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Research Article

Effect of Amoxicillin/Aqueous Extract of *Cussonia* arborea Hochst. (Araliaceae) on the Survival of *Escherichia coli* and *Staphylococcus aureus* Bacteria Isolated from Groundwater in Yaoundé (Central Region of Cameroon)

Luciane Marlyse Moungang[1]*, Christophe Colombe Simo Fotso[2,3], Antoine Tamsa Arfao[1], Sophia Loeticia Ngoumnaï[4], Olive Vivien Noah Ewoti[1], Yves Poutoum Yogne[1], Golda Reine Zame Meva'a[1], Lazare Sidjui Sidjui[3,5], Stéphanie La Blanche Guetchueng Tamdem[5] and Moïse Nola[1]

[1]*Faculty of Sciences, Department of Animal Biology and Physiology, Laboratory of Hydrobiology and Environment, University of Yaoundé I, Cameroon*

[2]*Faculty of Sciences, Department of Chemistry, University of Buea, Cameroon*

[3]*Faculty of Sciences, Department of Organic Chemistry, Laboratory 278, University of Yaoundé I, Cameroon*

[4]*Faculty of Medicine and Biomedical Science, Department of Pharmacognosy and Pharmaceutical Chemistry, University of Yaoundé I, Cameroon*

[5]*Institute of Medical Research Plant Studies,Yaoundé, Cameroon*

***Corresponding Author:** Luciane Marlyse Moungang, Faculty of Sciences, Department of Animal Biology and Physiology, Laboratory of Hydrobiology and Environment, University of Yaounde I, Cameroon. **E-mail:** lumou2000@yahoo.fr / Phone: +237 674 165 604 / 693 474 779

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Abstract

Herbal medicine is being increasingly used against infections in many parts of the world. The plants belonging to the genus *Cussonia* are among the most exploited and several of these species have been described as having antibacterial and antifungal activities. The antibiotics which have been for several decades for the treatment of bacterial diseases are losing their effectiveness more and more leading to the emergence multiresistant strains of microorganisms. These create the need to search for new alternatives especially in medicinal plants. The aim of this study is to evaluate the effect of combination of amoxillin and aqueous extract of *Cussonia arborea* on some bacteria isolated from ground water.

Methodology: The whole plant of *Cussonia arborea* was collected from Kon-Yambata centre region, Cameroon and was authenticated at the National Herbaruim, Yaounde with Voucher specimen number 32094/HN and kept. The leaves were dried in Shade for three weeks and shredded and ground into fine powder using an electric blender. The powder obtained was macerated for 48 h in distilled water. Phytochemical screening was performed on the extract obtained. Concentrations of 0.1, 0.5, and 2mg/mL and amoxicillin (0.5mg) were used to determine the various antibacterial activities. The methods used are spreading on the surface of agar media and microdilution technic. The medium were utilized on two bacteria strain isolated from well water sources in the city of Yaounde (Central region of Cameroon): *Escherichia coli* and *Staphylococcus aureus.*

Result: The percentage yield after extracting the leaves of *Cussonia arborea* with water was 11.42%. Phytochemical screening revealed the presence of the following classes of secondary metabolites alkaloids, polyphenols, anthraquinones, flavonoids, triterpenes and cardiotonic heterosides. The different extracts showed variable activities (4.63 to 100% inhibition) on strains in the planktonic

state. The aqueous extract showed antibacterial activity with MICs ranging from 12.5 to 25 mg/mL. The best activity was on *Staphylococcus aureus* with an MIC of 12.5 mg/mL. The extract showed bactericidal power on all of the strains tested. The different amoxicillin/extract combinations were found to be synergistic (fractional bacterial abundances between 0 and 0.5) on *Staphylococcus aureus*. On *E. coli*, a synergy of action (AF ≤ 0.5) was observed after 12 h and indifferences (0.5 <AF ≤ 4) were observed at the initial titer and after a contact time of 6h.

Conclusion: This suggests that the traditional use of *Cussionia arborea* could be alternatives for the treatment of infectious diseases and also contribute to the reduction of the flow of bacteriopollutants in water. It's important to take into account the role of its association with amoxicilline in the spread of resistance of microorganisms to antibacterial agents.

Keywords: *Cussonia arborea;* Amoxicillin/Aqueous Extract Association; Antibacterial Activity; Water

Introduction

Microorganisms are widely distributed in the environment and the majority of them are found in terrestrial or aquatic environments [1]. Indeed, water, in addition to its importance in the daily life of man for his food and non-food needs (personal hygiene and various household tasks), also constitutes a means of dissemination of pathogens causing diseases in humans. Access to drinking water is an obstacle to improving the health of populations, despite the water supply and sanitation programs put in place, since one in three people in the world (i.e. 2.4 billion people) still live without adequate sanitation and sub-Saharan Africa the most affected [2].

