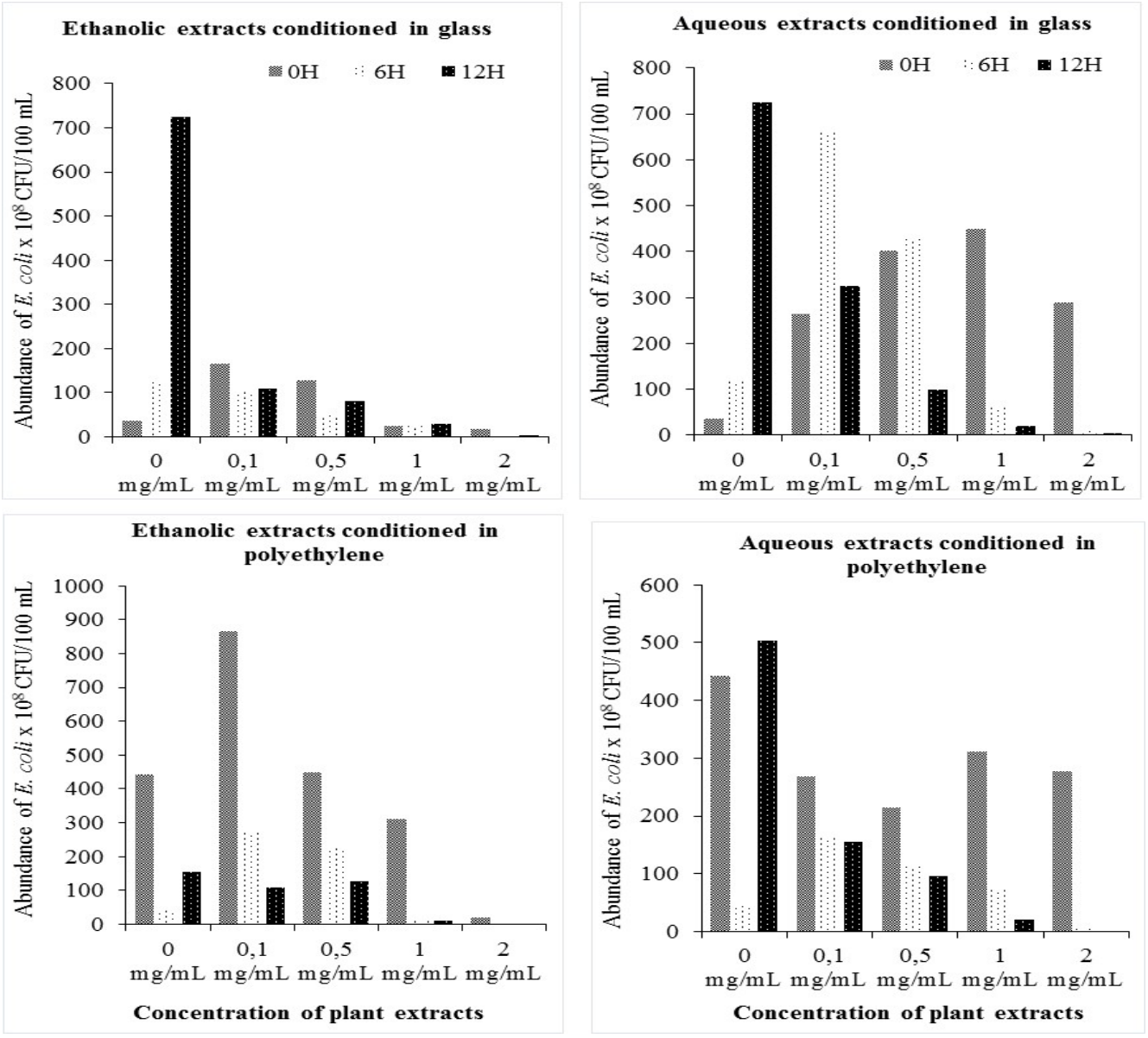

Figure 2: Effect of aqueous and ethanolic extracts on the growth of *E. coli* conditioned in glass and polyethylene at room temperature (23 ± 2 °C)

Effect of Aqueous and Ethanolic Extracts on the growth of *E. coli* at 37℃

Using the glass conditioning, the bacterial abundance of *E. coli* in the presence of ethanolic extract ranged from 1.0×10^{8} to 8.36×10^{10} CFU/100mL; whereas, that of the aqueous extract ranged from $3x10^{8}$ to $8.37x10^{10}$ CFU/100mL.

For the polyethylene conditioning, the bacterial abundance of the *E. coli* in the presence of ethanolic extract ranged from 4.0×10^{8} to 9.36×10^{10} CFU/100mL. And the aqueous extract ranged from $4x10^{8}$ to $6.37x10^{10}$ CFU/100mL.

