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Evaluation of *Acacia sieberiana* var. Woodii (Fabaceae) Aqueous Extract as a Natural Bactericidal Agent for Water Treatment against Pathogenic Bacteria

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Abstract

Contaminated water poses a risk to public health, particularly in regions relying on untreated sources. This study investigates the antibacterial potential of aqueous leaf extracts from *Acacia sieberiana* against *Streptococcus faecalis* and *Salmonella typhi* in simulated water systems. Bacterial suspensions were exposed to four extract concentrations (0.5 - 2 g/L) at 7˚C and 23˚C for 3, 6, 9 and 24 hours. MIC, and MBC were then determined. The extract lowered CFU values in a dose and time dependent manner. Complete inhibition of *Salmonella typhi* was achieved at ≥1.5 g/L within 3 hours. The aqueous extract of *Acacia sieberiana* significantly reduces the cultivability of the bacterial abundances of the two strains tested. In addition, *S. typhi* was the most sensitive bacterium to this plant extract. The temperature of the medium did not significantly influence bacterial inhibition ($P > 0.05$). The aqueous extract of *Acacia sieberiana* exhibited a bactericidal effect on the cultivability of these straing with MICs varying from 7.81 to 15.62 mg/mL. The results of the present study support the use of *A. sieberiana* in the treatment of pathogenic infections as well as reduction of the flux of bacteriopollutants in water.

Keywords

Aqueous Extract, Antibacterial Activity, *Acacia sieberiana*, Pathogenic Bacteria, Water Treatment

1. Introduction

In Cameroon, as in other emerging African countries, shortages of drinking water forces many households to drink spring and groundwater, which often at times harbor potential microbiological contaminant [1]. The microbiological contaminants may be due to the presence of protozoa, viruses, and/or pathogenic bacteria [2]. The bacteria known to be responsible for water contamination are of the genus *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Vibrio* and *Campylobacter* among others [3]. For decades, simple disinfection methods have been used. These include water disinfection by solar irradiation or Solar Water Disinfection (SODIS); treatment with chemical disinfectants such as: oxidants and chlorinated halogens, the residues of which have repercussions on health; filtration; boiling: which modifies the organoleptic properties of the water, thereby making it to become thick and unpleasant to drink.

In recent years, water disinfection methods using plant extracts have been proposed as a new alternative for water treatment at the household level. The use of plants for therapeutic purposes has been a common practice for millennia. And in Cameroon as well as in other countries, plants used in traditional medicine against infectious diseases have been the subject of numerous studies [4]-[6]. Although plant extracts have antibacterial (bactericidal) activities, they are still underused in the treatment of water intended for human consumption. *Acacia sieberiana* var. Woodii also known as *Vachellia sieberiana* is a species of tree belonging to the Fabaceae family. It is grown in Cameroon and is native to the savannahs of Africa. The plant is used to treat schistosomiasis, haemorrhage, orchitis, colds, diarrhoea, gonorrhoea, kidney problems, syphilis, ophthalmia, rheumatism, respiratory disorders, biharzia and tapeworm infection [7]. The pods are emollient, while the roots treat stomach-ache, urethral problems, edema and dropsy. The preliminary phytochemical screening of *Acacia sieberiana* indicated the presence of tannins, saponins, cardiac glycosides, anthraquinones and flavonoids [8]-[10]. Pharmacological studies on *Acacia sieberiana* revealed its effectiveness towards *Shigella sonnei*, *Staphylococcus epidermidis*, *Enterobacter cloacae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium avium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Salmonella* Paratyphi. In continuation, of our work on this species, the present study aims to evaluate the aqueous leaves extract of *A. sieberiana* towards two bacteriopollutants (*S. feacalis and S. typhi*), in water.

2. Materials and Methods

2.1. Plant Material

The fresh leaves of *Acacia sieberiana var. Woodii* was harvested at Kaele in the Far North region of Cameroon in October 2019. The sample was identified by a botanist at Cameroon National Herbarium (HNC) by comparison of the specimen with the existing samples in Cameroon Herbarium database and was registered

with the voucher specimen number 49882/HNC.

2.2. Preparation of Aqueous Extracts of *Acacia sieberiana*

The extractions were carried out using the following method [11]. The air dried leaves of *Acacia sieberiana* were ground to fine of powder (414.7 g) and mixed with 3000 mL of distilled water for 48 h at room temperature. The obtained macerate was filtered and then dried in an oven at a temperature of 45˚C ± 2˚C until total evaporation of the water. The steps involved in this extraction process are shown in Figure 1. From three independent extractions, the average dry extract mass obtained was 177.81 g, corresponding to an extraction yield of 42.88%.

