

Evaluation of the Efficacy of *Albizia zygia* Extracts on Bacterial Inhibition in Aquatic Microcosm

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Abstract

The objective of this study is the phytochemical analysis and the determination of the antibacterial activity of aqueous and hydro-ethanolic extracts obtained from the leaves and bark of the trunk of Albizia zygia, against Escherichia coli and Salmonella typhi bacteria in aquatic microcosms. Phytochemical screening was performed as described by Pareck. The results obtained show that the hydro-ethanolic and aqueous extracts of Albizia zygia trunk bark recorded higher extraction yields (26.71% and 33.2% respectively) compared to the aqueous and hydro-ethanolic extracts of leaves of the same plant. Secondary metabolites with antibacterial activities such as anthraquinones, anthocyanins, flavonoids, polyphenols, tannins and saponins were found in both types of extracts. Flavonoids and anthocyanins were relatively more abundant than the other chemical constituents. The highest cellular inhibition rate of Escherichia coli was 99.88%, obtained after 9 hours of exposure in the hydro-ethanolic extract solution of trunk bark at the concentration 1.5 g/L. The Salmonella typhi rate was 99.95% after 9 hours of exposure of bacterial cells to the hydro-ethanol extract of the bark of the trunk at the concentration 1.5 g/L. This rate increased proportionally with the bacterial-extract contact time. The temperature of the medium did not significantly influence bacterial inhibition (P > 0.05). The obtained results justify the use of the plant Albizia zygia in the reduction of the flow of bacterio-pollutants contained in water intended for consumption.

Keywords

Albizia zygia Extract, Phytochemical Screening, Bacterial Inhibition, Aquatic Microcosms

1. Introduction

Today, one third of humanity lives in a situation known as "water stress" with less than 1700 m³ of freshwater available per capita per year (WHO, 2009). Despite the small amount of freshwater directly accessible to humans, its quality is strongly influenced by pollution, which can be physical, chemical or biological (Vilaginès, 2003). Biological pollution is due to the presence of micro-organisms such as protozoa, fungi, viruses and bacteria in water (Festy et al., 2003). The presence of pathogenic bacteria in drinking water poses serious health problems due to the persistence of waterborne diseases. Among these waterborne diseases, we have typhoid fever, which is a serious foodborne illness caused by the Salmonella typhi bacterium, which is present in water contaminated by fecal matter. Each year, typhoid affects between 11 and 20 million people globally and results in 128,000 to 161,000 deaths (Molly et al., 2023). To reduce mortality from waterborne diseases, contaminated water must therefore be treated before consumption. For decades, simple disinfection methods have often been used (chemical disinfection with health effects, SODIS method, filtration and boiling). More recently, water disinfection methods using plant extracts have been proposed as a new alternative to water treatment at the household level. In Cameroon, as elsewhere, several plants used in traditional medicine against infectious diseases have been extensively researched. Studies have been carried out on the evaluation of the synergistic effect of different pH values and different infusion concentrations of Artemisia annua on Enterococcus faecalis growth in aquatic microcosms under dark and lighting conditions (Mobili et al., 2015). Studies conducted by Tamsa et al. (2018) identified the main factors involved in the inhibition of Enterococcus faecalis in the presence of an aqueous extract of Eucalyptus microcorys in an aquatic microcosms. In addition, Metsopkeng et al. (2019) evaluated in microcosm the survival of Aeromonas hydrophila and Enteropathogenic Escherichia coli (EPEC), in the presence of Moringa oleifera aqueous seeds extract at concentrations ranging from 1 to 40 g/L, and under incubation temperatures of 4°C and 23°C. Furthermore, Moungang et al. (2022) demonstrated the usefulness of Cussonia arborea for treating bacterial and infectious diseases.

Albizia zygia is used in the traditional treatment of diarrhea and venereal diseases (Ndjakou et al., 2007). However, although recent work has shown that plant extracts have antibacterial activities, there is still little information on the synergistic effect of temperature and incubation time of *Albizia zygia* extracts on *Escherichia coli* and *Salmonella typhi* bacteria. Few data are also available on which

parts of the plant have the best antibacterial efficacy against these two microorganisms. Plant extracts are still under-exploited in the treatment of water for human consumption.