This insufficiency leads the populations to turn to other alternative sources of water supply of questionable microbiological quality such as water from lentic environments (wells, springs, boreholes) and rivers. Thus, studies carried out by [3] in a few wells, boreholes and rivers in the city of Douala-Cameroon and by [4] on a few sources and boreholes in the Mvog-Betsi district in Yaoundé - Cameroon, revealed a link between the contamination of the various water supply points and the water-borne diseases recorded in the hospitals of the said zones, in particular gastroenteritis. The situation is increasingly alarming due to the emergence of strains of microorganisms that are resistant to most of the available antibiotics as a result of their excessive and uncontrolled use [5].

In order to remedy this situation, in recent years, various studies have focused on the search for new therapies from various natural resources, in particular medicinal plants [6]. The richness of plants in secondary metabolites and their accessibility justify their use. Traditional medicine is used by nearly 80% of the African population and sometimes at the same time resort to conventional drugs [7]. Studies carried out on the associations between plant extracts and reference antibiotics in bacterial strains exhibiting beta-lactamases have found that the activity of the antibiotics had been restored and/or potentiated in the presence of plant extracts in certain strains [8,9]. The present work aims to evaluate the effect of the association of aqueous extracts of *Cussonia arborea* with amoxicillin on the survival of *Escherichia coli* and *Staphylococcus aureus* isolated from groundwater.

Material and Methods

Plant material

The choice of the plant was guided on the basis that there exist Cameroonian species but has been very little scientific data. The leaves whole plant was collected in Kon-Yambeta - Cameroon, in the Central Cameroon region. The plant was subsequently taken to the National Herbarium of Cameroon where it was authenticated and Voucher specimen kept with the reference number 32094/HNC. The leaves of *C. arborea* were cleaned (sorting, dusting), cut up, and then dried in the shade at room temperature (18 - 25 ° C) for three weeks. The dry leaves were then pulverized using a mechanical grinder until a more or less fine powder was obtained and stored in air tight containers.

Operating mode

About 1700 g of powder was obtained and introduced into a 6 L flask. 5 L of distilled water was added into the flask while stirring so as to ensure the complete soaking of the powder. After 48 h of maceration, the filtrate was obtained by filtration through muslin cloth in order to remove solid particles. The operation was repeated two (02) times with renewal of solvent every 48 h. The different filtered fractions were combined and dried in an oven at 45 °C. The extraction yield was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Mass of crude extract}}{\text{Initial powder mass}} \times 100$$

Qualitative phytochemical screening

Phytochemical screening was carried out on the extract in order to determine the chemical substances (secondary metabolites) present in the plant. Secondary metabolites are divided into three main families, namely: alkaloids, phenolic and terpene compounds. The phytochemical tests were carried out according to the methods described by [10-12].

Bacterial isolation and storage

Bacteriological analyzes were carried by both qualitative and quantitative methods. The bacterial species used were *E. coli* and *S. aureus*. These bacterial species were chosen because of their importance in hygiene and public health in indicating the microbiological quality of water intended for consumption [13]. They were isolated from well and spring water in the equatorial region of Cameroon (Central Africa). *E. coli* and *S. aureus* were isolated on specific culture media, namely Endo agar (Bio-Rad) and Chapman mannite agar (Bio-Rad) respectively by the membrane filtration technique.

Their identification was made according to standard method [14]. For the preparation of bacterial stocks, a colony forming unit (CFU) of each strain from standard agar medium was inoculated into 100 mL of nutriment broth (Oxford) for 24h at 37ºC. After cells were harvested by centrifugation at 8000 rev/min for 10 min at 10ºC and washed twice with NaCl (8.5 g/L) solution. Each pellet was re-suspended in 50 mL of NaCl solution. After homogenization, 1 mL of this was then transferred into 500 mL of sterile NaCl solution (0.85%) contained in the Erlenmeyer flask and the stocked.

Experimental protocol

Determination of the antibacterial activity of the extract and the antibiotic (amoxicillin)

The antibacterial activity was evaluated by two methods: the method of spreading until exhaustion on the surface of the agar culture medium and by the method of microdilution. The different culture media used were Mueller Hinton Broth Agar (MHB), Regular Agar (PCA), Endo Agar and Mannitol Salt Agar.

Preparation of bacterial inoculum

100 µL of the bacterial storage suspension were taken and subcultured on ordinary agar (PCA) in a petri dish. A colony was isolated after 24 h of incubation at 37 °C. The colony was removed using a sterile Platinum loop and subcultured on an ordinary agar sloped into tubes. After 18 h of incubation at 37 °C, the bacterial suspensions were prepared by taking from this culture. The colonies which were introduced into sterile physiological water until a turbidity corresponding to point 0.5 of the Mc Farland scale corresponding to the concentration of $1.5x10^8$ CFU/mL which constituted the stock suspension [15]. The stock suspension was then diluted to an appropriate strength for the evaluation of antibacterial activities.

