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Activities of *Albizia Zygia* (Fabaceae) Extracts on *Salmonella Typhi* Bacteria in Aquatic Microcosm: Influence of Some Abiotic Factors

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ABSTRACT

The present study aims at evaluating the activity of aqueous and hydro-ethanolic extracts of Albizia zygia (A. zygia) on Escherichia coli cultures in aquatic microcosms. Bacteria cells were exposed to different concentrations (0.5g/l, 1g/l and 1.5g/l) of aqueous and hydro-ethanolic extracts of A. zygia leaves and/or trunk bark for 3h, 6h and 9h at incubation temperatures of 7°C and 23°C. Phytochemical screening showed the presence of metabolites such as anthraquinones, anthocyanins, flavonoids, polyphenols, tannins and saponines in the two types of extracts. From these experiments we note that bacteria abundance varies according to the concentration of the extracts and the incubation time. In most cases lower abundances are recorded at 23°C in the presence of hydro-ethanolic extract from the trunk bark.

The highest rate of cellular inhibition (99.95%) was recorded after 9 hours of exposure of bacteria cells to the hydro-ethanolic extracts from trunk bark at a concentration of 1.5g/l at a temperature of 7°C. The temperature of the environment did not significantly influence bacterial inhibition (P>0.05). In general, the hydro-ethanolic extracts from trunk barks were more effective.

Keywords: *A. zygia, phytochemical screening, water quality, bacterial inhibition*

HIGHLIGHTS

- Bacterial abundances undergo a temporal variation depending on the concentration of extracts and the duration of exposure.
- The presence of extracts of *A. zygia* significantly reduces the cultivability of the bacterium under consideration in water.
- Cell cultivability inhibition rates increase gradually with increasing bacteria-extract contact time.
- Hydro-ethanolic and aqueous extracts of *A. zygia* can be used as alternative methods in water disinfection.

INTRODUCTION

Nearly one billion people still lack access to safe drinking water, half of whom live in the African and Pacific regions (WHO 2009). In Cameroon in particular, insufficient means of local municipalities does not permit the optimal distribution of drinking water, as a result, populations are forced to drink water from wells and springs, ignoring its quality (Nola *et al*. 1998). This quality can be influenced by several types of pollution, which is due to the presence of microorganisms such as protozoa, fungi viruses and bacteria in water. Human and animal wastes that may get into water sources through surface run off may contain pathogenic bacteria that cause diarrhea (Kirui *et al*. 2015).The presence of bacteria in water exposes users to short-term risk because they are partly responsible for waterborne diseases; diarrheal diseases are among the most deadly infectious diseases in the world, especially in developing countries in the tropical zone (Faridabano *et al*. 2007). For decades, simple disinfection methods have been used. These include chemical disinfection, whose residues have short or medium-term health effects (Gamage & Zhang 2010), and other methods such as physical disinfection of water by solar irradiation or Solar Water Disinfection (SODIS), filtration and boiling, which modifies the organoleptic properties of water and makes it unpalatable. In

recent years, water disinfection methods using plant extracts have been proposed as a new alternative for household water treatment. The use of plants for therapeutic purposes has been a common practice for thousands of years. Moreover, several plants used in traditional medicine have been the subject of numerous studies. Several studies have focused on this topic and have shown that aqueous extracts of *Lantana Camara, Cymbogon citritus, Hibiscus rosa-sinensis, Eucalyptus microcorys, Moringa oleifera* and *Artemisia annua* have bactericidal effects in aquatic environment (Mobili *et al*. 2015; Metsopkeng *et al*. 2019 and Tamsa Arfao *et al*. 2018). Previous studies have shown that in an aquatic environment containing *Eucalyptus microcorys* extracts, the cultivability of *Salmonella typhi* is significantly impacted and the observed inhibitions are due to the presence of chemical compounds such as flavonoids, terpenoids, phenolic compounds and alkaloids (Tamsa Arfao *et al*. 2018). However, there is still little information on the synergistic effect of *A. zygia* extract concentration, temperature and incubation time on *S. typhi* cells in aquatic microcosm. Little data is also available on the choice of plant parts with better antibacterial efficacy.

This work aims at evaluating the activity of aqueous and hydro-ethanolic extracts of *A. zygia* on the cultivability of *S. typhi* in aquatic microcosm.

Material and Methods

Material

Plant Material

The plant material consists of leaves and the trunk bark of *A. zygia*, collected in the Center Region (Cameroon). The authentication was carried out at the National Herbarium of Cameroon under the number 2338/SRFK. The leaves and bark of the trunk were washed, cut and dried under sun, then ground with a crushing machine.