2. Materials and Methods

2.1. Plant Material Collection

The plant material consists of fresh leaves and bark of the trunk of *Albizia zygia*, collected in the Central region (Cameroon). Authentication was carried out at the National Herbarium of Cameroon (HNC) by comparison with the existing samples in their database under the identification number: 2338/SRFK. After cleaning, the leaves and barks were dried for one month in the laboratory at room temperature and ground to powder using a grinding machine. Figure 1 is a photograph of fresh and dried leaves and bark from the trunk of *Albizia zygia* in the laboratory.



Figure 1. *Albizia zygia* (a) Fresh leaves; (a') Dry leaves; (b) Fresh trunk bark and (b') Dry trunk bark.

2.2. Bacterial Strains

The bacterial strains chosen for this study were Gram-negative bacteria; they were *Escherichia coli* and *Salmonella typhi*. They were chosen because of their role in determining the quality of drinking water (WHO, 2004). These two species were isolated from Yaoundé waterways.

2.3. Preparation of Extracts

Extractions were performed according to the method recommended by Romani et al., (2006).

2.3.1. Preparation of Aqueous Extracts

Aqueous extracts were prepared by maceration of 300 g of leaf crushed and 250 g of trunk bark crushed in 5000 mL and 4000 mL of distilled water respectively. After 48 hours, the extracts were filtered and then dried in an oven at a temperature of $45^{\circ}C \pm 2^{\circ}C$ until total evaporation of the water. The steps involved in this extraction process are shown in Figure 2.



Figure 2. Extraction protocol for aqueous extract.

2.3.2. Preparation of Hydro-Ethanol Extracts

To prepare the hydro-ethanolic extracts, 500 g of the leaf crush and 400 g of the trunk bark crush were macerated in 6500 mL and 4500 mL of the ethanol/distilled water mixture in the proportions 70/30, respectively. After 48 hours, the extracts were filtered and then oven dried at a temperature of $45^{\circ}C \pm 2^{\circ}C$ until total evaporation of the solvent. The hydro-ethanol extracts are obtained according to the steps shown in **Figure 3**.

2.3.3. Yield of Extraction (y)

The yield of the different extracts 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.

$$y(\%) = \frac{m_1}{m_2} \times 100$$

 m_1 is the weight of the dry extract in g. m_2 is the dry weight of plant material in g.



Figure 3. Extraction protocol for hydro-ethanol extracts.

2.3.4. Phytochemical Analysis of Extracts

The study of the chemical profile allows detecting the different chemical families constituting a 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 *Albizia zygia*, according to the method described by Pareck & Chanda (2007).

2.3.5. Preparation of the Different Extract Solutions

The extract concentrations were 0.5 g/L, 1 g/L and 1.5 g/L. The homogenized extracts were filtered successively on filter paper, sterile cotton, sterile Whatman paper, and finally cellulose nitrate membrane of 0.45 μ m porosity (Tamsa et al., 2018).

2.4. Preparation of Bacterial Suspensions and Inoculations

Fresh bacterial culture grew (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 (BaCl₂ and 1% H₂SO₄). The concentration of bacteria in the stock suspension was about 2×10^8 UFC/mL (Pasteur, 1987). Subsequently, 0.5 mL of the bacterial suspension was taken with a sterile

pipette and introduced into the test tubes containing 10 mL of *Albizia zygia* extract solution of known concentration.

The experiments were performed using the aqueous extract divided into 2 batches representing the two bacterial species. The first batch consisted of 48 sterile 25 mL test tubes each containing 10 mL of Albizia zygia extract solution at different concentrations (control tube 0 g/L, 0.5 g/L, 1 g/L and 1.5 g/L) and 0.5 mL of Escherichia coli suspension adjusted to a density of 0.5 Mac Farland (BaCl₂ and H₂SO₄ 1%). The second batch consisted of 48 tubes for Salmonella typhi. Each batch consisted of doublets of 24 tubes representing the part of the plant to be studied (24 tubes for the aqueous extracts of the leaves and 24 others for the aqueous extracts of the bark of the trunk). Each doublet was divided into two groups, the first of which was incubated in a refrigerated chamber at 7°C and the second at 23°C. The same process was applied for the hydro-ethanol extract. Temperatures of 7°C and 23°C were used to simulate the ambient water storage temperature in most households in the equatorial region and 4°C was used to simulate the storage temperature in refrigerators for those consuming fresh water. Figure 4 shows the solutions of Albizia zygia extracts in test tubes.