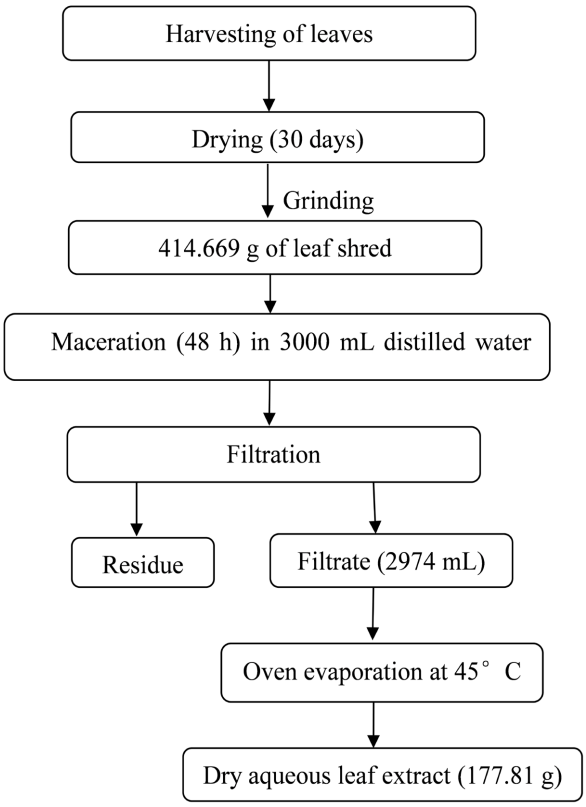

Figure 1. Extraction protocol for aqueous extract.

2.3. Yield of Extraction (R)

The yield of the extract is defined as the ratio of the quantity of plant material extracted to the quantity of plant material used. It is calculated according to the following equation:

$$\mathbf{R}(\%) = \frac{\mathbf{Mob}(\mathbf{g})}{\mathbf{Mi}(\mathbf{g})} \times 100 .$$

R is Yield,
Mob is weight of the dry extract in g,
Mi is the dry weight of plant material in g.

2.4. Phytochemical Analysis of Extract

The study of the chemical profile allows the detection of different classes of compounds present in the plant. The tests are based on the intensity of the precipitate and the turbidity. Thus, we proceeded to the characterization of secondary metabolites present in the plant *A. sieberiana*, according to the method described by Pareck and Chanda [12].

2.5. Bacteria Isolation

The bacteria *S. faecalis and S. typhi* were isolated from the Olezoa River (Yaounde-Cameroon) using Bile Aesculin Agar medium and Wilson-Blair agar respectively contained in Petri dishes, and using plate count technics. The Petri dishes were incubated at 37˚C for 24 hours. After incubation times, colonies on Wilson-Blair agar were blue and those on Bile Aesculin Azide were slightly opalescent black colonies with a light black halo. They were cultured on ordinary agar poured slanted into test tubes and incubated at 37˚C for 24 hours to obtain pure strains [13].

2.6. Preparation of Extract Solutions at Different Concentrations

The paste obtained was used for the preparation of the extract solutions. Four extract concentrations of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L were prepared by dissolving the different masses in sterile physiological water (NaCl 0.85%). Each concentration was separately filtered on whatmann paper, followed by filtration under vacuum on a cellulose nitrate membrane with a porosity of 0.45 µm [14]. This facilitated the elimination of any bacterial load that may exist [15]. 0.1 mL of each filtrate was inoculated onto ordinary agar to ensure the sterility of the solutions; and the solutions were stored in a refrigerated chamber at a temperature of 7˚C for subsequent manipulations. This operation was performed under sterile conditions around a Bunsen burner.

2.7. Preparation of Bacterial Suspensions and Inoculation

Fresh bacterial culture grown on nutrient agar were harvested using a sterile platinum loop and introduced into a test tube containing 10 mL of sterile physiological water. The homogenized bacterial suspension was vortexed and adjusted to a density of 0.5 Mac Farland ($BaCl_2$ and 1% H_2SO_4). The concentration of bacteria in the stock suspension was around 1.5×10^8 CFU/mL. Four dilutions for *S. faecalis* and two for *S. typhi* were made from this stock suspension, with the aim of reducing the bacterial load [16]. Subsequently, 0.5 mL of the bacterial suspension was withdrawn using a sterile pipette and introduced into the control flasks containing 100 mL of physiological water and into the flasks containing 100 mL of solution of aqueous extract of *A. sieberiana*.