Selection and Isolation of the Bacterial Strain

The study is focused on *S.typhi* bacteria. It was selected because of its great importance in hygiene and public health and its strong presence as a pathogenic indicator in drinking water. It was isolated from the Olezoa watercourse (Yaounde, Centre Region – Cameroon) and then identified by standard techniques (APHA2005).

Methods

Preparation of Aqueous and Hydro-Ethanolic Extracts of *A. zygia*

Aqueous extracts

300g of crushed leaves and 250g of crushed trunk bark were mixed with 5000ml and 4000ml of distilled water respectively for 48h and stirred morning and evening. The obtained macerate was successively filtered with the help of cotton and Whatman paper n°4. The volumes of the obtained filtrates were reduced with the help of rotary evaporator at a temperature of 60°C. The collected paste was freeze-dried to produce the aqueous extract. After this process, 62g (leaves) and 83g (trunk bark) of the aqueous extracts were obtained.

Hydro-Ethanolic Extracts

For hydro-ethanolic extracts, 500g of crushed leaves and 400g of crushed trunk bark were macerated respectively in 6500ml and 4500ml of ethanol/distilled water mixtures in 70/30 proportions for 48h and stirred morning and evening. The obtained macerate was successively filtered with the help of cotton and Whatman paper n°4. The volumes of the obtained filtrates were reduced with the help of rotary evaporator at a temperature of 60°C. The collected paste was freeze-dried to produce the hydro-ethanol extract. After drying, 77.04g (leaves) and 106.85g (trunk bark) of ethanolic extracts were obtained.

Preparation of the Different Extract Concentration Ranges

The extract concentrations were 0.5g/l, 1g/l and 1.5g/l. The homogenized extracts were successively filtered through filter paper, sterile cotton, sterile Whatman paper, cellulose nitrate membrane with a porosity of 0.45 (Tamsa Arfao *et al*., 2018).

Phytochemical screening of plant extracts

In order to determine the different classes of potentially bioactive compounds present in the extracts of *A. zygia*, phytochemical screening was performed according to the method of Odebeyi and Sofowara (1978).

Experimental protocol

The experiments were performed in 2 stages. The 1st was done by using hydro-ethanolic extract and the 2nd by using the aqueous extract. During the extract concentration for each experiment, 2 groups of test tubes, A and B,

were used. Each group made up of 4 sterile test tubes of 25ml each containing 10ml of *A. zygia* extract solution at different concentrations (0g/l control tube, 0.5g/l, 1g/l and 1.5g/l) and 0.5ml of the bacterial suspension adjusted to a density of 0.5 Mac Farland ($BaCl_2$ and H_2SO_4 1%).

Group A samples were incubated at 23°C and those of Group B at 7°C. 23°C was used to simulate the ambient water storage temperature in most households in the equatorial region and 4° C was used to simulate storage temperature in refrigerators for those who consume fresh water.

Bacteriological analyses were carried out under sterile conditions next to a burner flame. They were carried out after 3, 6 and 9 hours of incubation by the surface spreading technique until exhaustion (APHA 2005). Previous studies have shown that a plant extract becomes active after 3 hours of bacterial-extract contact (Tamsa Arfao *et al*. 2018). After homogenization, 100µl of the sample to be analysed was spread on the surface of the specific culture medium (Wilson-Blair agar) cast in Petri dish. The incubation time was 24 hours at 37°C. The number of viable and cultivable germs isolated was counted directly on Petri dishes using an OSI colony counter. Bacterial abundances were expressed in Colony Forming Units (CFU) and were reported to100 ml of inoculum (CFU/100 ml).

Data Analysis

The temporal variations of cell densities, expressed in decimal logarithmic units (log CFU/100 ml) are illustrated by histograms using Excel 2016 software. The percentages of inhibition (PI) of *A. zygia* extracts on bacterial cells were evaluated using the formula described by Garcia-Ripoll *et al*. (2009)

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

PI = Percentage of Inhibition, No = Initial bacterial abundance (Control tube), Nn = Bacterial abundance after the action of the extract.

Results

Phytochemical Screening of *A. zygia*

Based on the intensity of staining, it was noted that the major chemical constituents identified from the aqueous extract of *A. zygia* leaves are flavonoids and anthocyanins. Tannins, anthraquinones, polyphenols and saponins are found in small quantities. In the aqueous extract of the trunk bark, the major chemical constituents identified are flavonoids, catechic tannins, anthocyanins, alkaloids and saponins. Polyphenols, gallic tannins, anthraquinones, sterols and triterpenes are found in small quantities.