Figure 4. Test tubes containing different concentrations of Albizia zygia extracts.

2.5. Antimicrobial Analysis

The antimicrobial activity was carried out under aseptic condition focused on the quantitative aspect. They were carried out after 3, 6 and 9 hours of incubation, using the technique of spreading on the surface of the agar culture medium until exhaustion (Pasteur, 1987). After homogenization, 100 μ L of the sample to be analyzed was spread on petri dish containing solidified Endo agar and Hektoen agar for *E. coli* and *S. typhi* respectively. Petri dishes containing *E. coli* were incubated at 44°C while those containing *S. typhi* were incubated at 37°C for 24 hours. Viable and culturable organisms were counted by direct Petri dish counting using an OSI colony counter. Bacterial abundances were expressed in Colony Forming Units (CFU) and reported to 100 mL of inoculum (CFU/100 mL).

2.6. Data Analysis

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 *Albizia zygia* extracts on bacterial 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

Nn = Bacterial abundance after the action of the extract.

3. Results

3.1. Yields of Plant Extracts

The results of the yields of the aqueous and hydro-ethanolic extracts of the leaves and bark of the trunk of *Albizia zygia* are shown in **Figure 5**. According to the results obtained, the highest extraction yield was observed with the aqueous extract of the bark of the trunk of the plant *Albizia zygia* (33.2%).





3.2. Phytochemical Screening

Phytochemical analyses of the prepared extracts allowed us to characterize the presence of some chemical substances in the extracts (**Table 1**). Polyphenols, flavonoids, tannins, anthocyanins, and anthraquinones were present in the aqueous and hydro-ethanolic extracts of the leaves and bark of *Albizia zygia* trunk. On the other hand, alkaloids, sterols, and triterpenes were absent in the aqueous and hydro-ethanolic extracts of *Albizia zygia* leaves.

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

Table 1. Screening of chemical constituents of aqueous and hydro-ethanolic extracts of leaves and bark of *Albizia zygia* trunk.

+: presence; -: absent.

According to the intensity of color, the chemical constituents in the aqueous extract of *Albizia zygia* leaves were noted to be flavonoids, anthocyanins, tannins, anthraquinones, polyphenols and saponins. In the aqueous extract of the trunk bark, the chemical constituents identified were flavonoids, tannins, alkaloids and saponins, polyphenols, anthraquinones, anthocyanins, sterols and triterpenes.

On the other hand, the hydro-ethanol extract of *Albizia zygia* leaves, was found to content also flavonoids, anthocyanins and tannins, polyphenols, anthraquinones and saponins. The chemical constituents identified in the hydro-ethanolic extract of *Albizia zygia* trunk bark are flavonoids, alkaloids, polyphenols, tannins, anthocyanins, saponins, anthraquinones, sterols and triterpenes.

3.3. Antibacterial Activity of the Extracts

3.3.1. Percentage Inhibition of Bacterial Cells in the Presence of Aqueous Leaf Extract

At the temperature of 7°C, the percentages of inhibition of *Escherichia coli* cells fluctuated between 19.15% and 37.74%, between 50.81% and 93.39% and between 57.34% and 94.93% at the extract concentrations of 0.5 g/L, 1 g/L and 1.5 g/L respectively. For *Salmonella typhi* these percentages ranged from 20.06% - 36.04%, 29.65% - 65.76%, and 76.99% - 93.99% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations respectively. At 23°C, *Escherichia coli* cell inhibition percentages ranged from 23.13% to 57.88%, 40.01% to 78.53%, and 71.92% to 94.60% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. Those of *Salmonella typhi* fluctuated between 16.15% and 30.70%, 35.08% and 66.42%, and 60.85% and 95.34% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. *Salmonella typhi* appeared to have the highest inhibition rate (95.34%) after 6 h of exposure in 1.5 g/L aqueous extract of *Albizia zygia* leaves incubated at 23°C (**Figure 6**).