2.8. Antimicrobial Analysis

The antimicrobial activity was carried out under aseptic condition focused on the

quantitative aspect. They were carried out after 3, 6, 9 and 24 hours of incubation, using the technique of spreading on the surface of the agar culture medium until exhaustion [16]. After homogenization, 100 µL of the sample to be analyzed was spread on the surface of the specific culture medium poured into a Petri dish (Bile Esculin Azide agar for *S. faecalis* and Wilson-Blair agar for *S. typhi*). The incubation periods were 24 hours for both *S. faecalis* and *S. typhi*, at a temperature of 37˚C. The counting of isolated viable and cultivable germs was done by direct counting on a Petri dish using an OSI brand colony counter. Bacterial abundances were expressed in Colony Forming Units (CFU) and expressed in mL of inoculum (CFU/mL).

2.9. Determination of the Minimum Inhibitory Concentrations (MIC) of the Extract

The MIC of the extract was determined by the liquid microdilution method using the M07-A9 protocol described by CLSI [17] with a few modifications. It is the lowest concentration that inhibits any visible growth of a microorganism after incubation at 37˚C for 18 to 24 hours [18].

In each well of microplates (96 wells), was introduced a volume of 100 µL of Mueller Hinton broth. In the first wells of each column (numbered 1 to 12) was introduced a volume of 100 µL of extract at concentrations ranging from 40 to 9.5 mg/mL. The successive serial dilutions of ratio 2 allowed us to obtain a range of concentrations from 20 - 1.25 mg/mL. The concentration of the crude extract was fixed at 2000 mg/mL, and the concentration ranges of this extract fluctuated between 3.90 - 500 mg/mL. A volume of 100 µL of inoculum was introduced into each well, for a final volume of 200 µL per well. Plates were covered and sealed with parafilm and incubated at 37˚C for 18 hours. The negative control consisted of wells containing exclusively Mueller Hinton broth and inoculum. The positive control consisted of a reference antibiotic (Gentamicin).

2.10. Determination of Minimum Bactericidal Concentrations (MBC)

As concerns the MBCs, a volume of 150 µL of culture broth was introduced into new plates, and completed to 200 µL by adding a volume of 50 µL of the contents of the wells of greater or equal concentration. The plates were incubated at 37˚C for 48 hours, followed by visualization with INT. The concentrations at which no pink coloring was observed were taken as bactericidal and the smallest of these was noted as MBC. The tests were carried out in triplicate.

After determination of the MIC and MBC, the MBC/MIC ratios were calculated. This ratio permits the characterization of the activity of a given antibiotic [19], where, MBC/MIC ≤ 4: bactericidal effect; MBC/MIC > 4: bacteriostatic effect; MBC/MIC > 32: the bacterium is tolerant to the extract.

2.11. Data Analysis

All experiments were performed in triplicate (n = 3). Data were analyzed using

non-parametric statistical tests (Spearman's rank correlation and Kruskal-Wallis H test) due to non-normal distribution of variables. Exact P-values were reported throughout.

The variations of cell densities were expressed in logarithmic unit (log CFU/mL) as histograms using Excel 2016 software. The percentages of inhibition (PI) of the aqueous extract of *A. sieberiana* on bacterial cells were evaluated using the formula described by Garcia-Ripoll and other authors [20].

$$\mathbf{PI} = \frac{\mathbf{N_0} - \mathbf{N_n}}{\mathbf{N_0}} \times 100$$

PI = Percentage of inhibition, $\mathbf{N_0}$ = Initial bacterial abundance, $\mathbf{N_n}$ = Bacterial abundance after the action of the extract.

On one hand, the relationships between cell abundances and different concentrations of the extract as well as cell abundances and the different temperature ranges on the other hand, were evaluated by Spearman's "r" correlation tests at each incubation period. Comparisons of the means of the data were performed using the Kruskal-Wallis H test. These analyses were performed using SPSS 18.0 software.

3. Results

3.1. Yield of Plant Extract

The yield of the aqueous extract of the leaves of *A. sieberiana* was 42.88%.

3.2. Phytochemical Screening

Phytochemical analyses allowed us to characterize the presence of some chemical substances in the extract. According to the intensity of color, the chemical constituents identified in the aqueous extract of *A. sieberiana* leaves were alkaloids, tannins, triterpenes, flavonoids, saponins and steroids. Anthocyanins, anthraquinones, glycosids and coumarines were absent (Table 1).