Preparation of extract and antibiotic solutions

The extract was prepared in volumes of 500 mL with concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.1 mg/mL. Amoxicillin solution was prepared at a concentration of 0.5 mg/mL. Solutions required for synergy were obtained from combining the amoxicillin solution with the extract solution.

Determination of the antibacterial activity of *C. arborea* extract

Effect of *C. arborea* extracts on the survival of microorganisms according to the different contact times

For each bacterial species used, 5 flasks of 350 mL designated A, B, C, D and E were used. Flask A containing 50 mL of physiological water (NaCl: 8.5 g/L) was used as a control. The remaining vials B, C, D and E each contained 50 mL of the extract at concentrations of 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL and 2 mg/mL, respectively.

Subsequently, 0.5 mL of bacterial suspension was introduced, depending on the bacteria tested, into the vials. This constituted the initial instant T_0. Thus, after timing periods of 0, 6, and 12 h chosen; 100 µL of sample were taken from each vial and inoculated on specific agar culture media.

Determination of the minimum inhibitory concentration (MIC) of the extract

The MIC of the extract was determined by the liquid microdilution method using the M07-A9 protocol described by [15] with some modifications. This is the smallest concentration that will inhibit any visible growth of a microorganism after incubation at 37°C for 18 to 24 h [15].

In each well (96 wells) of microplates, a volume of 100 µL of Müeller Hinton broth was introduced. Then in the first wells were

introduced a volume of 100 µL of the stock solution at a concentration of 400 mg/mL of the aqueous extract. Successive serial dilutions allowed us to obtain a range of concentrations from 1.56 mg/mL to 100 mg/mL. A volume of 100 µL of inoculums (2×10^6 CFU/mL) was then introduced into each well, up to a final volume of 200 µL per well. Plates were covered and sealed with parafilm paper and incubated at 37°C for 18-24 h. Columns 4 and 8 served as negative control for Müeller Hinton broth. Line F served as a positive control for bacterial strains and contains Müeller Hinton broth and the bacterial inoculums. The positive control consisted of a reference antibiotic (Ampicillin) whose concentration range varied between 4 µg/mL and 256 µg/mL. After incubation, the bacterial growth was estimated using iodonitrotetrazolium chloride (INT), the principle of which is based on the capture of protons emitted by dehydrogenases (enzymes present on the membrane of bacteria) of bacteria alive after having metabolized glucose. The enzymes are reduced and reddens the medium after about 30 minutes of re-incubation [9]. For this 40 µL of the INT solution was introduced into each of the wells of the microplate. MICs were defined as the smallest concentrations for which we did not observe a pink coloration. The tests were repeated in triplicates.

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

In order to determine the MBCs, a volume of 150 µL of culture broth was introduced into new plates, and then the volume was made up to 200 µL by adding a volume of 50 µL of the contents of the wells of greater or equal concentration at the MIC. These plates were then incubated for 24 h at 37 ° C followed by visualization at INT. All the concentrations at which we did not observe a pink coloration were bactericidal and the smallest of these was noted as MBC. The tests were carried out in triplicates.

After determining the MICs and MBCs, the MBC/MIC ratios were calculated.

Effect of the amoxicillin/*C. arborea* extract combination on the survival of microorganisms according to the different contact times

For each bacterial species used, 5 vials of 350 mL (A', B', C ', D' and E') were used for this study. Vial A' containing 100 mL of the 0.5 mg/mL amoxicillin solution was used as a control. The remaining vials B', C', D' and E' each contained 50 mL of the extract at concentrations of 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL and 2 mg/mL respectively. Then 50 mL of the antibiotic solution at a fixed concentration of 0.5 mg/mL was added to the vials of series B', C', D 'and E' for a final volume of 100 mL. Subsequently, 1 mL of bacterial suspension was introduced into the vials. This moment represented the initial time of contact T_0. After contact periods of 0, 6, and 12 h; 100 µL of sample were taken from each vial and inoculated on specific agar culture media.

Data analysis

The temporal variations of the abundances of the bacterial cells expressed in CFU/100 mL were represented by histograms. The percentages of inhibition (PI) after the action of the extracts, the antibiotic or the antibiotic/extract combination on different strains were calculated according to the following formula described by Garcia-Ripoll [16]:

No = Abundance of bacterial cells in physiological water (control)

Nn = Abundance of bacterial cells after the action of the extract, the antibiotic or the combination of extract and antibiotic.

The degrees of connection between the bacterial abundances and the periods of contact for each concentration of extract on the one hand, and between the bacterial abundances and the concentrations of extract for each period of contact, were evaluated by the correlation tests "r" from Spearman. Comparisons of data means were performed using the Kruskal-Wallis H test.

$$FA=\frac{\text{Cell abundance obtained after the action of the antibiotic / extract combination}}{\text{Cell abundance obtained after the action of the antibiotic}}$$

All of these analyzes were performed using SPSS software version 16.0.