Figure 6. Percentage of inhibition of bacterial cells after 3, 6, and 9 hours of incubation in different concentrations of aqueous extract of plant leaves.

3.3.2. Percentage of Inhibition of Bacterial Cells in the Presence of Aqueous Extract of Trunk Bark

The percentage of metabolically non-culturable *Escherichia coli* cells exposed at 7°C ranged from 22.34% to 57.77%, 53.50% to 77.85% and 89.73% to 92.19% at extract concentrations of 0.5 g/L, 1 g/L and 1.5 g/L respectively. For *Salmonella typhi* these percentages fluctuated between 44.75% and 70.99%, between 72.17% and 82.38% and between 80.64% and 94.09% at 0.5 g/L, 1 g/L and 1.5 g/L extract concentrations respectively. At the incubation temperature of 23°C, the percentages of inhibition of *Escherichia coli* cells ranged from 12.68% to 51.02%, from 45.31% to 88.14%, and from 75.38% to 92.12% at extract concentrations of 0.5 g/L, 1 g/L, and 1.5 g/L, respectively. For *Salmonella typhi* these percentages ranged from 32.11% - 91.68%, 55.54% - 68.97%, and 84.42% - 91.96% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations respectively. The highest cell inhibition rate (94.09%) was recorded with *Salmonella typhi* after 9 h of incubation in 1.5 g/L aqueous extract of *Albizia zygia* trunk bark incubated at 7°C (**Figure 7**).



Figure 7. Percentage of inhibition of bacterial cells after 3, 6, and 9 hours of incubation in different concentrations of the aqueous extract of the plant trunk barks.

3.3.3. Percentage Inhibition of Bacterial Cells in the Presence of Hydro-Ethanol Extract of Leaves

At temperature 7°C, the percentage of non-culturable *Escherichia coli* cells ranged from 46.72% to 95.11%, 63.36% to 94.14%, and 77.33% to 97.58% at extract concentrations of 0.5 g/L, 1 g/L, and 1.5 g/L respectively. For *Salmonella typhi* these inhibition percentages ranged from 82.96% to 98.67%, 94.30% to 98.23%, and 98.14% to 99.72% at extract concentrations of 0.5 g/L, 1 g/L, and 1.5 g/L respectively. At 23°C, the percent inhibition of *Escherichia coli* cells fluctuated between 25.23% and 99.07%, 27.89% and 99.43%, and 87.79% and 99.88% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. For *Salmonella typhi* these percentages ranged from 46.13% to 99.08%, 50.80% to 99.69%, and 69.02% to 99.85% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. *Escherichia coli* is the species that appears to record the highest inhibition rate (99.88%) after 9 h of exposure in the highest concentration (1.5 g/L) of hydro-ethanol extract of *Albizia zygia* leaves (**Figure 8**).



Figure 8. Percentage of inhibition of bacterial cells after 3, 6, and 9 hours of incubation in the different concentrations of the hydro-ethanolic extract of the plant leaves.

3.3.4. Percentage of Inhibition of Bacterial Cells in the Presence of Hydro-Ethanol Extract of Trunk Bark

The percentage of inhibition of metabolically non-culturable *Escherichia coli* cells exposed at 7°C fluctuated between 90.60% and 98.91%, between 91.74% and 99.01% and between 94.15% and 98.91% at the extract concentrations of 0.5 g/L, 1 g/L and 1.5 g/L respectively. For *Salmonella typhi* the inhibition percentages fluctuated between 71.87% and 99.02%, between 98.67% and 99.55%, and between 98.91% at extract concentrations of 0.5 g/L, 1 g/L, and 1.5 g/L respectively. At 23°C, the percentages of *Escherichia coli* cells ranged from 92.44% - 98.94%, 90.64% - 98.66%, and 97.32% - 99.66% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. For *Salmonella typhi*, these percentages ranged from 86.37% to 99.73%, 99.41% to 99.87%, and 99.59% to 99.95% at 0.5 g/L, 1 g/L, and 1.5 g/L extract concentrations, respectively. The highest cell inhibition rate (99.95%) was recorded with *Salmonella typhi* after 9 h of exposure in the highest concentration (1.5 g/L) of hydro-ethanol extract of trunk bark incubated at 23°C (**Figure 9**).