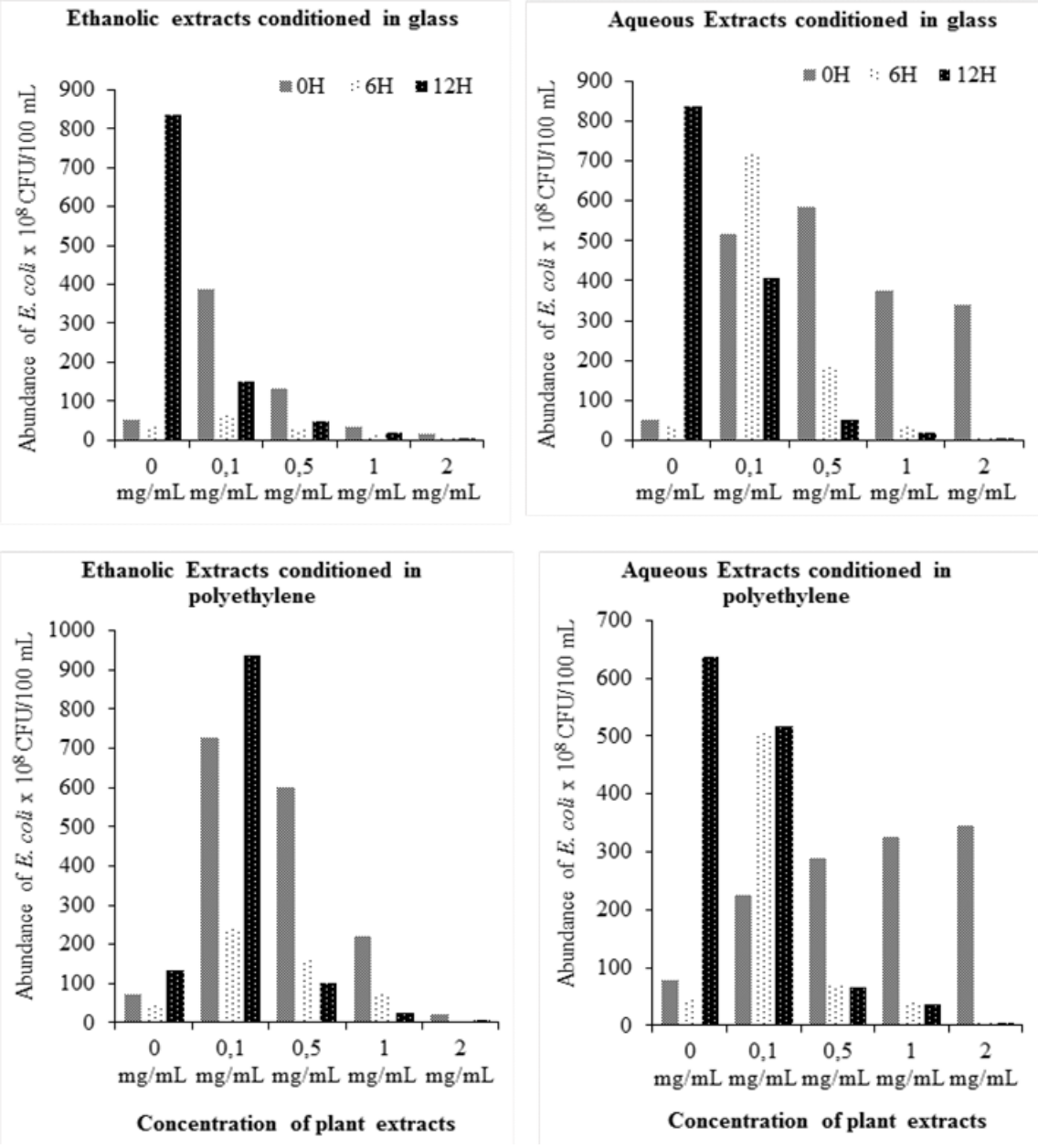

Figure 3: Effect of aqueous and ethanolic extracts on the growth of *E. coli* conditioned in glass and polyethylene at 37℃

Effect of Aqueous and Ethanolic Extracts on the growth of *Salmonella typhi* at 7°C

Conditioned in glass, the bacterial abundance of *Salmonella typhi* reached $6.37x10^{10}$ CFU/100mL and $3.5x10^{10}$ CFU/100mL respectively, in the presence of ethanolic and aqueous extract. For the polyethylene conditioning, the bacterial density of *Salmonella typhi* reached $4.37x10^{11}$ CFU/100mL and $4x10^{10}$ CFU/100mL respectively, in the presence of ethanolic and aqueous extract.