To assess the effect of antibiotic/extract combinations, fractional bacterial abundances (FA) were determined according to the modified formula described by [17].

Synergy is established when AF ≤ 0.5; indifference when AF is between 0.5 and 4; finally, the antagonism for any AF ≥ 4 [17].

Results and Discussion

Results

Extraction yield and phytochemical screening of the extract

After extracting the plant with water, we obtained an extraction yield of 11.42%. The phytochemical screening of the aqueous extract of the leaves of *C. arborea* shows the presence of alkaloids, polyphenols, anthraquinones, flavonoids, triterpenes and cardiotonic heterosides. Anthocyanins, Tannins, saponins and sugars are absent.

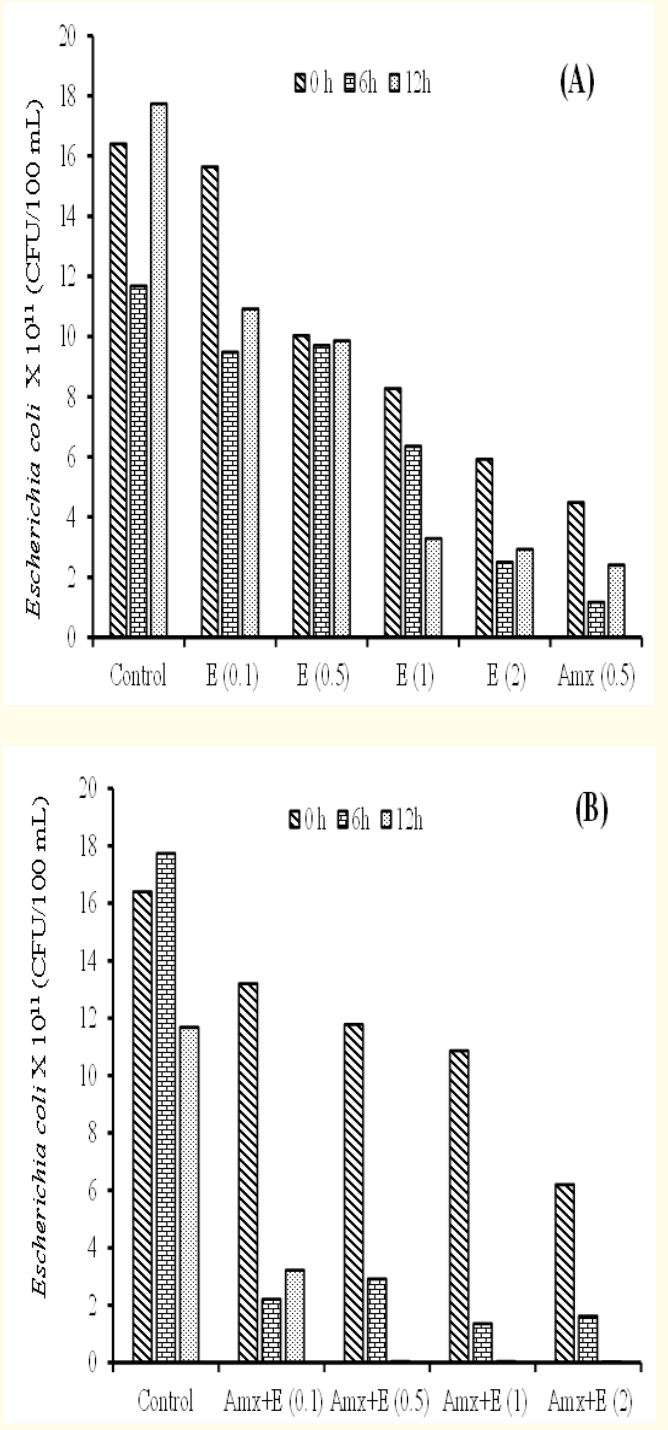
Temporal variation of the abundances of *E. coli* and *S. aureus* in the presence of the aqueous extract of *C. arborea* leaves, of the antibiotic and in the presence of the combination of aqueous extract/antibiotic

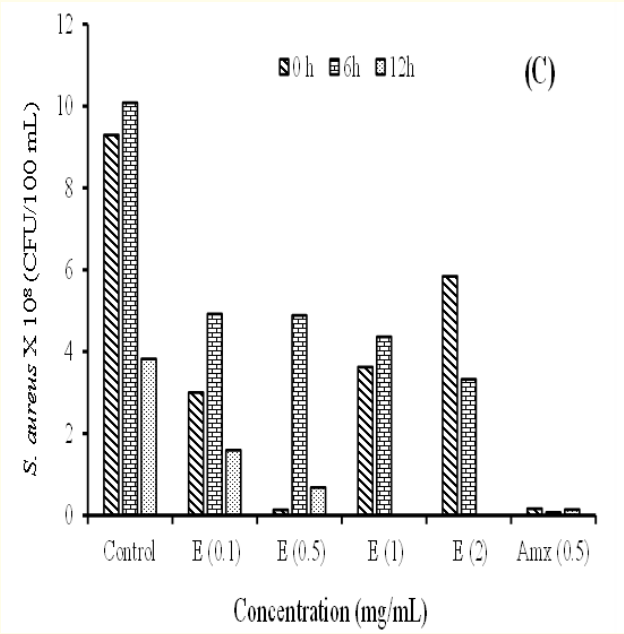
The densities of *E. coli* in the presence of the aqueous extract of *C. arborea* leaves ranged from 2.5×10^{11} to 15.64×10^{11} CFU/100 mL. In the presence of amoxicillin, they ranged from 1.16×10^{11} to 4.48×10^{11} CFU/100 mL. In general, a decrease in bacterial abundances is noted in the presence of extracts of *C. arborea* and amoxicillin compared to those of the control which varied between 11.68×10^{11} and 17.74×10^{11} CFU/100 mL.