Figure 9. Percentage of inhibition of bacterial cells after 3, 6, and 9 hours of incubation in the different concentrations of the hydro-ethanolic extract of the bark of the plant trunk.

3.3.5. Comparison between the Average Abundances of *Escherichia coli* and *Salmonella typhi* Exposed in Each Concentration of Extract

The mean abundances of *Escherichia coli* and *Salmonella typhi* exposed to the aqueous extracts were compared with the mean abundances of *Escherichia coli* and *Salmonella typhi* exposed to the hydro-ethanolic extracts of *Albizia zygia* using the Kruskal-Wallis H test (**Table 2**). These bacterial abundances were found to differ significantly (P < 0.05) from one extract to another.

Table 2. Comparison between the average abundances of *E. coli* and *S. typhi* in each extract.

Bacterial species	Aqueous extracts	Hydro-ethanolic extracts
E. coli	0.027*	0.018*
S. typhi	0.022*	0.011*

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

3.3.6. Comparison of the Mean Abundances of *E. coli* and *S. typhi* Exposed in the Aqueous and/or Hydro-Ethanolic Extracts of Each Plant Part

The mean abundances of *E. coli* and *S. typhi* exposed in the aqueous and/or hydro-ethanolic extracts of the leaves and/or bark of the trunk of *Albizia zygia* were compared using the Kruskal-Wallis H test (**Table 3**). It was found that these bacterial abundances differed significantly (P < 0.05) from one part of the plant to another for each type of extract.

Table 3. Comparison between the average abundances of *E. coli* and *S. typhi* exposed in the aqueous and/or hydro-ethanolic extracts of each part of the plant.

Types of extract	Aqueous extracts		Hydroethanolic extracts	
Bacterial species	Leaves	Trunk bark	Leaves	Trunk bark
E. coli	0.035*	0.019*	0.021*	0.015*
S. typhi	0.026*	0.018*	0.013*	0.009*

* = significant difference for P < 0.05.

4. Discussion

The result of the extraction yields (**Figure 5**), shows that the aqueous extract of the trunk barks presents the highest extraction yield (33.2%). However, the statistical analysis does not show a significant difference (P > 0.05) with the hydroethanol extract of the plant leaves which would have the lowest yield (15.4%). These results are different from Oluyemi et al. (2014) obtained with the aqueous, ethanolic and methanolic extracts of the same plant. This difference could be explained by the fact that the extraction yield is related to the extraction methods applied, the genetic properties of the species used (Bruneton, 1999), the harvesting conditions of the plant material and the geographical origin (Narayana et al., 2001).

The phytochemical screening of the plant revealed the presence of the main families of chemical compounds likely to confer antimicrobial properties. These include flavonoids, anthocyanins, tannins, polyphenols, anthraquinones and saponins present in aqueous and hydro-ethanolic extracts of *Albizia zygia*. These results are similar to those of Regina et al., (2016) who had reported the presence of these compounds in the aqueous and hydro-ethanolic extracts of the same plant. However, the results of Regina et al., (2016) did not show the presence of anthocyanins and polyphenols in the aqueous and hydro-ethanolic extract of *Albizia zygia*. Nevertheless, these results are different from those observed by Oluyemi et al., (2014) who noted the presence of flavonoids, tannins, anthraquinones, cardiac glycosides, alkaloids and saponins in the ethanolic, methanolic and aqueous extract of *Albizia zygia* leaves.