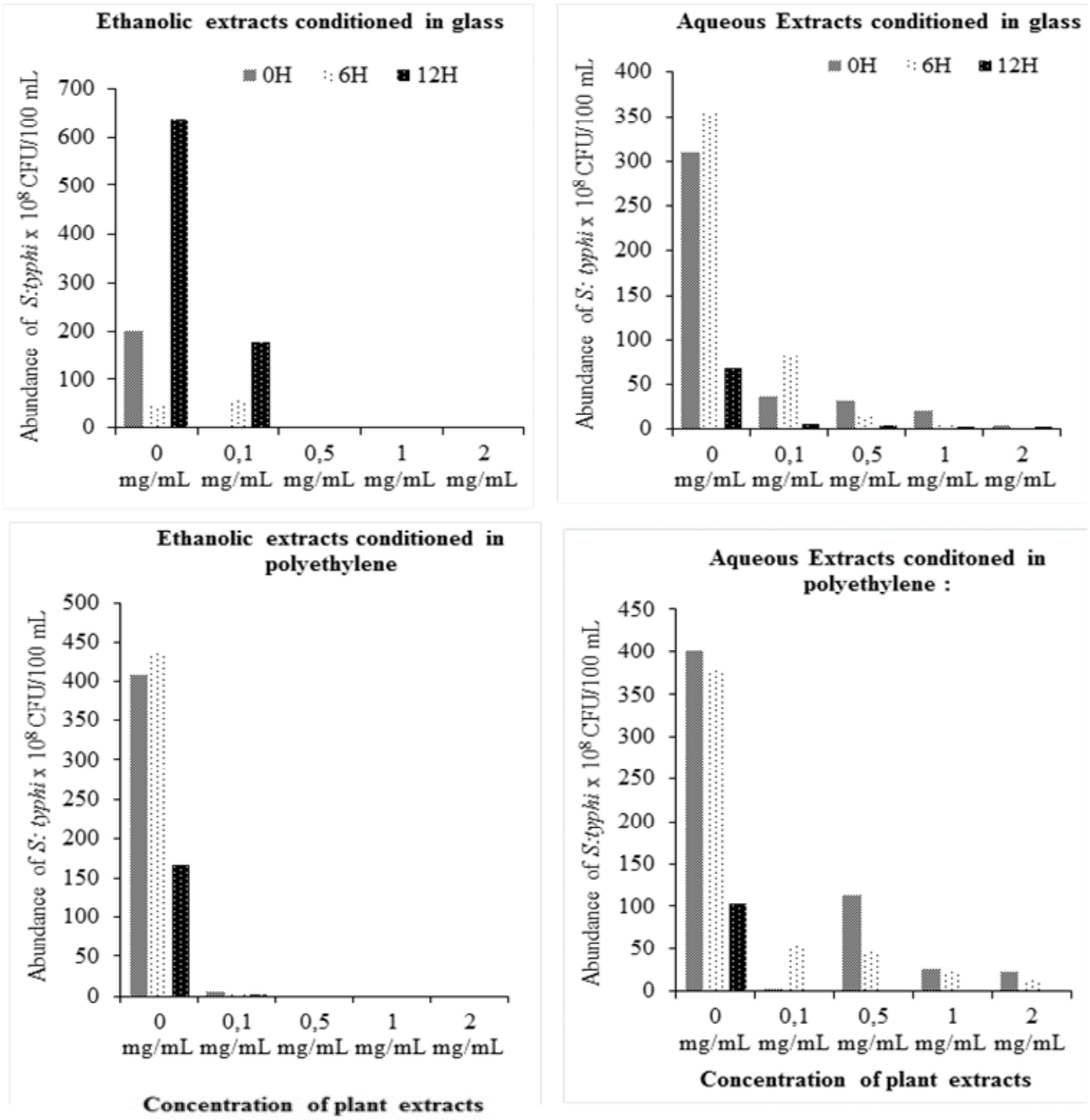

Figure 4: Effect of aqueous and ethanolic extracts on the growth of *Salmonella typhi* conditioned in glass and polyethylene at 7°C

Effect of Aqueous and Ethanolic extracts on the growth of *Salmonella typhi* at room temperature (23 ± 2 °C)

Conditioning in glass, the bacterial abundance of *Salmonella typhi* in the presence of ethanolic extract reached 1.29×10^{10} CFU/100mL; while that of the aqueous extract reached 3.50×10^{10} CFU/100mL.

Conditioned in polyethylene, the bacterial density of *Salmonella typhi* reached 4.69×10^{10} CFU/100mL and 1.15×10^{10} CFU/100mL respectively, in the presence of ethanolic and the aqueous extract.