Regarding the hydro-ethanolic of *A. zygia* leaves, the major chemical constituents identified are flavonoids, anthocyanins, and catechic tannins. Polyphenols, gallic tannins, anthraquinones and saponins are found in small quantities.

The major chemical constituents identified in the hydro-ethanolic extract of *A. zygia* trunk bark are flavonoids, alkaloids, polyphenols, catechic tannins, anthocyanins and saponins. Gallic tannins, anthraquinones, sterols and triterpenes are found in small quantities. The different chemical constituents of the leaves and the *A. zygia* trunk bark and relative abundance are summarized in Table 1.

Table 1: *Screening of chemical constituents of aqueous and hydro-ethanolic extracts of A. zygia (leaves and trunk bark).*

Types of extract		Aqueous extracts		Hydro-ethanolic extracts	
Chemical compounds sought		**Assessment of the relative abundance of leaves**	**Assessment of the relative abundance of trunk bark**	**Assessment of the relative abundance of leaves**	**Assessment of the relative abundance of trunk bark**
Sterols and triterpenes		-	+	-	+
Polyphenols		+	+	+	++
Flavonoids		++	++	+++	+++
Tannins	catechic	+	++	++	++
	gallic	+	+	+	+
Anthraquinones		+	+	+	+
Anthocyanins		++	++	++	++
Alkaloids		-	++	-	+++
Saponins		+	++	+	++
Lipids		-	-	-	-

+++: Abundant; ++: Fairly abundant; +: Not very abundant; -: Absent or traces

Variations of *S. typhi* Cell Abundances in the Presence of Hydro-Ethanolic Extracts of *A. zygia*.

In the solution containing the hydro-ethanolic extract of leaves at 7°C, the densities of *S. typhi* ranged from 4.39 to 5.27 (log (CFU/100 ml)), from 3.91 to 5.23 (log (CFU/100 ml)), and from 3.60 to 4.80 (log (CFU/100 ml)) respectively at the extract concentrations of 0.5g/l, 1g/l and 1.5g/l. At 23°C cell densities fluctuated between 5.39 and 5.74 (log (CFU/100 ml)), between 5.35 and 5.55 (log (CFU/100 ml)), and between 4.59 and 5.31 (log (CFU/100 ml)) respectively at extract concentrations of 0.5g/l, 1g/l and 1.5g/l.

In the solution containing the 7°C hydro-ethanolic extract of the trunk barks, the densities of *S. typhi* ranged from 4.02 to 5.43 (log (CFU/100 ml)), from 3.30 to 5.08 (log (CFU/100 ml)), and from 2.23 to 5.00 (log (CFU/100 ml)) at the extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C the cell densities fluctuated between 4.23 and 5.12 (log (CFU/100 ml)), between 3.60 and 4.62 (log (CFU/100 ml)), and between 3.01 and 4.46 (log (CFU/100 ml)) respectively at extract concentrations of 0.5g/l, 1g/l and 1.5g/l (Figure 1).