Although quantitative analysis of secondary metabolites was not performed in this study, previous works reported significant levels of tannins (23.5 %), flavonoids (15.2 %) and saponins (8.7 %) in *A. sieberiana* leaf extracts [8] [9], supporting their potential contribution to antimicrobial activity.

Table 1. Screening of chemical constituents of aqueous extract of leaves of *A. sieberiana*.

Types of extract	Aqueous extract of leaves
Chemical compound sought	Assessment of the relative abundance
Alkaloids	+
Tanins	+
Triterpenes	+
Flavonoids	+
Anthraquinones	−

Continued

Anthocyanins	−
Glycosides	−
Saponins	+
Stéroids	+
Coumarines	−

Légende: (+) = Presence of metabolites. (−) =Absence of metabolites.

3.3. Antibacterial Activity of *A. sieberiana* Extract on the Tested Bacterial Strains

The temporal variations of the cell abundances of the bacterial species studied are represented by histograms. They are expressed in decimal logarithmic units (CFU/mL). In the control tubes, were physiological water (NaCl: 0.85%). Bacterial densities varied from one incubation period to another. In the presence of the aqueous extract of *A. sieberiana*, there is a decrease in bacterial abundance. These abundances fluctuate depending on the concentration of the plant extract, temperature and incubation time.

3.4. Temporal Variations of the Abundances of *Streptococcus faecalis* in the Presence of the Aqueous Extract of the Leaves of *Acacia sieberiana*

In physiological water during the 24 hours period, the densities of *S. faecalis* varied between 8.50 and 8.86 (log (CFU/mL)) and between 8.50 and 8.74 (log (CFU/mL)) respectively at temperatures 7˚C and 23˚C. The densities of these cells experience an overall decrease in the presence of the extract (Figure 2). In the solution containing the extract at 7˚C, the cell densities oscillated between 8.42 and 8.71 (log (CFU/mL)), 8.40 and 8.59 (log (CFU/mL)), 8.34 and 8.53 (log (CFU/mL)) and 8.08 and 8.30 (log (CFU/mL)) respectively at extract concentrations of 0.5 g/L, 1 g /L, 1.5 g/L and 2 g/L. In the solution containing the extract of *A. sieberiana* at 23˚C cell densities fluctuated between 8.40 and 8.54 (log (CFU/mL)), 8.16 and 8.37 (log (CFU/mL)), 8.06 and 8.38 (log (CFU/mL)) and 8.02 and 8.34 (log (CFU/mL)), respectively at extract concentrations of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L. Figure 2 represents the temporal variation of the densities of *S. faecalis* cells in physiological water (NaCl: 0.85%) and in solutions containing the aqueous extract of the leaves of *A. sieberiana* at different concentrations.

3.5. Temporal Variations of the Abundances of *Salmonella typhi* in the Presence of the Aqueous Extract of the Leaves of *Acacia sieberiana*

Figure 3 shows the temporal variation of the densities of *S. typhi* cells in physiological water (NaCl: 0.85%) and in solutions containing the aqueous extract of the leaves of *A. sieberiana* at different concentrations. In physiological water, the densities of *S. typhi* varied between 4.58 and 4.81 (log (CFU/mL)) and 4.30 and 4.71

(log (CFU/mL)) respectively at temperatures 7 and 23˚C. The densities of these cells decrease overall in the presence of the extract (Figure 3). In the solution containing the extract at 7˚C, these cell densities varied between 1.83 and 3.65 (log (CFU/mL)), 0.70 and 2.17 (log (CFU/mL)), 0 and 2.18 (log (CFU/mL)) and 0 and 2.04 (log (CFU/mL)) respectively at extract concentrations of 0.5 g/L, 1 g/L, 1 .5 g/L and 2 g/L. In the solution containing the extract of *A. sieberiana* incubated at 23˚C cell densities reached 3.03 (log (CFU/ mL)), 2.27 (log (CFU/ mL)), 2.18 (log (CFU/ mL)) and 1.90 (log (CFU/ mL)) respectively at extract concentrations of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L.