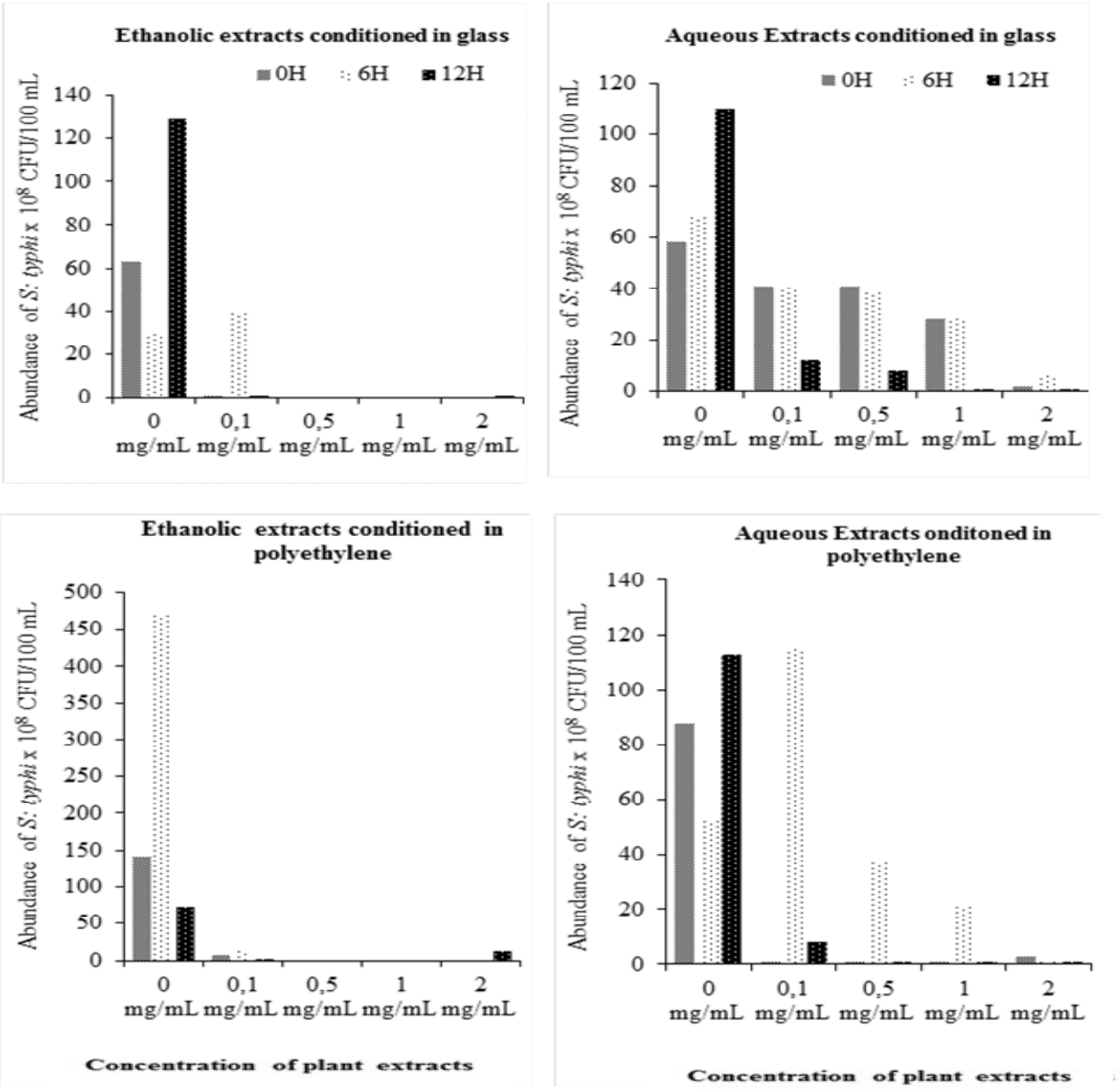

Figure 5: Effect of aqueous and ethanolic extracts on the growth of *Salmonella typhi* conditioned in glass and polyethylene at room temperature (23 ± 2 °C)

Effect of Aqueous and Ethanolic Extracts on the growth of *Salmonella typhi* at 37°C

Conditioned in glass, the bacterial abundance of *Salmonella typhi* in the presence of ethanolic extract reached 2.08×10^{10} CFU/100mL, and the aqueous extract ranged from $1x10^{8}$ to $3.50x10^{10}$ CFU/100mL. For the polyethylene conditioning, the bacterial density reached 3.1×10^{10} CFU/100mL and $2.72x10^{10}$ CFU/100mL respectively, in the presence of ethanolic and the aqueous extract.