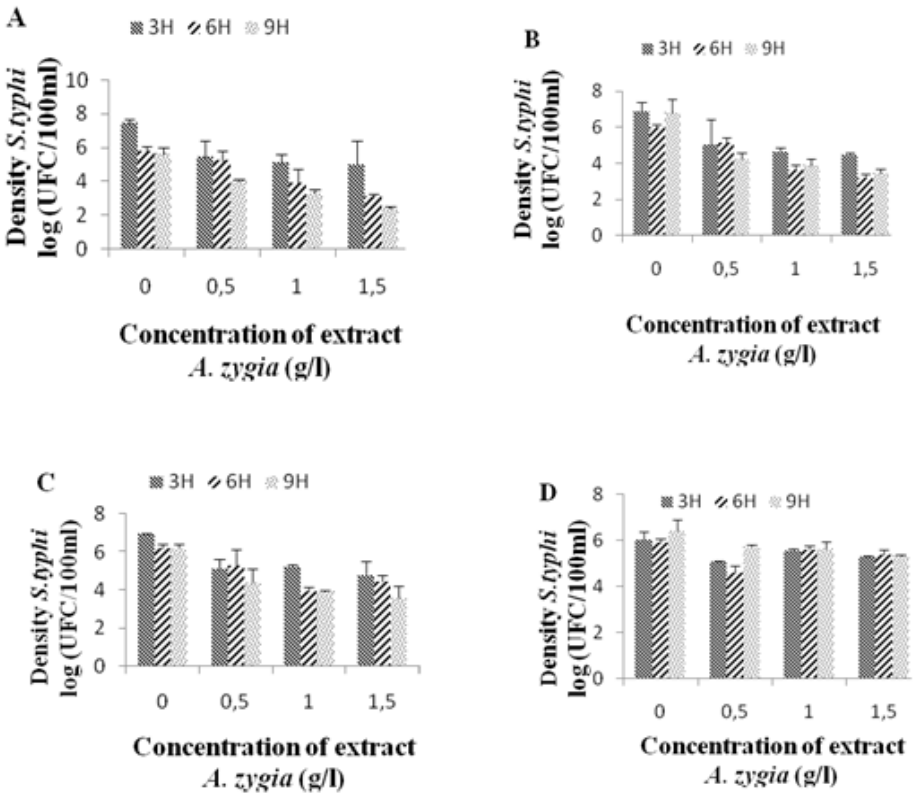

Figure 1: *Temporal variations of S. typhi cell densities in the presence of hydro-ethanolic extract of A. zygia trunk barks (A: 7°C, B: 23°C) and leaves (C: 7°C, D: 23°C) at different incubation temperatures.*

Variation of *S. typhi* Cell Abundances in the Presence of Aqueous Extracts of *A. zygia*

In the solution containing the aqueous extract of leaves at 7°C, the densities of *S. typhi* ranged from 7.36 to 7.64 (log (CFU/100 ml)), from 7.09 to 7.46 (log (CFU/100 ml)), and from 6.28 to 6.93 (log (CFU/100 ml)) respectively at the extract concentrations of 0.5g/l, 1g/l and 1.5g/l. At 23°C cell densities fluctuated between 7.46 and 7.69 (log (CFU/100 ml)), between 7.14 and 7.53 (log (CFU/100 ml)), and between 5.31 and 6.74 (log (CFU/100 ml)) respectively at extract concentrations of 0.5g/l, 1g/l and 1.5g/l.

In the solution containing the 7°C hydro-ethanolic extract of the trunk barks, the densities of *S. typhi* ranged from 7.13 to 7.31 (log (CFU/100 ml)), from 6.92 to 7.01 (log (CFU/100 ml)), and from 6.27 to 6.85 (log (CFU/100 ml)) at the extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C the cell densities fluctuated between 6.47 and 7.44 (log (CFU/100 ml)), between 7.07 and 7.21 (log (CFU/100 ml)), and between 6.46 and 6.77 (log (CFU/100 ml)) respectively at extract concentrations of 0.5g/l, 1g/l and 1.5g/l (Figure 2).

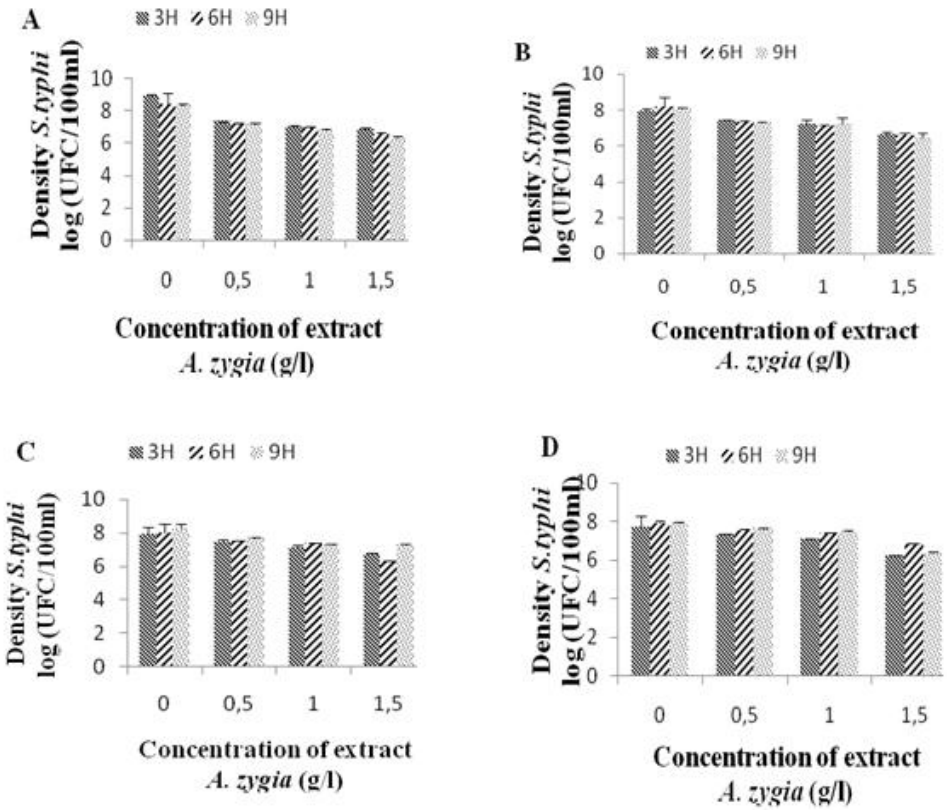

Figure 2: *Temporal variations of S. typhi cell densities in the presence of aqueous extract of A. zygia trunk barks (A: 7°C, B: 23°C) and leaves (C: 7°C, D: 23°C) at different incubation temperatures.*

Percentage of cell inhibition

The percentage of inhibition was calculated to avoid the direct impact of *A. zygia* extracts at different concentrations on the survival of *S. typhi*, under the influence of incubation temperature and exposure time.