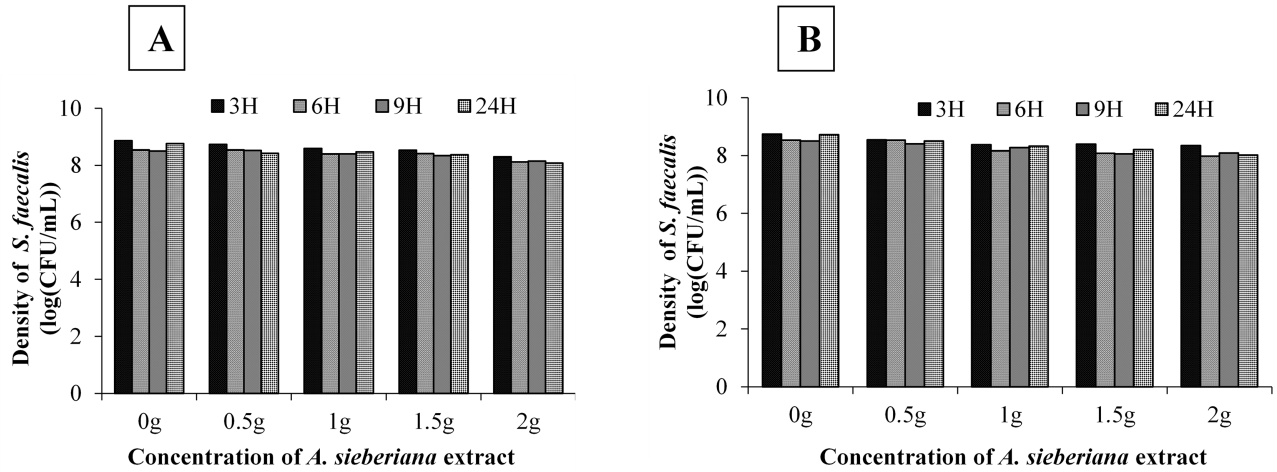

Figure 2. Temporal variations and standard deviations of cell densities of *Streptococcus faecalis* in the presence of the aqueous extract of *Acacia sieberiana* leaves at different incubation temperatures ((A) 7˚C; (B) 23˚C).

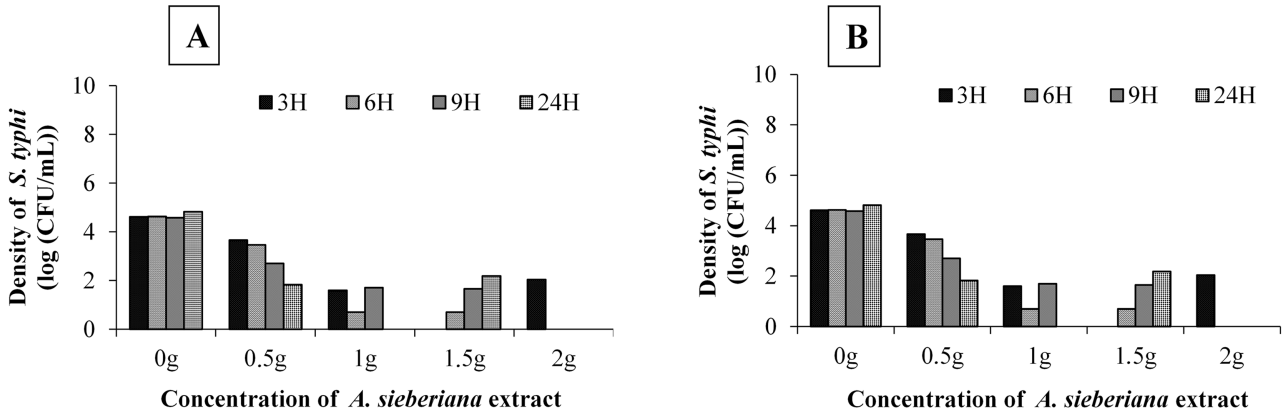

Figure 3. Temporal variations and standard deviations of cell densities of *Salmonella typhi* in the presence of the aqueous extract of *Acacia sieberiana* leaves at different incubation temperatures ((A) 7˚C; (B) 23˚C).

3.6. Evaluation of the Percentages of Inhibition of Bacterial Cells in the Presence of the Aqueous Extract of the Leaves of *Acacia sieberiana*

In the aqueous extract, the cultivability of the bacterial cells were relatively inhibited. The percentages of inhibition of *S. faecalis* and *S. typhi* under various incu-

bation temperatures are presented in Table 2. In the majority of cases, the percentages of inhibition of bacterial cells increase with the concentration of the extract and the incubation period.

At the temperature 7˚C, the percentages of inhibition of *S. faecalis* cells fluctuated between 17.89 and 48.62%, 27.77 and 54.28%, 25.56 and 59.56%, and 62.37 and 79.05% at extract concentrations of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L respectively. Those of *S. typhi* reached 99.89% and 99.98% at extract concentrations of 0.5 g/L and 1 g/L respectively; and they reached 100% at extract concentrations of 1.5 g/L and 2 g/L.