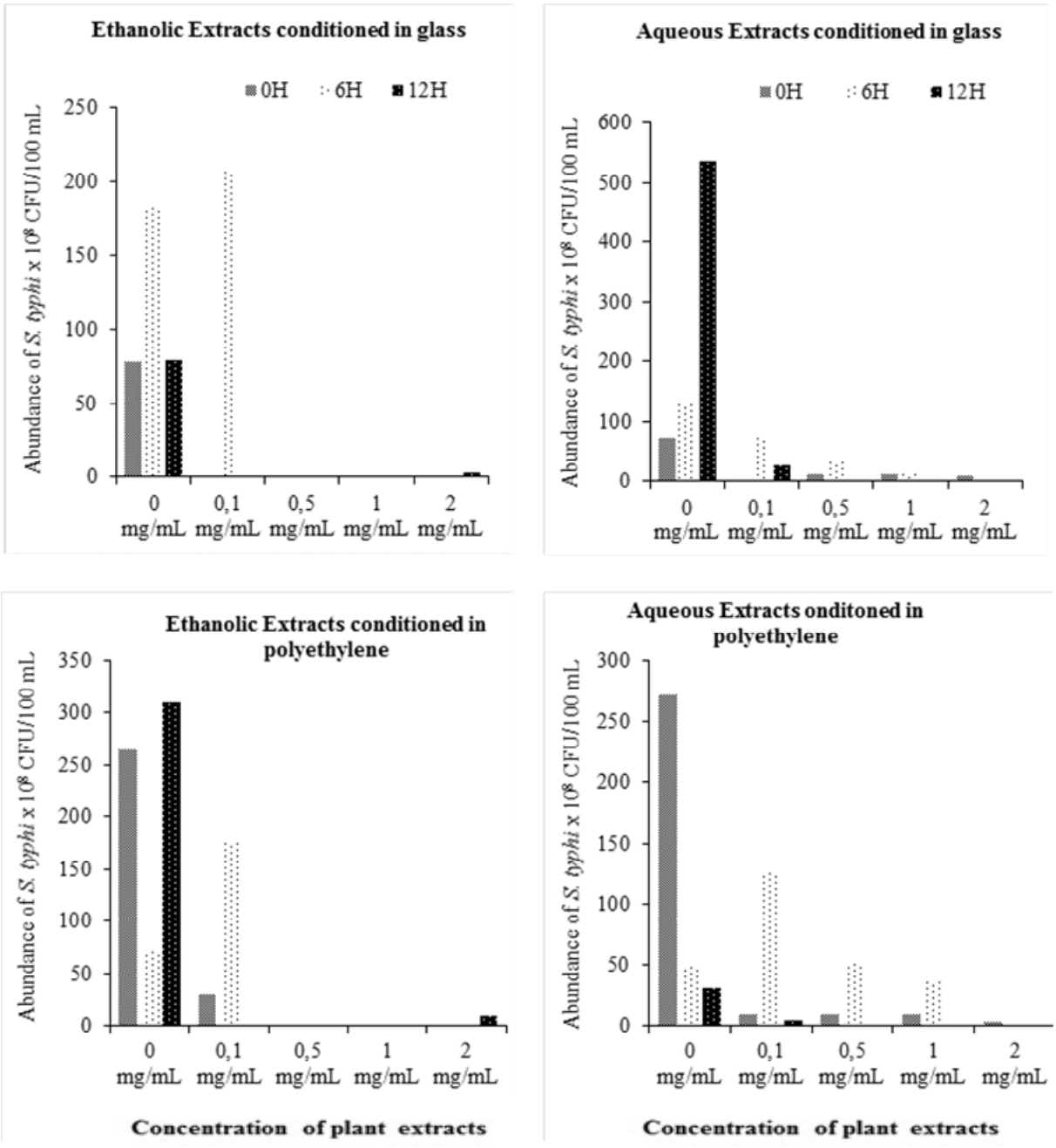

Figure 6: Effect of aqueous and ethanolic extracts on the growth of *Salmonella typhi* conditioned in glass and polyethylene at 37°C

Percentage of cells inhibition

The percentage of cells inhibition was calculated in order to evaluate the direct impact of *Sida rhombifolia* extracts at different concentrations on the survival of bacterial cells, under the influence of incubation temperature, exposure time and the nature of the container.

Percentage of cell inhibition of *E. coli* in the presence of the extracts conditioned in glass at different temperature

At 7°C, the inhibition percentages ranged from 0 to 99.6% in the aqueous and ethanolic extract. The greatest inhibition was observed at the concentration of 2 mg/mL.

At room temperature (23 ± 2 °C), the inhibition percentages reached 91.6% in the aqueous extract. As for the ethanolic extract, it reached 99.4%. The greatest inhibition was observed in ethanolic extract at the concentration of 2 mg/mL.

The percentages at 37°C of growth inhibition of *E. coli,* ranged from 0 to 99.6% in the aqueous and ethanolic extracts. The greatest inhibition occurred at 2 mg/mL (table 3).