When amoxicillin is combined with the aqueous extract, the densities of *E. coli* fluctuated between 0.03×10^{11} and 13.2×10^{11} CFU/100 mL. The abundances of *E. coli* in the presence of amoxicillin ranged from 1.16×10^{11} to 4.48×10^{11} CFU/100 mL. In general, there is a decrease in bacterial densities in the presence of the various combinations of the extract with amoxicillin.

Densities of *S. aureus* reached 5.84×10^{8} CFU/100 mL in the presence of the aqueous extract. In the presence of amoxicillin they ranged from 0.07×10^{8} to 0.14×10^{8} CFU/100 mL. In general, a decrease in the densities of *S. aureus* was observed in the presence of extracts of *C. arborea* and amoxicillin compared to those of the control which oscillated between 3.82×10^{8} and 10.08×10^{8} CFU/100 mL.

When amoxicillin was combined with the aqueous extract, the abundances of *S. aureus* reached 0.01×10^{11} CFU/100 mL. In general, there is a sudden decrease in bacterial abundance in the presence of the various combinations of the extract with amoxicillin from the first period of contact. The different results obtained are shown in figure 1.





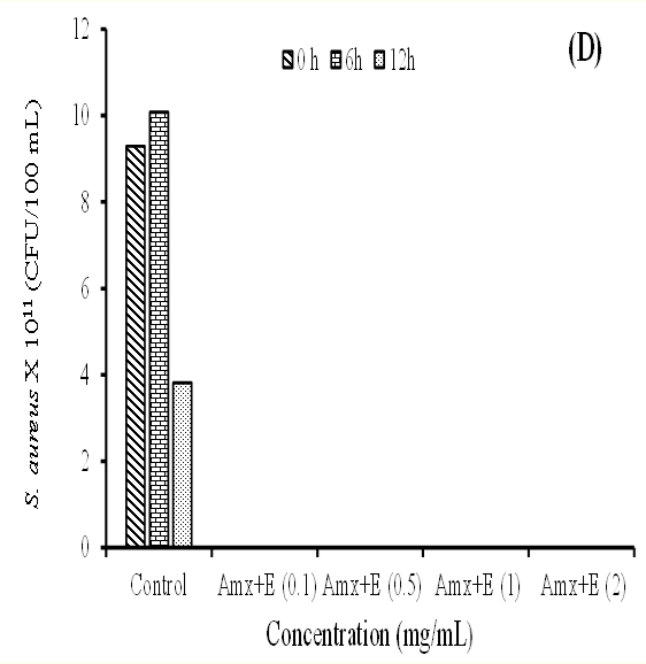


Figure 1: Temporal variation in abundances of *E. coli* and *S. aureus* in the presence of the aqueous extract of *C. arborea* and amoxicillin (A and C) and the various amoxicillin/extract combinations (B and D).

Minimum inhibitory and bactericidal concentrations of the extract

Minimum inhibitory concentrations of the extract (MIC)

The aqueous extract used showed antibacterial activity on the bacteria tested with MICs varying between 12.5 and 25 mg/mL. The extract showed better activity against *S. aureus* with an MIC of 12.5 mg/mL. The minimum inhibitory concentrations of the aqueous extract of the leaves of *C. arborea* on the strains tested are presented.

Strains tested	Aqueous extract	Amoxicillin
E. coli	25	> 0.00256
S. aureus	12.5	> 0.00256

Table 1: Minimum inhibitory concentrations (MIC) in mg/mL of the aqueous extract of the leaves of *C. arborea* on the bacterial strains tested.

Minimum bactericidal concentrations of extracts (MBC)

The extract's MBC was determined and the MBC/MIC ratios calculated. The MBC/MIC ratios were 2 on the strains tested. Thus, the extract exhibited a bactericidal effect on the two bacterial strains used. The results of the CMBs obtained and the CMB/CMI ratios calculated are presented in table 2.