At 23˚C, the percentage inhibition of *S. faecalis* cells fluctuated between 19.61% - 79.87% at extract concentrations of 0.5 -2.0 g/L, respectively. Those of *S. typhi* oscillated between 96.20 and 99.38%, and 99.06 and 99.96 at extract concentrations of 0.5 g/L, 1 g/L respectively. They achieved 100% at extract concentrations of 1.5 g/L and 2 g/L. *S. typhi* is the species that seems to record the highest inhibition rates. At all the concentrations of the extract and during all the incubation times, the percentages of inhibition of *S. typhi* were very high, reaching inhibition rates of 100% at concentrations greater than or equal to 1.5 g/L at 3 hours of exposure compared to those of *S. faecalis* where the highest inhibition rates were observed at 2 g/L after 24 hours of exposure in the extract.

Table 2. Percentages of inhibition of *S. faecalis* and *S. typhi* after 3 h, 6 h, 9 h and 24 h of exposure in the solution of the aqueous extract of the leaves of *Acacia sieberiana* at a concentration of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L.

		S. faecalis				*S. typhi*			
		Extract concentrations of *A. sieberiana*							
Incubation temperatures	Incubation times	0.5 g/L	1 g/L	1.5 g/L	2 g/L	0.5 g/L	1 g/L	1.5 g/L	2 g/L
7˚C	3 h	17.89	27.77	25.56	62.37	88.85	99.86	99.77	100
	6 h	28.90	46.63	53.67	72.57	93.22	99.90	99.88	100
	9 h	31.28	48,31	56.32	77.01	98.67	99.96	99.98	100
	24 h	48.62	54.28	59.56	79.05	99.89	99.98	100	100
23˚C	3 h	19.61	42.05	57.53	60.53	96.20	99.06	100	100
	6 h	29.03	56.25	63.51	60.92	96.31	96.36	100	100
	9 h	36.74	57.11	64.15	71.76	97.74	99.92	100	100
	24 h	39.68	60.56	70.08	79.87	99.38	99.96	100	100

3.7. Minimal Inhibitory (MIC) and Bactericidal (MBC) Concentrations of the Crude Extract of *A. sieberiana*

The results showed antibacterial activity on the germs tested with MICs ranging from 7.81 to 15.62 mg/mL respectively for *S. typhy and S. faecalis*. The crude extract revealed better bacterial activity on *S. typhi* with an MIC of 7.81 mg/mL.

The MBCs were determined and the MBC/MIC ratios calculated. The MBC/MIC ratios ranging from 0.2 to 1 respectively for *S. faecalis and S. typhy*. The results showed that the aqueous extract of *A. sieberiana* presented a bactericidal effect on

the two bacterial strains used.

Table 3 shows the results of the MICs, MBCs and the MBC/MIC ratios (in mg/mL) of the crude extract of *A. sieberiana* as well as those of the reference antibiotic (Gentamicin) on *S. faecalis* and *S. typhi.*

Table 3. Antibacterial activity of the crude extract and the antibiotic (Gentamicin): MIC, MBC, Ratio (R) MBC/MIC (in mg/mL).

	Bacteria							
	S. faecalis				*S. typhi*			
	MIC	MBC	R	Effects	MIC	MBC	R	Effects
Crude extract	15.62	3.9	0.2	*Bactericidal*	7.81	7.81	1	*Bactericidal*
Gentamicin	7.81	15.62	2	*Bactericidal*	3.90	7.81	2	*Bactericidal*

3.8. Spearman's "r" Correlation between the Different Variables Considered

The correlation test was used to evaluate the degree of connection between the bacterial abundances of the control (absence of the extract) and the bacterial abundances in the presence of the extract at various concentrations, incubation times and temperatures.

3.9. Correlation between Bacterial Abundances and Concentrations of the Aqueous Extract of *Acacia sieberiana* Leaves

Spearman's "r" correlation coefficients between the bacterial abundances and the concentrations of the aqueous extract of *A. sieberiana* were calculated and are tabulated in Table 4. The results indicates that the increase in the concentration of the extract significantly influenced ($P < 0.01$ and $P < 0.05$) the decrease in the abundance of cultivable cells.

Table 4. Correlation between bacterial abundances and concentrations of the aqueous extract of *Acacia sieberiana* leaves.