Table 3: Percentage of *E. coli* inhibition conditioned in glass at different temperatures

Temperature	Extracts and Period of contact		Concentration of plant extracts (mg/mL)			
7°C	**Extracts**	**Periods of contact (hrs)**	**0.1**	**0.5**	**1**	**2**
	EE	0	0	0	72.2	80.7
		6	0	0	0	73.7
		12	42.2	86.9	94.8	99.6
	AE	0	0	0	0	0
		6	0	0	0	0
		12	78.84	90.7	95.3	99.6
Room temperature (23 ± 2 °C)	**EE**	0	0	0	33.3	91.6
		6	17.7	61.2	77.4	16.9
		12	84.8	88.9	95.5	99.4
	AE	0	0	0	0	0
		6	0	0	33.3	91.6
		12	17.7	61.2	77.4	16.9
37°C	**EE**	0	0	0	33.9	71.7
		6	0	20	62.8	91.5
		12	81.8	94.0	97.6	99.9
	AE	0	0	0	0	0
		6	0	0	0	85.7
		12	51.4	93.9	97.6	99.9

Percentage of cell inhibition of *E. coli* in the presence of the extracts conditioned in polyethylene at different temperature

The percentages at 7°C of growth inhibition of *E. coli*, were calculated for each type of extract, in polyethylene conditioning and in different contact period (Table 4). As concerns the aqueous extract, the inhibition percentages ranged from 0 to 99.6%. For the ethanolic extract, the inhibition percentages ranged from 0 to 99.4%. The greatest inhibition for the aqueous extract was observed at the concentration of 2 mg/mL.

At room temperature (23 ± 2 °C), the inhibition percentages reached 99.8% in aqueous extract. As for the ethanolic extract, it reached 99.3%. The greatest inhibition was observed at the concentration of 2 mg/mL in the aqueous extract.

The inhibition percentages at 37°C, reached 99.4% in aqueous and ethanolic extract, and the greatest inhibition was observed at the concentrations of 2 mg/mL.

Table 4: Percentage of *E. coli* inhibition conditioned in polyethylene at different temperatures

Temperature	Extracts and Period of contact		Concentration of plant extracts (mg/mL)			
7°C	**Extracts**	**Periods of contact (hrs)**	**0.1**	**0.5**	**1**	**2**
	EE	0	0	21.9	0	0
		6	29.8	71.4	93.1	97.8
		12	11.8	60.8	89	99.4
	AE	0	0	22.1	71.4	93.1
		6	29.8	71.4	93.1	87.8
		12	11.8	60.8	89	99.6
Room temperature (23 ± 2 °C)	**EE**	0	0	0	0	30
		6	0	0	75.5	97.7
		12	30.7	19.2	93.6	99.3
	AE	0	39.3	51.6	29.4	37.1
		6	0	0	0	86.3
		12	68.9	80.1	95.8	99.8
37°C	**EE**	0	0	0	0	72.6
		6	0	0	0	91.7
		12	0	22.7	80.3	99.4
	AE	0	0	0	0	0
		6	0	0	12.5	88.9
		12	18.8	89.7	94.1	99.4

Percentage of cell inhibition of *Salmonella typhi* in the presence of the extracts conditioned in glass at different temperature

The aqueous extract conditioned in glass at 7°C, resulted in 76.7 to 99.7% inhibition. As for the ethanolic extract, it reached 100%. The greatest inhibition was observed at the concentration of 2mg/mL in ethanolic extract.

At room temperature (23 ± 2 °C), the aqueous and ethanolic extract had 100% inhibition, at the concentration of 2mg/mL.

The inhibition percentages reached 99.8% and 100% respectively, at 37°C, for both the aqueous and ethanolic extract, with the greatest inhibition observed at the concentration of 2mg/mL in ethanolic extract.

Table 5: Percentage of *S. typhi* inhibition conditioned in glass at different temperatures

Temperature	Extracts and Period of contact		Concentration of plant extracts (mg/mL)			
7°C	**Extracts**	**Periods of contact (hrs)**	**0.1**	**0.5**	**1**	**2**
	EE	0	99.9	100	100	100
		6	0	100	100	100
		12	72.4	100	100	100
	AE	0	88.1	89.7	93.9	99.7
		6	76.7	96.3	98.9	99.7
		12	91.1	94.1	98.5	98.5
Room temperature (23 ± 2 °C)	**EE**	0	98.4	100	100	100
		6	0	100	100	100
		12	99.2	100	100	100
	AE	0	29.3	29.3	51.7	96.6
		6	39.7	42.6	58.8	91.1
		12	89	92.7	100	100
37°C	**EE**	0	98.7	100	100	100
		6	0	100	100	100
		12	98.7	100	100	97.4
	AE	0	98.6	82.2	83.6	86.3
		6	44.2	72.9	89.1	97.7
		12	95	99.6	99.8	99.8

Percentage of cell inhibition of *Salmonella typhi* in the presence of the extracts conditioned in polyethylene at different temperature

The aqueous extract at 7°C, showed 76.7 to 99.7% inhibition. As for the ethanolic extract, it ranged from 0-100%. The greatest inhibition was observed at the concentration of 2 mg/mL in ethanolic extract.

At room temperature (23 ± 2 °C), the inhibition percentages ranged from 0-99% for the aqueous extract. As for the ethanolic extract, it ranged from 0-100%. The greatest inhibition was observed at the concentration of 2 mg/mL in ethanolic extract.