In the aqueous and hydro-ethanolic extract of trunk bark, secondary metabolites such as flavonoids, alkaloids, polyphenols, tannins, anthocyanins, saponins, anthraquinones, sterols and triterpenes were obtained. These results corroborate those of Wilfred et al., (2017) and Ganiyat & Adeniyi (2013) who already reported the presence of all these secondary metabolites except anthocyanins in the methanolic and hexanolic extract of *Albizia zygia* stem barks. Indeed, alkaloids, sterols and triterpenes are present only in the stem bark. Polyphenols, flavonoids, tannins, anthraquinones and saponins were found in both parts of the plant selected for the study. This variation in secondary metabolites observed could be related to the degree of maturation of the plant at harvest. In this regard, Falleh et al., (2008) and Podsedek (2007) noted that high temperature, solar exposure, maturity at harvest and storage conditions affect the biosynthesis of secondary metabolites.

Both the aqueous and hydro-ethanolic extracts exhibited antimicrobial activity against the studied bacterial strains. According to the results obtained from the antibacterial activity of the extracts, the percentages of inhibition of *Escherichia coli* varied between 12.68% and 99.88%, while in *Salmonella typhi* they varied between 16.15% and 99.95%. The extracts showed a good antibacterial activity which would be due to the various chemical compounds present in the plant. Indeed, phenolic compounds, flavonoids, triterpenes, alkaloids, tannins, and saponosides present in the considered extracts are recognized for their toxicity towards some microorganisms (Loguercio et al., 2005; Raven et al., 2007). The mechanism may be related to the inhibition of hydrolytic enzymes (proteases, carbohydrase...), which causes the inactivation of microbial adhesins, transport proteins and cell envelope Cheurfa et al., (2017). It is in this sense that Cheurfa et al., (2017) reported that different classes of polyphenols, mainly tannins and flavonoids, can increase the toxicity of extracts towards microorganisms. The inactivation of microorganisms is modulated by the bacterial-extract contact time, with long contact times acting on unreached targets in contrast to relatively short contact times. This analysis could explain the origin of the abundant cell death recorded after 6 and 9 hours of contact of the bacteria with the extracts. It appears from this study that the hydro-ethanol extract of the trunk bark was the most active with an inhibition rate of 99.95% when compared to the two bacterial species considered. The activity of the aqueous extract would be less effective on the species considered than that of the hydro-ethanolic extract. This difference in activity would probably be due to the phytochemical composition which differs between the two types of extracts. It is possible that the solvents used during the extraction process are at the origin of this difference in activity of the extracts of the Albizia zygia plant. Undoubtedly, the solvents used may not have been able to retain the same molecules in equal proportions in the two types of extracts (aqueous and hydro-ethanolic) because of their polarities. The inhibition rate increased with the concentration of the extract, as a negative and significant correlation (P < 0.05) was observed between cell densities and the concentration of the Albizia zygia extracts. These results are similar to those of Tassou et al., (2000) and Tsuchiya & Iinuma (2000) who showed that the higher the concentration of extract, the greater the amount of potassium ions and proteins that leak out of the cell and that this leakage could cause structural and functional damage to the plasma membrane.

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. *Escherichia coli* and *Salmonella typhi* in the aqueous and hydro-ethanol extract solutions of the leaves and trunk bark would appear to be insensitive to variations in incubation temperatures.

5. Conclusion

The present study aimed to evaluate in aquatic microcosm, the activity of *Albizia zygia* extracts on the cultivability of *Escherichia coli* and *Salmonella typhi* bacteria. Secondary metabolites with antibacterial activity such as anthraquinones, anthocyanins, flavonoids, polyphenols, tannins and saponins were highlighted in both types of extracts. These secondary metabolites were qualitatively more abundant in the hydro-ethanol extract. The results show that the presence of *Albizia zygia* extracts significantly reduces the cultivability or growth of the bacteria considered in water. Overall, the hydro-ethanolic extract was found to be more

effective than the aqueous extract. In addition, the most convincing results were obtained with the hydro-ethanolic extracts of the trunk bark. *Salmonella typhi* was the most sensitive bacterium to the extracts of *Albizia zygia*.

The plant of *Albizia zygia* contains chemical compounds that may confer antimicrobial properties. These compounds could be used in the formulation of new products for the treatment of water for human consumption. Further study will be the isolation and structural characterization of the bioactive components of *Albizia zygia*.