Bacteria species	Extract concentrations of *A. sieberiana*			
	0.5 g/L	1 g/L	1.5 g/L	2 g/L
S. faecalis	−0.183*	−0.206*	−0.258*	−0.388*
S. typhi	−0.404*	−0.374*	−0.509**	−0.521**

* = significative correlation at $P < 0.05$; ** = significative correlation at $P < 0.01$; ddl: 15.

3.10. Correlation between Bacterial Abundances and Incubation Times at Each Incubation Temperature

Spearman's "r" correlation coefficients between bacterial abundances and incubation times at each incubation temperature of the aqueous extract of the leaves of *A. sieberiana* were calculated and presented in Table 5. It shows that the increase in incubation time led to a significant decrease in the abundances of *S. faecalis* and *S. typhi* with increasing incubation temperature ($P < 0.01$ and $P < 0.05$).

Table 5. Correlation between bacterial abundances and incubation times at each incubation temperature.

Bacteria species	Incubation temperatures	Incubation times			
		3 h	6 h	9 h	24 h
S. faecalis	7˚C	−0.320*	−0.412*	−0.468*	−0.415*
	23˚C	−0.295*	−0.398*	−0.460*	−0.462*
S. typhi	7˚C	−0.306*	−0.399**	−0.604**	−0.775**
	23˚C	−0.013*	−0.310*	−0.696**	−0.641**

* = significative correlation at $P < 0.05$; ddl: 15.

3.11. Correlation between Bacterial Abundances and Incubation Temperatures at Each Concentration of the Aqueous Extract of *Acacia sieberiana* Leaves

Spearman's "r" correlation coefficients between bacterial abundances and incubation temperatures at each concentration of the aqueous extract of *A. sieberiana* leaves were calculated and presented in Table 6. The result demonstrates that irrespective of the concentration of the extract for majority of cases, the variation of the incubation temperature would have practically no effect on the bacterial abundances of *S. faecalis* and *S. typhi*. The only exception was for 1 g/L concentration, where the increase in incubation temperature leads to a significant drop ($P < 0.05$) in the density of the bacterium *S. typhi* (Table 6).

Table 6. Correlation between bacterial abundances and incubation temperatures at each concentration of the aqueous extract of *Acacia sieberiana* leaves.

Extract concentrations	Bacteria species	
	S. faecalis	*S. typhi*
	7˚C	23˚C
0.5 g/L	−0.081	−0.287
1 g/L	−0.597	−0.502*
1.5 g/L	−0.190	−0.187
2 g/L	−0.136	−0.232

* = significative corrélation at $P < 0.05$; ddl: 15.

3.12. Comparison between Average Bacterial Densities and Different Concentrations of the Extract at Different Incubation Temperatures

The comparison between the average densities of the bacteria analyzed and the different concentrations of the extract at each incubation temperature was made using the Kruskal-Wallis H test, the values of the significance coefficients are presented in Table 7. It appears that these bacterial abundances differ significantly ($P < 0.01$) from one concentration to another, independently of the incubation temperatures.

Table 7. Comparison between the average bacterial densities and the different concentrations of the extract at different incubation temperatures.

	Bacteria species			
	S. faecalis		*S. typhi*	
Incubation temperatures	7˚C	23˚C	7˚C	23˚C
Concentrations	0.009**	0.005**	0.006**	0.009**

** = significative corrélation at $P < 0.01$; ddl: 4.

4. Discussion

4.1. Phytochemical Screening

The phytochemical analysis of the aqueous extract of *Acacia sieberiana* revealed the presence of several secondary metabolites that are likely responsible for its biological activity. Alkaloids, tannins, triterpenes, flavonoids, saponins, and steroids were identified in the leaf extract. These findings are consistent with previous reports on Fabaceae family plants, such as *Cassia sieberiana* [21]. However, other studies using methanolic extracts of *A. sieberiana* have not detected flavonoids, saponins, or steroids [22], while extracts from *A. senegal*, a species of the same genus, also lacked tannins, saponins, and flavonoids [23]. Such variations in metabolite composition may be attributed to factors including soil characteristics, climate, extraction solvents and methods, or the physiological maturity of the plant at the time of harvest. Environmental parameters such as temperature, sunlight, and storage conditions are also known to influence the biosynthesis of secondary metabolites [24] [25].