The aqueous extract at 37°C reached 99.8% inhibition, while ethanolic extract was 100%. The greatest inhibition was observed at the concentration of 2 mg/mL in ethanolic extract.

Table 6: Percentage of *S. typhi* inhibition conditioned in polyethylene at different temperatures

Temperature	Extracts and Period of contact		Concentration of plant extracts (mg/mL)			
7°C	**Extracts**	**Periods of contact (hrs)**	**0.1**	**0.5**	**1**	**2**
	EE	0	99.9	100	100	100
		6	0	100	100	100
		12	72.4	100	100	100
	AE	0	88.1	89.7	93.9	99.7
		6	76.7	96.3	98.9	99.7
		12	91.1	94.1	98.5	98.5
Room temperature (23 ± 2 °C)	**EE**	0	98.4	100	100	100
		6	0	100	100	100
		12	99.2	100	100	100
	AE	0	29.3	29.3	51.7	96.6
		6	39.7	42.6	58.8	91.1
		12	89	92.7	99	99
37°C	**EE**	0	98.7	100	100	100
		6	0	100	100	100
		12	98.7	100	100	97.4
	AE	0	98.6	82.2	83.6	86.3
		6	44.2	72.9	89.1	97.7
		12	95	99.6	99.8	99.8

Minimum inhibitory and bactericidal concentrations of extracts

Minimum inhibitory concentrations of extracts (MICs)

The extracts showed antibacterial activity on the bacterial strains tested with MICs ranging from 3.125 to 25 mg/mL. The ethanolic extract (EE) showed best results on *Salmonella typhi* with a MIC of 3.125 mg/mL. The aqueous extract (AE) demonstrated MIC values of 12.5 mg/mL and 6.25 mg/mL for *Escherichia coli* and *Salmonella typhi*, respectively (Table 7).

Table 7: MICs in mg/mL of different extracts on the bacterial strains tested

Bacterial strains	EE	AE	Ampicillin
E. coli	25	12.5	>0.00256
S. typhi	3.125	6.25	>0.00256

Minimum bactericidal concentrations of extracts

The MBCs of the different extracts were determined and the ratio MBC/MIC was calculated. It varied from 1 to 8 among the 2 strains tested. The various extracts had bactericidal effects on the strains, with the exception of the aqueous extract (AE) on *E. coli* which showed a bacteriostatic effect. The results of MBCs obtained and the ratio MBC/MIC are presented below on table 8.

Table 8: Minimum bactericidal concentrations (MBCs) in mg/mL of different extracts on the strains tested and MBC/MIC ratio of extracts

Bacterial strains	Aqueous Extract (AE)	Ethanolic extract (EE)	Ampicilline (CMB)	Rapport MBC/MIC	
				AE	EE
E. coli	25	100	UD	8	4
S. typhi	50	100	UD	< 2	< 2

Correlation and comparisons between analysed variables

The coefficient of correlation <<r>> of Spearman between bacterial abundances and extract concentration were calculated for each period of contact and condition type.

We notice a reduction of bacterial abundance negatively correlated to the increased of the ethanolic plant extract concentration at all conservation temperatures. The greatest significant difference was noticed with the glass conditioning tested on *S. typhi* at 7°C. The bacterial abundance greatly reduced with respect to the increase in the concentration of the aqueous plant extract at all conservation temperatures. The greatest significant difference was noticed with the glass conditioning tested on *E. coli* and *S. typhi*at 7°C. The bacterial abundance reduced with respect to the increase in the concentration of the ethanolic plant extract at 7°C and (23 ± 2 °C). The greatest significant difference was noticed with the glass conditioning tested on *S. typhi*at ambient temperature. A reduction was noticed for the bacterial abundance negatively correlated on all the varied parameters. The greatest significant difference was found on the extracts in glass conditioning conserved at 37°C on *S. typhi*.

Comparison between the bacterial abundance and the concentration of the extracts versus the different incubation temperatures and conditioning

The comparison of the mean bacterial abundance versus the temperature showed no significant variations and same went for that of the mean bacterial abundance versus the different extracts concentrations.

The comparison of the mean bacterial abundance versus the different types of extracts showed no significant variations and same for that of the mean bacterial abundance versus the different types of conditioning.

The Kruskal-Wallis "H" test revealed for each species significant variations in abundances with increased extract concentrations except with the case of aqueous extract on *S. typhi*.

Discussion

Increase in antimicrobial drug resistances has caused researchers to find alternative means of tackling this public health problem. Medicinal plants have proven their effectiveness in the treatment of infectious diseases. However, studies have proven that some factors can influence the activities of plant extracts. The ethanolic and aqueous extracts from the stems of *Sida rhombifolia* yielded 2.56% and 2.8% respectively. These results differs with those of Gupta et *al.* 2009, who obtained yields of 16.92% and 1.85% for the aqueous and ethanolic extract respectively, from the aerial parts of *Sida rhombifolia* [22]. In addition, it varies the results of Assam JP *et al* in 2010, who acquired a yield of 38.60% with the hydro-methanolic extract of the whole plant [23]. The differences in affinity of the secondary metabolites with the extraction solvents could somehow account for this.