Strains tested	Aqueous extract (MBC)	Amoxicillin (MBC)	MBC/MIC ratios
E. coli	50	ND	2
S. aureus	25	ND	2

Table 2: Minimum Bactericidal Concentrations (MBC) in mg/mL of the various extracts on the bacterial strains tested and MBC/MIC ratios of the extract.

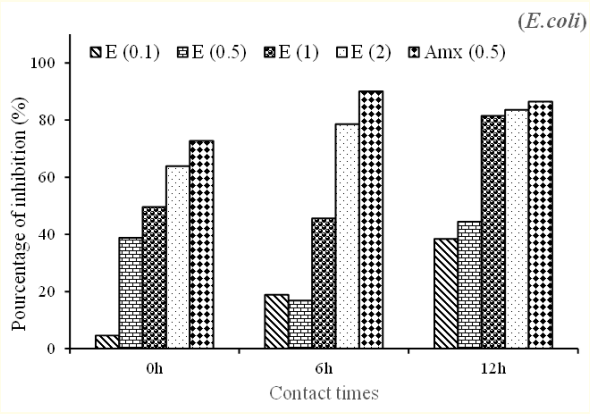
ND: not determine.

Percent inhibition (%) of *E. coli* and *S. aureus* in the presence of the aqueous extract of the leaves of *C. arborea* and the antibiotic

Growth inhibition percentages for different bacterial species were calculated for the extract and amoxicillin and in the presence of the amoxicillin/extract combination for each contact period. With the aqueous extract, the percentages of growth inhibition of *E. coli* oscillated between 4.63 and 83.54% for a better inhibition

observed at the concentration of 2 mg/mL after 12 h of contact. With amoxicillin, the inhibition percentages ranged from 72.68 to 90.07% (Figure 2).

The percentages of inhibition of the growth of *S. aureus* in the presence of the aqueous extract reached 100% for total inhibitions observed at concentrations of 1 and 2 mg/mL after 12 h of contact. With amoxicillin, these percentages varied between 96.34 and 99.31% (Figure 2).



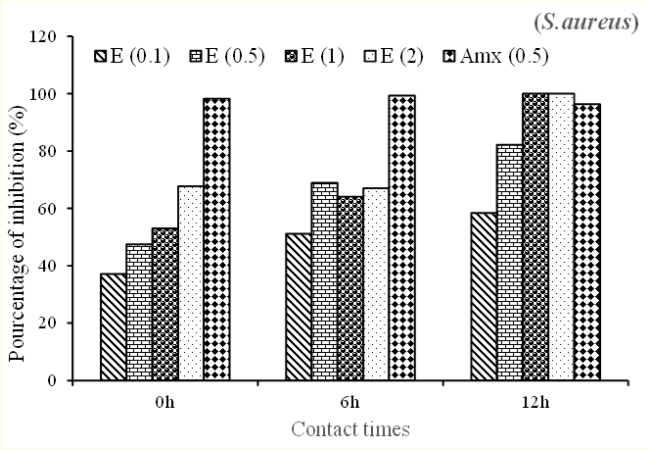
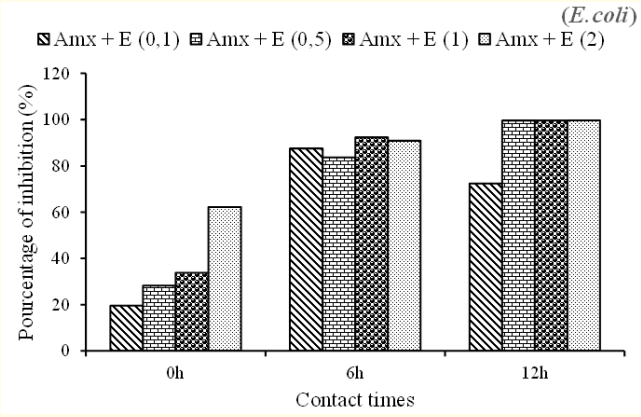


Figure 2: Percentage inhibition (%) of the abundances of *E. coli* and *S. aureus* in the presence of the aqueous extract.

Percent inhibition (%) of *E. coli* and *S. aureus* in the presence of the amoxicillin/extract combination

With the amoxicillin/extract combination, the percentages of growth inhibition of *E. coli* oscillated between 19.51 and 99.74% for better inhibition observed at the concentration of 2 mg/mL of the extract after 12 h of contact. Total inhibitions (100%) and at all contact periods were obtained with *S. aureus* cells. The best growth inhibitions were observed from 0 h of contact (Figure 3).