Conflicts of Interest

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

References

- Bruneton, J. (1999). *Pharmacognosy, Phytochemistry, Medicinal Plants* (3rd ed., 1120 p.). Paris, Technique et Documentation.
- Cheurfa, M., Allem, R., Zabel, K., Aichouni, W., & Medjkane, M. (2017). Study of the Effects of Extracts from the Roots of *Glycyrrhiza glabra* L. and *Zizyphus lotus* L. on Some Human Pathogenic Bacteria. *Phytothérapie*, *17*, 15-21.
- Falleh, H., Ksouri, R., Chaieb, K., Karray, B. N., Trabelsi, N., Boulaaba, M., & Abdelly, C. (2008). Phenolic Composition of *Cynara cardunculus* L. Organs, and Their Biological Activities. *Biology Reports*, 331, 372-379. <u>https://doi.org/10.1016/j.crvi.2008.02.008</u>
- Festy, B., Hartemann, P., Ledrans, M., Levallois, P., Payment, P., & Tricard, D. (2003). Water Quality. *Environment and Public Health—Foundations and Practices*, 333-368.
- Ganiyat, K., & Adeniyi, O. (2013). Phytochemical Screening, Antioxidant, V Antimicrobial and Toxicity Activities of Polar and Non-Polar Extracts of *Albizia zygia* (DC). *Stem-Bark Annual Review & Research in Biology, 3,* 1020-1031.
- Garcia-Ripoll, A., Amat, A., Arques, A., Vicente, R., Ballesteros, M. M., Sanchez, P. J. et al. (2009). Confirming *Pseudomonas putida* as a Reliable Bioassay for Demonstrating Biocompatibility Enhancement by Solar Photo-Oxidative Processes of a Biorecalcitrant Effluent. *Journal of Hazardous Materials, 162,* 1223-1227. https://doi.org/10.1016/j.jhazmat.2008.06.010
- Loguercio, A., Battistin, A., Agueda, C. V. A., Réia, H. A., & Niura, M. (2005). Antibacterial Activity of Hydro-Alcoholic Extract of Jambolão (*Syzygium cumini* (L.) Skells) Leaves. *Rural Science*, *35*, 371-376. <u>https://doi.org/10.1590/S0103-84782005000200019</u>
- Metsopkeng, S. C., Lontsi, D. C., Noah, E. O. V., Moungang, L. M., Nana, P. A., Tamsa, A. A. et al. (2019). *Moringa oleifera* Seeds Extract Activity on Enteropathogenic *Escherichia coli* and *Aeromonas hydrophyla* Cells in Aquatic Microcosm. *Journal of Applied Biotechnology*, 2, 13-30. <u>https://doi.org/10.5296/jab.v7i2.14917</u>
- Mobili, O., Lontsi, D. C., Tamsa, A. A., Nougang, M., Allahdin, O., Noah, E. O. V. et al. (2015). Synergistic Effect of Light, pH and *Artemisia annua* Extract on *Enterococcus faecalis* in Aquatic Microcosms. *International Journal of Applied Microbiology and Biotechnology Research*, 3, 62-72.
- Molly, H., Jenny, W., Anna, A. M., Carol, T., Kira, A. C., Yoann, N., Kristen, H., Matthew, M., Amanda, T., Adwoa, D. B., & Lucy, B. (2023). Typhoid Fever Surveillance, Incidence Estimates, and Progress toward Typhoid Conjugate Vaccine Introduction-Worldwide, 2018-2022. *Morbidity and Mortality Weekly Record*, 72, 171-176.