Percentage of Inhibition of *S. typhi* Cells in the Presence of Hydro-Ethanolic Extract of Leaves and Trunk Barks

At 7°C, the percentage of inhibition of *S. typhi* cells in the presence of hydro-ethanolic extract of leaves ranged from 82.96 to 98.67%, 94.30 to 98.23%, and 98.14 to 99.72% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C, these percentages ranged from 46.13 to 99.08%, 50.80 to 99.69% and 69.02 to 99.85% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively.

At 7°C, the percentage of inhibition of *S. typhi* cells in the presence of hydro-ethanolic extract of trunk barks fluctuated between 71.87 and 99.02%, 98.67 to 99.55% and 98.91 to 99.91% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C, these temperatures ranged from 86.37 to 99.73%, 99.41 to 99.87%, and 99.59 to 99.95% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. The highest rate of cell inhibition (99.95%) was recorded with *S. typhi* after 9 hours of exposure in the highest concentration (1.5g/l) of hydro-ethanol extract of trunk bark incubated at 23°C (Table II).

Table II: *Percentage inhibition (standard deviation from the mean) of S. typhi after 3, 6 and 9 hours of exposure in the hydro-ethanol extract solution of leaves and trunk bark of 0.5g/l, 1g/l and 1.5g/l concentration.*

		Extract concentration						
		Leaves				Trunk bark		
Incubation temperature	**Incubation period**	0.5g/l	1g/l	1.5g/l		0.5g/l	1g/l	1.5g/l
7°C	3H	98.67 (0.4)	98.23 (1.2)	99.33 (0.4)		99.02 (0.3)	99.55 (0.2)	99.63 (0.5)
	6H	87.68 (5.4)	94.30 (3.7)	98.14 (1.4)		71.87 (4.7)	98.65 (1.2)	98.91 (1.2)
	9H	82.96 (4.2)	94.32 (3.6)	99.72 (0.1)		97.58 (1.6)	99.54 (0.1)	99.42 (0.6)

23°C	3H	88.88 (4,0)	67.21 (1.9)	81.02 (1.1)		98.55 (1.3)	99.41 (0.4)	99.59 (0.2)
	6H	46.13 (14.2)	50.80 (5.7)	69.92 (2.8)		86.37 (3.9)	99.69 (0.2)	99.84 (0.0)
	9H	99.08 (0.1)	99.69 (0.4)	99.85 (0.0)		99.73 (0.0)	99.87 (0.0)	99.95 (0.0)

Percentage of Inhibition of *S. typhi* in the Presence of Aqueous Extract of Leaves and Trunk Bark

The percentages of inhibition of *S. typhi* cells in the presence of aqueous extract of leaves incubated at 7°C fluctuated between 20.06 and 36.04%, 29.65 to 65.76% and 76.99 to 93.99% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C, these percentages ranged from 16.15 to 30.70%, 35.08 to 66.42%, and 60.85 to 95.34% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively.

At 7°C, the percentage of inhibition of *S. typhi* cells in the presence of trunk bark extract fluctuated between 44.75 and 70.99%, 77.17 to 82.38% and 80.64 to 94.09% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. At 23°C, these temperatures ranged from 32.11 to 91.68%, 55.54 to 68.97%, and 84.42 to 91.96% at extract concentrations of 0.5g/l, 1g/l and 1.5g/l respectively. The highest rate of cell inhibition (94.09%) was recorded with *S. typhi* after 9 hours of exposure in the highest concentration (1.5g/l) of trunk bark extract incubated at 7°C. Table III represents the different percentages of cell inhibition.