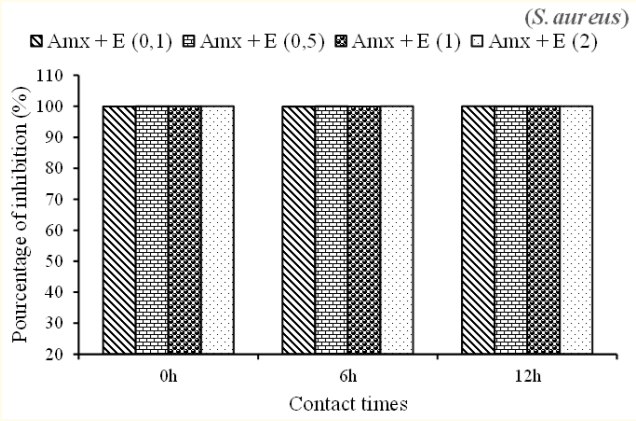


Figure 3: Percentage inhibition (%) of the abundances of *E. coli* and *S. aureus* in the presence of the aqueous extract/antibiotic combination.

Fractional bacterial abundances of amoxicillin/extract combination

The fractional bacterial abundances obtained for amoxicillin/extract combination on *S. aureus* and *E. coli* is shown in table 3.

From the results presented in this table, it emerges that the fractional bacterial abundances reached 0.14 for *S. aureus* and a synergy of action (AF ≤ 0.5) was observed after all periods of contact. For *E. coli* they varied from 0.01 to 2.95. Thus a synergy of action (AF ≤ 0.5) was observed after 12 h of incubation; and indif-

Cells	Contact times	Concentration of extract and antibiotic (mg/mL) and fractional bacterial abundances				
		Amx+E (0.1)	Amx+E (0.5)	Amx+E (1)	Amx+E (0.1)	Amx (0.5)
E. coli	0 h	2.95	2.63	2.42	1.38	1
	6 h	1.91	2.51	1.16	1.40	1
	12 h	1.34	0.02	0.02	0.01	1
S. aureus	0 h	0.02	0.00	0.00	0.00	1
	6 h	0.14	0.00	0.04	0.00	1
	12 h	0.00	0.00	0.00	0.00	1

Table 3: Fractional bacterial abundances (FA) of amoxicillin/aqueous extract combinations on *E. coli* and *S. aureus.*

ferences (0.5 <AF ≤ 4) were observed at the initial time and after 6 h of incubation (Table 3).

Correlations between the different variables analyzed

The Spearman "r" correlation coefficients between bacterial abundances and incubation times for each amoxicillin/extract combination are shown in table 4.

	E. coli			*S. aureus*		
	0 h	6 h	12 h	0 h	6 h	12 h
Extract concentration	-1.00**	-0.80**	-1.00**	-1.00**	-0.40	-0.95**
Amx + Extract concentration	-1.00**	-0.60**	-0.95	-0.78**	-0.63*	Nd

Table 4: Correlation between bacterial abundances and extract concentrations as well as amoxicillin/extract concentrations for each incubation period.

* Significant correlation at P < 0.05, ** Very significant correlation at P < 0.05; dof: 11.

It appears that with increasing incubation times, we observe a decrease in bacterial abundance. There are significant and negative correlations with the extract alone; as well as with the amoxicillin/extract combination at all extract concentrations for both bacterial species. Based on the results presented in this table, no significant and negative difference was observed between the abundances of *S. aureus* after 12 h of contact with the amoxicillin/extract combinations.

Discussion

This study demonstrated the antibacterial efficacy of the extract of the leaves of *C. arborea*. This plant has bactericidal properties that vary in effectiveness relatively depending on cell type and environmental conditions.

The aqueous maceration of the leaves of *C. arborea* gave us a yield of 11.42%. These results are similar to those of [18] which obtained a yield of 31.60% with the methanolic extract and of 16.00% with the aqueous extract of the leaves of *C. arborea*. The difference observed between these results could be explained by the extraction method used in this study.

The phytochemical profile carried out on the extract of the leaves of *C. arborea* revealed the presence of a few families of compounds that may be able to give them their biological activities. Classes of secondary metabolites such as alkaloids, polyphenols, anthraquinones, flavonoids, triterpenes and cardiotonic heterosides were present. Anthocyanins, Tannins, saponins and sugars were absent.

Indeed, polyphenolic products, in particular flavonoids and tannins are known for their toxicity vis-à-vis microorganisms. The mechanism by which these molecules act is certainly complex and may involve many modes of action. In the enzymatic field, the mechanism of toxicity may be associated with the inhibition of hydrolytic enzymes (proteases and carbohydrolases), or with other

interactions for the inactivation of microbial adhesins, transport and envelope proteins [19]. These molecules also inhibit extracellular microbial enzymes, the sequestration of substrates necessary for microbial growth or the neutralization of elements such as iron [20]. In addition, flavonoids lacking a free hydroxide group have a higher bactericidal action than those that have this group, with a hydroxide group there is an increase in the chemical affinity for the lipid membrane. The targets of flavonoids are the cell membrane [19]. The antimicrobial activity depends on the presence of a phenolic compound and probably on that of numerous secondary metabolites; on the position and the number of hydroxide groups it contains [21,22].