4.2. Activity of *A. sieberiana* Extract on Bacterial Cells

Very few studies have been carried out on *A. sieberiana* compared to other species of the same genus. It is with this in mind that the antibacterial activity of the aqueous extract of the leaves of this plant was evaluated on two bacterial species. A temporal variation of cell densities in the presence of the aqueous extract of *A. sieberiana* has been observed. This variation is dependent not only on the concentration of the plant extract, but also on the incubation time and the incubation temperature. The quantitative analysis of bacterial abundances in the control solutions, after each incubation period and that of the abundances of bacterial cells after exposure in the extract solutions, made it possible to show that the variation in cellular abundances would be linked to the action of the extract of *A. sieberiana* on the culturability of these cells. Indeed, the concentration of the extract would have a very considerable impact on the cultivability of bacterial cells. The higher it is, the greater the inhibition of bacterial densities. These strong inhibitions would probably be due to the secondary metabolites present in the aqueous extract of the plant. By combining the results of cell culturability and those of phytochemical screening, we can think that the antibacterial activities would be linked to the presence of alkaloids, tannins, triterpenes, flavonoids, saponins and steroids found

in the aqueous extract of the leaves of *A. sieberiana*. Polyphenolic compounds such as alkaloids, flavonoids, and tannins are recognized for their microbial toxicity, and both tannins and saponins have demonstrated antimicrobial activity [26]-[29]. A significant negative correlation ($P < 0.05$) was found between extract concentration and bacterial abundance, indicating that increasing extract levels correspond to decreased bacterial density. This could result from leakage of potassium ions and proteins from bacterial plasma membranes due to membrane disruption, as suggested in prior studies [30] [31].

Furthermore, prolonged exposure to certain phytochemicals may lead to toxic accumulation in bacterial cells, contributing to their death over time [32].

The extract's bactericidal effect can also be attributed to its active secondary metabolites. Phenolic compounds, flavonoids, triterpenes, alkaloids, tannins, and saponins are known to damage microbial cells by interacting with and denaturing proteins or enzymes, either directly at active sites or indirectly through steric hindrance [28] [33]. Another key property of these metabolites is their hydrophobicity, which facilitates integration into and destabilization of microbial membranes, thereby increasing permeability [34]. This results in leakage of intracellular contents, including ions and essential molecules, which can ultimately lead to cell death if critical thresholds are exceeded [35] [36]. These effects are magnified over longer contact times, which may explain the progressive decline in bacterial cell viability from 3 to 24 hours of incubation.

A significant negative correlation was again found between bacterial abundance and extract concentration at each incubation time. Regarding incubation temperature, our results indicated that it did not significantly affect bacterial inhibition. This observation aligns with previous work using aqueous and hydroethanolic extracts of *Albizia zygia*, where temperature had no major effect on the inhibition of *E. coli* and *S. typhi* [37]. The lack of temperature sensitivity may be due to the slower metabolic activity of mesophilic bacteria under such conditions, reducing their responsiveness to antimicrobial agents [38].

The survival of some bacterial cells even in the presence of the extract may be explained by resistance mechanisms. Bacteria can synthesize enzymes that degrade or inactivate antimicrobial compounds, including antibiotics [39].

Statistical analyses using the Kruskal-Wallis H test ($P < 0.01$) confirmed that extract concentration had a significant inhibitory effect on both bacterial species across all incubation temperatures.

The present findings demonstrate that *A. sieberiana* aqueous extract exhibits bactericidal properties in aquatic environments. Similar antibacterial effects have been reported for extracts from other plants, such as *Eucalyptus microcorys*, which also contain saponins and tannins [40].

Although the present findings highlight the antibacterial potential of *Acacia sieberiana* extracts for water treatment, it is important to consider possible toxicity and undesirable changes in taste, odor, or color that could limit household acceptability. Certain phytochemicals, such as saponins and tannins may impart bitter-

ness or astringency. Therefore, before recommending household-scale applications, future studies should assess the safety of residual compounds through toxicological analyses, evaluate sensory impacts, and establish standardized dosing protocols that balance antimicrobial efficacy with user acceptability.

5. Conclusion

The presence of the aqueous extract of *A. sieberiana* significantly reduced the cultivability of *S. faecalis* and *S. typhi* densities. The percentages of inhibition of these bacterial species increase relatively with the concentrations of the extract. In addition, these percentages of inhibition gradually increase with the increase in the bacteria-extract contact time. Furthermore, the temperature did not significantly influence the bacterial inhibition. The crude extract revealed a bactericidal effect on the cultivability of the two bacterial strains tested, with MICs of 7.81 - 15.62 mg/mL. The results of the present study support the use of *A. sieberiana* in the treatment of pathogenic infections as well as reduction of the flux of bacteriopollutants in water.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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