Table III: *Percentage inhibition (standard deviation from the mean) of S. typhi after 3, 6 and 9 hours of exposure in the aqueous extract solution of leaves and trunk bark of 0.5g/1, 1g/1 and 1.5g/1 concentration.*

		Extract concentration						
		Leaves				**Trunk bark**		
Incubation temperature	**Incubation period**	0.5g/l	1g/l	1,5g/l		0,5g/l	1g/l	1,5g/l
7°C	3H	36.04 (2.7)	65.76 (3.4)	76.99 (5.8)		44.75 (2.2)	72.17 (4.4)	80.64 (8.5)
	6H	20.06 (5.0)	29.65 (4.1)	81.1 (6.7)		70.99 (7.4)	82.38 (7.7)	92.19 (4.0)
	9H	22.06 (1.4)	33.02 (6.3)	93.99 (0.6)		51.47 (2.7)	81.64 (8.3)	94.09 (5.2)
23°C	3H	30.70 (13.3)	66.42 (9.4)	86.73 (8.3)		32.11 (0.6)	59.97 (18.3)	89.78 (14.9)
	6H	25.98 (4.7)	46.51 (4.0)	95.34 (0.4)		46.59 (7.8)	68.97 (12.8)	84.42 (12.0)
	9H	16.15 (2.5)	35.08 (2.1)	60.85 (3.2)		91.68 (4.1)	55.54 (3.5)	91.96 (2.4)

Relationships between the Parameters Considered

Spearman's correlation coefficients« *r* » between bacterial densities and the concentration of *A. zygia* extracts at each incubation time were calculated and presented in table IV. It showed that the cell densities of *S. typhi* decrease significantly ($P < 0.05$ and $P > 0.01$) as the concentration of aqueous and hydro-ethanolic extracts of *A. zygia* increased during each exposure. The exception was with the aqueous and hydro-ethanolic extracts of leaves where a non-significant correlation was realized after 6 and 9 hours of incubation respectively.

Table IV: *Spearman's correlation « r » between bacterial densities and concentrations of aqueous and hydro-ethanolic extracts of A. zygia leaves and trunk bark during each exposure time.*

	Bacterial species	**Part of the plant**	**Incubation period (hours)**		
			3h	6h	9h
Aqueous extracts	*S. typhi*	Leaves	-0.973**	-0.0973**	-0.679
		Trunk bark	-0.976**	-0.976**	-0.830*
Hydro-ethanolic extracts	*S. typhi*	Leaves	-0.837*	-0.712	-0.994**
		Trunk bark	-0.878**	-0.976**	-0.927**

= significant correlation to P < 0.05* * = significant correlation to P < 0.01* *ddl: 3*

The degrees of association between *S. typhi* densities and incubation periods for each concentration of aqueous and hydro-ethanolic extracts of *A. zygia* leaves and trunk bark were evaluated. The correlation coefficients are presented in table V. It was discovered that, in most cases, increasing the exposure time resulted in a significant decrease in the abundance of *S. typhi* ($P < 0.05$ and $P < 0.01$).

Table V: *Correlation between bacterial densities and incubation periods for each concentration of aqueous and hydro-ethanolic extract from the leaves and trunk bark of A. zygia*

	Bacterial species	Part of the plant	Concentrations of extracts		
			0.5g/l	***1g/l***	***1.5g/l***
Aqueous extracts	*S. typhi*	Leaves	-0.956**	-0.956**	-0.120
		Trunk bark	-0.837*	-0.359	-0.837*
Hydro-ethanolic extracts	*S. typhi*	Leaves	-0.485	-0.970**	-0.847*
		Trunk bark	-0.717	-0.837*	-0.974**

**= significant correlation to P < 0.05 ** = significant correlation to P < 0.01 ddl: 5*

Comparison between the Mean Abundances of *S. typhi* Cells Exposed to Each Type of Extract

The mean abundances of *S. typhi* cells exposed to aqueous extracts were compared with the mean abundances of *S. typhi* exposed to hydro-ethanolic extracts of Kruskal-Wallis H test. The values of the significance coefficients are presented in Table VI.

Table VI: *Comparison between the mean abundances of S. typhi cells exposed to each type of extract*

Bacterial species	Aqueous extracts	Hydro-ethanolic extracts
S.typhi	0.022*	0.011*

**= significant difference to P < 0,05*

It appears that these bacterial abundances differ significantly ($P < 0.05$) from one extract to another.

Comparison between the average abundances of *S. typhi* exposed to aqueous and/or hydro-ethanolic extracts of each plant part

The mean abundances of *S. typhi* cells exposed to aqueous and/or hydro-ethanolic extracts of leaves and/or trunk bark of *A. zygia* were compared using the Kruskal-Wallis H test. The values of the significance coefficients are presented in table VII. These bacterial abundances seem to differ significantly ($P < 0.05$) for each type of *A. zygia* extract.