Flavonoids, alkaloids, tannins, triterpenoids, essential oils, saponins, glycosides and phenols have been reported as active classes of secondary metabolites possessing antibacterial activities [24]. The phytochemical profiling of *Sida rhombifolia* indicated the presence of phenols, flavonoids, coumarins, triterpenes, saponins and tannins in both the ethanolic and aqueous extracts. The results are similar to that of Abat in 2017 [25]; and differs from those of Assam *et al.* 2010, in which extracts of the whole plant indicated the presence of alkaloids and steroids [23]. Harvesting at different periods of the day in different geographical locations could account for the variation in the constituents. Also, bio-diversified soil textures, solvent used for extraction and extraction method, climate, stage of plant maturation and different plant parts could play important roles in the phytochemistry.

Concerning the difference in the nature of conditioning the ethanolic extract showed remarkable activity (max = 100% inhibition) especially after 6 and 12 hours of contact period with *Salmonella typhi* strains conditioned in glass. This was slightly different from those of *E. coli* (max = 99.9%) conditioned in glass. However, no significant differences were observed ($p \leq 0.05$) between bacterial abundances at different contact periods for all bacterial strains. The aqueous extracts showed slightly different results compared to the ethanolic extracts. A maximum of 99.8% and 99.6% were observed for *Salmonella typhi* and *E. coli*, respectively, in the glass conditioning. Generally, the correlations were different but not very significant on the tested strains ($p \leq 0.05$).

In regards to the temperature of conservation, the ethanolic extract showed the best results at 37°C (99.9%) on *E. coli* and at all temperatures for *S. typhi (*100%*).* The aqueous extracts showed the best results at temperatures 7°C and 37°C (99.6%) on *E. coli,* while that of *S. typhi* was at 37°C (99.8%).

The ethanolic extract at concentrations 2 mg/mL (99.9%) and 0.5-2 mg/mL (100%) demonstrated significant activities against *E. coli* and *S. typhi,* respectively. At concentrations 2 mg/mL (99.8%) and 0.1-2 mg/mL (99%), the aqueous extracts showed significant activities on *E. coli* and *S. typhi,* respectively.

As concerns the contact period, at 12 hours the ethanolic extract showed good results on *E. coli (*99.9%*).* At all periods, especially 12 hours, 100% activity was observed for *S. typhi.* The aqueous extract at 12 hours showed significant results on *E. coli (*99.8%*)* and *S. typhi* (99.8%), respectively.

The variations in bacterial abundances in the presence of the extracts were studied for their antibacterial activity. The decrease in bacterial abundances was significant and negatively correlated ($p < 0.001$) to the increase in concentrations of *E. coli* and *S. typhi* strains. The ethanolic and aqueous extracts of the stems of *Sida rhombifolia* showed varying antibacterial activity with respect to the strains tested with minimum inhibitory concentrations ranging from 1.562 to 50 mg/mL. The ethanolic and aqueous extracts had MIC values of 25 and 12.5 mg/mL on E. coli; and 3.125 and 6.25 mg/mL on *S. typhi.* These proves, the ethanolic extract was more active than the aqueous extract. This high activity observed in the ethanolic extract could be due to the presence of greater flavonoid content [25]. Both extracts also showed great bactericidal effects (CMB/CMI ratio between 1 and 4); though, the aqueous extracts showed bacteriostatic effects on *E. coli* (MBC/MIC ratio = 8). In addition, the ethanolic extract gave a ratio of 4 for *E. coli* and < 2 for *S. typhi.* These results, are almost similar to that of Debalke *et al*. (2018), who proved that, the aqueous-ethanolic extract of the aerial parts of *Sida rhombifolia* had a ratio of 4 on *Escherichia coli* compared to 1 on *Salmonella typhi* [24]. These findings indicates, the secondary metabolites of different classes in the plant extract could be responsible for the antimicrobial activities.

Conclusion

The various extracts presented variable activities on planktonic *E. coli* and *S. typhi* strains isolated from well water in Yaounde. The ethanolic extract showed a remarkable activity (100% inhibition) on *S. typhi* tested after six and twelve hours of contact. Also, both the aqueous and ethanolic extracts, exhibited variable activities with MIC values ranging from 6.25 to 50 mg/mL. The ethanolic extract showed good antibacterial activity on all strains tested with a significant bactericidal effect, compared to the aqueous extract. These results confirmed the traditional use of *Sida rhombifolia* in the treatment of infectious diseases especially waterborne diseases caused by *E. coli* and *S. typhi*.

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