https://doi.org/10.15585/mmwr.mm7207a2

- Moungang, L. M., Sidjui, S.L., Bayaga, H. N., Mfouapon, J. N., Nguélé, O., Gonsu, H. K., & Ngameni, B. (2022). Phytochemical Screening and *in Vitro* Evaluation of the Antibacterial Activity of Organic Extracts from the Root Bark of *Cussonia arborea* (Araliaceae). *Journal of Applied Biotechnology*, *9*, 1-7. <u>https://doi.org/10.5296/jab.v9i2.19102</u>
- Narayana, K. R., Reddy, M. S., & Chaluvadi, M. R. (2001). Bioflavonoids Classification, Pharmacological, Biochemical Effects and Therapeutic Potential. *Indian Journal of Pharmacology, 33*, 2-16.
- Ndjakou, L. B., Vonthron, S. C., Fongang, S. R., Tantangmo, F., Ngouela, S., Kaiser, M. et al. (2007). *In Vitro* Antiprotozoal Activities and Cytotoxicity of Some Selected Cameroonian Medicinal Plants. *Journal of Ethnopharmacology*, *111*, 8-12. <u>https://doi.org/10.1016/j.jep.2006.10.036</u>
- Oluyemi, O., Olufunke, O. A., & Oluwole, M. D. (2014). Evaluation of Antimicrobial Activities of *Albizia zygia* DC Leaf Extracts against Some Clinically Important Pathogens. *Journal of Natural Sciences Research*, *4*, 98-105.
- Parekh, J., & Chanda, S. (2007). *In Vitro* Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plant. *Turkish Journal of Biology*, *31*, 53-58.
- Pasteur, D. (1987). Laboratory Media and Reagents. Microbiology and Immunology (3rd ed.).
- Podsedek, A. (2007). Natural Antioxidants and Antioxidant Capacity of Brassica Vegetables: A Review. LWT, 40, 1-11. <u>https://doi.org/10.1016/j.lwt.2005.07.023</u>
- Raven, P., Evert, R., & Eichhorn, S. (2007). *Biologia Vegetal* (7th ed., 728 p.). Editora Guanabara Koogan S.A.
- Regina, A. O., Asante, K. I., Safo, D. O., Tuffour, I., Oforiattah, E., Takuhiro, U., & Nyarko, K. A. (2016). Cytotoxic Effects of *Albizia zygia* (DC) J. F. Macbr, a Ghanaian Medicinal Plant, against Human T-Lymphoblast-Like Leukemia, Prostate and Breast Cancer Cell Lines. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*, 392-396.
- Romani, A., Pinelli, P., Cantini, C., Cimato, A., & Heim, L. D. (2006). Characterization of Violetto di Toscana, a Typical Italian Variety of Artichoke (*Cynara scolymus* L.). *Journal* of Food Chemistry, 95, 221-225. <u>https://doi.org/10.1016/j.foodchem.2005.01.013</u>
- Tamsa, A. A., Lontsi, D. C., Noah, E. O. V., Moussa, D., Yaouba, A., Tchikoua, R., & Nola, M. (2018). Use of the Aqueous Extract of *Eucalyptus microcorys* for the Treatment in Microcosm, of Water Containing *Enterococcus faecalis*. Hierarchisation of Cells' Inhibition Factors. *H2Open Journal*, *1*, 47-56. <u>https://doi.org/10.2166/h2oj.2018.002</u>
- Tassou, C. C., Koutsoumanis, K., & Nychas, G. J. E. (2000). Inhibition of Salmonella enteritidis and Staphylococcus aureus in Nutrient Broth by Mint Essential Oil. Food Research International, 33, 273-280. <u>https://doi.org/10.1016/S0963-9969(00)00047-8</u>
- Tsuchiya, H., & Iinuma, M. (2000). Reduction of Membrane Fluidity by Antibacterial Sophoraflavanone G Isolated from *Sophora exigua*. *Phytomedicine*, *7*, 161-165. <u>https://doi.org/10.1016/S0944-7113(00)80089-6</u>
- Vilaginès, R. (2003). *Water, Environment and Public Health* (236 p.). Edition Lavoisier, Paris.

WHO (2004). Guidelines for Drinking Water Quality (1050 p.). Recommandation, Geneva.

WHO (2009). World Health Statistics (149 p.).

Wilfred, O. O., Philip, F. U., Emmanuel, O. E., & Sampson, C. N. (2017). Comparative Phytochemical, Antioxidant and Antimicrobial Properties of *Ficus capensis, Aristolochia ringens, Albizia zygia* and *Lannea welwitschii. Dhaka University Journal of Pharmaceutical Sciences, 16*, 147-157. <u>https://doi.org/10.3329/dujps.v16i2.35249</u>