Table VII: *Comparison between the average abundances of S. typhi exposed to aqueous and/or hydro-ethanolic extracts of each plant part*

Type of Extract	Aqueous extracts		Hydro-ethanolic extracts	
	Leaves	Trunk bark	Leaves	Trunk bark
S.typhi	0.026*	0.018*	0.013*	0.009*

**= significant difference to P < 0,05*

DISCUSSION

Phytochemical screening of aqueous extracts of *A. zygia* revealed the presence of some secondary metabolites (table 1). The aqueous extract of leaves revealed the presence of flavonoids, anthocyanins, tannins, polyphenols, anthraquinones and saponins. Flavonoids and anthocyanins were relatively more abundant than the other chemical constituents. The results are similar to those of Regina *et al*. (2016) which had indicated the presence of these compounds. However, Regina et *al*'s results do not indicate the presence of anthocyanins and polyphenols in the aqueous and hydro-ethanolic extract of *A. zygia*.

In the aqueous extract of trunk bark, secondary metabolites such as flavonoids, alkaloids, polyphenols, tannins, anthocyanins, saponins, anthraquinones, sterols and triterpenes were obtained. Flavonoids, anthocyanins, alkaloids, saponins and catechic tannins are relatively more abundant than other chemical constituents. This result is similar to that of Assong (2017) who in addition to these compounds had reported the presence of others such as coumarins, resins and mucilages except alkaloids, sterols and triterpenes in the root bark of the *Albizia* genus plant to which the *A. zygia* species belongs.

Phytochemical screening of hydro-ethanolic extracts of *A. zygia* also revealed the presence of some secondary metabolites. Molecules such as flavonoids, anthocyanins, tannins, polyphenols, anthraquinones and saponins were obtained in the hydro-ethanolic extract of leaves. This result is different from that observed by Oluyemi *et al.* (2014) which indicated the presence of flavonoids, tannins, anthraquinones, cardiac glycosides, alkaloids and saponins in ethanolic, methanolic and aqueous extract of *A. zygia* leaves. Molecules such as flavonoids, alkaloids, polyphenols, tannins, anthocyanins, saponins, anthraquinones, sterols and triterpenes were identified in the hydro-ethanolic extract of the trunk bark. Wilfred *et al*. (2017) & Ganiyat *et al*. (2013) already revealed the presence of all these secondary metabolites except anthocyanins in the methanolic and hexanolic extract of *A. zygia* stem bark. Indeed, alkaloids, sterols and triterpenes are present only in the stem bark. Meanwhile polyphenols, flavonoids, tannins, anthraquinones and saponins were highlighted in the two parts of the plant selected for the study. This qualitative variation of the secondary metabolites observed could be related to the diversity of the soils, climate, solvent, extraction method and the level of ripening of the plant during harvest, In this regard, Podsedck (2007) and Falleh *et al*. (2008) noted that high temperature, exposure to sun, maturity during harvest and storage conditions affect the biosynthesis of secondary metabolites.

Effects of *A. zygia* Extracts on Bacterial Cells

A temporal variation of cell densities in the presence of aqueous and hydro-ethanolic extracts of *A. zygia* was observed. This variation depends not only on the concentration of the plant extract, but also on the temperature and duration of incubation and on the part of the plant considered. The quantitative analysis of the bacterial densities in the control solutions, after each incubation period and the densities of the bacterial cells after exposure in the various solutions of the extracts, made it possible to show that, the variation of the cellular densities would be related to the action of the extracts of *A. zygia* on the cultivability of these cells.

The percentages of *S. typhi* inhibition ranged from 16.15 to 99.95% (table III). The concentration of the extract would have a considerable impact on the cultivability of bacterial cells. The extract concentrations of 1g/l and 1.5g/l would be those with the highest efficiency resulting in a high rate of cell inhibition. The inhibition rate increases with the concentration of the extract. The high percentages of inhibition recorded during the experiment would probably be due to the secondary metabolites present in the aqueous and hydro-ethanolic extracts of the plant. Similar results were reported by Tamsa Arfao *et al*. 2013 who showed that the increase in inhibition percentages of *S. typhi* bacterial cells is strongly correlated with the increase in concentration of *Eucalyptus microcorys* plant extract. These authors also demonstrate that the percentage variations of inhibition according to the temperature and the duration of incubation would be explained by the variation of the antibacterial activity of the bioactive compounds in the plant extract. Combining the results of the cell cultivation and the phytochemical screening, it could be suggested that the antibacterial activities are related to the presence of phenols found in the extract of leaves and trunk bark of *A. zygia*. Polyphenols including flavonoids and tannins are known for their toxicity on microorganisms. Tannins and saponins possess antibacterial properties (Loguercio *et al*. 2005; Voravuthikunchai 2005; Raven *et al.* 2007 and Tamsa Arfao *et al*. 2017). A negative and significant correlation was observed between cell densities and the concentration of *A. zygia* extracts. These results are similar to those of Tassou *et al*. (2000) and Tsuchiya *et al*. (2000). According to the latter, the higher the extract concentration, the greater the amount of potassium ions and proteins that leak out of the cell; these leaks can cause structural and functional damage to the plasma membrane. From this study, it appears that the hydro-ethanolic extract of the trunk bark would be the most active in terms of inhibitory effect on *S. typhi*, with an inhibition rate of 99.95%. The aqueous extract would have a less effective antibacterial activity than the hydro-ethanolic extract. This difference in activity would probably be due to the phytochemical composition which differs between the two types of extracts. The bacterial inhibition observed throughout the study could be due to the accumulation of certain micronutrients from the plant extracts that would become toxic to the bacterial cells over time (Nola *et al*. 2010). The bacterial inhibition would also be due to the presence of secondary metabolites in the plant extract. Upadhyay *et al*. (2010) showed that aqueous and hydro-ethanolic extracts of leaves rich in phenolic compounds inhibited the growth

of *B. cereus* and *S. aureus*. Indeed, phenolic compounds, flavonoids, triterpenes, alkaloids, tannins, and saponosides present in the considered extracts are known for their toxicity towards some microorganisms (Loguercio *et al*. 2005; Raven *et al*. 2007). Tannins also have the ability to complex proteins leading to the inactivation of enzymes, either directly by binding to active sites, or indirectly by steric hindrance created by the attachment of tannin molecules on the enzyme (Zimmer *et al*. 1996).

Moreover, the main characteristic of secondary metabolites is their hydrophobicity. It allows their solubilization in membranes, which causes a destabilization of the structure and an increase in membrane permeability (Sikkema *et al*. 1994). These changes lead to a leakage of ions and intracellular compounds (Carson *et al*. 2002). If the loss of material is too great or if the released cytoplasmic elements are essential for the survival of the bacterium, this leads to cell death. This inhibitory effect is modulated by the bacterium-extract contact time, with long contact times acting on targets not reached by relatively short contact times. This analysis could explain the origin of the abundant cell death recorded after 6 and 9 hours of the bacteria with the extracts. Thus, a negative and significant correlation between bacterial densities and concentrations of aqueous and hydro-ethanolic extracts of leaves and trunk bark of *A. zygia* was noted during this study for each incubation time (table IV).

The results of the present study show that increasing the incubation temperature would not have a significant effect on the densities of the bacterial cells used. *S. typhi* in aqueous and hydro-ethanolic extract solutions of leaves and trunk bark would appear insensitive to variations in incubation temperatures. This insensitivity to temperature variations would be related to the fact that under these conditions, the metabolism of mesophilic bacteria is relatively slow and the toxic products are only very slowly metabolized (Lessard *et al*. 1983).

The Kruskal-Wallis H-test ($P < 0.05$) showed on the one hand that the hydro-ethanolic extract would have a stronger inhibitory effect than the aqueous extract and on the other hand, that the trunk bark extract would act more than the leaf extract.

The results indicate that the aqueous and hydro-ethanolic extracts of *A. zygia* have an antibacterial effect in the aquatic environment. Similar data was revealed by Sunday *et al*. (2008) and Tamsa Arfao *et al*. (2013) who showed that the aqueous extracts of *Lantana camara*, *Cymbogon citrates*, *Hibiscus rosa sinensis* and *Eucalyptus microcorys* have bactericidal effect in aquatic environment. Lutgen and Michels (2008) showed that by adding *Artemisia annua* tea to water contaminated with bacterial cells, a considerable reduction of the bacterio-contaminant load was observed at levels lower than those obtained after boiling water.

CONCLUSION

The present study aims at evaluating in aquatic microcosm, the activity of *A. zygia* extracts on the cultivation of *S. typhi* bacteria. The concentration of the extracts, the residence time of the bacteria in the extract solutions and the incubation temperature of the different extract solutions are the factors that were considered in the various experiments conducted.

Generally, the results show that the presence of *A. zygia* extracts significantly reduces the cultivability of the bacteria considered in the water.

Secondary metabolites with antibacterial activity such as anthraquinones, anthocyanins, flavonoids, polyphenols, tannins and saponins were found in both types of extracts. These secondary metabolites were qualitatively and quantitatively more abundant in the hydro-ethanolic extract.

The use of medicinal plants in water disinfection offers many research opportunities in a world where access to clean water remains a permanent concern for public authorities. The data obtained from this exploratory work permit us to consider the use of *A. zygia* extracts as an alternative process in water disinfection. However, practical application modalities should be recommended by complementary works.

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