Polyphenolic molecules may play a role in cell wall degradation, destabilization of the cytoplasmic membrane leading to the release of cellular components. They have a role in the synthesis of DNA and RNA [23], as well as proteins and lipids and in mitochondrial function, the creation of the cell wall is also influenced by these elements [24,25]. The flavonoids and alkaloids of *C. arborea* are known to possess antimicrobial activity [26,27]. The toxicity of flavonoids to microorganisms results from a non-specific interaction such as hydrogen bonds with proteins or enzymes of the cell wall, neutralization of metal ions, inhibition of bacterial metabolism, sequestration of substances necessary for cell growth [28]. Flavonoids such as myricetin inhibit the growth of multi-resistant bacteria such as *Burkholderia cepacia*, *Enterococcus* vancomycin-resistant, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* [18].

For each of the bacterial species used, the abundances of cultivable cells varied depending on the incubation period and the concentration of the extract. The antibacterial activity of *C. arborea* leaf extract and/or amoxicillin was evaluated in two bacterial species, one gram negative and the other gram positive. These different bacterial strains have been isolated in an aquatic environment. Temporal variations in the abundances of bacterial cells in the presence of the extract or of amoxicillin on the bacterial strains tested were observed. These variations were dependent on the concentrations of the extract and the incubation times of the bacterial cells in the different concentrations of the extract. Thus, the decrease in bacterial abundances was significantly and negatively correlated ($P < 0.01$) with the increase in the concentrations of the extract on the strains studied. This variation in cell abundance could be due to the accumulation of certain chemicals that can be toxic to bacteria [29].

The extract has been shown to be active at different concentrations on bacterial strains with percentages of inhibition varying from 0 to 100%. The extract showed remarkable activity (100% inhibition) with significant differences according to the different concentrations and incubation times ($P < 0.05$) on the two bacterial strains tested. This result could be linked to the presence in this extract of alkaloids and flavonoids which would act by a mechanism of toxicity on bacteria, either by inhibition of hydrolytic enzymes, or by disruption of the functions of membrane proteins, or by inhibition of DNA gyrase [30] of the species tested. The apparent inhibition can also be due to the presence of several classes of secondary metabolites such as flavonoids, triterpenoids, alkaloids, tannins, and saponins present in our extract. Indeed, the species *C. arborea* is known to produce many volatile compounds in large quantities, in particular isoprenoids (called terpenes) which accumulate in glands abundantly distributed in the leaf parenchyma and bark [31]. The variation of the percentages of inhibition as a function of the incubation period was noted (figures 2 and 3). This could be due to the variation in the antibacterial activities of the bioactive compounds present in the extract of *C. arborea*. The plant extract contains bioactive compounds whose configuration or properties change over time, such as flavonoids. In addition, the complexing properties (reversible and irreversible) of flavonoids may also explain the variations in the percentage of inhibition observed during the incubation times. The stability and reactivity of secondary metabolites such as flavonoids are generally linked to their molecular structures [32]. The reactivity of flavonoids dissolved in water leads to molecular instability and this can vary with changes in environmental properties.

Parameters such as light, pH, temperature, nature of solvent, presence of enzyme and oxidant can act on flavonoids [32]. The fluctuation of the percentage inhibition appears to vary depending on the bacterial species as well as the concentration of the extract, the temperature and the incubation time. The bactericidal activity of an antibacterial substance or an antibiotic can be time-dependent (time-dependent bactericidal antibiotic) or concentration-dependent (bactericidal antibiotic concentration dependent) [33]. It has also been suggested that the antimicrobial agent is differentiated based on selective toxicity and that the bacteriostatic agent is often an inhibitor of protein synthesis and acts by binding to ribosomes. If its concentration is reduced, it would be released from the ribosome and bacterial growth would begin [34].

The minimum inhibitory concentrations of the extract ranged from 12.5 to 25 mg/mL. This extract exhibited good activity on the bacterial species tested with bactericidal effects (MBC/MIC ratio = 2) on *S. aureus*. This could be explained by the presence in the aqueous extract of alkaloids and flavonoids which are endowed with significant antimicrobial activity [35]. These results corroborate those of [36]; who had found that the aqueous extract of the bark of the trunk of *C. arborea* had significant activity on *E. coli* and *S. aureus* unlike the ethanolic and hydroethanolic extracts which showed little or no activity [36]. Thus the secondary metabolites present in the extract considerably influence the antibacterial activity observed [35].

The fractional bacterial abundances of the amoxicillin/extract combination were highly variable depending on the bacterial species tested. The amoxicillin/aqueous extract combination exhibited a synergistic action on the two bacterial species tested and indifferences ($0.5 < AF \leq 4$) were observed at the initial time and after 6 h of incubation on *E. coli*. Very significant and negative correlations ($P < 0.05$) were observed from the initial time point at all concentrations for both bacterial species. This could be explained by the fact that amoxicillin, which interacts with penicillin-binding proteins by inhibiting the formation of peptidoglycan, would have seen its activity potentiated by the extract on the two bacterial species studied. The differences observed between Gram-negative strains and Gram-positive strains could be due to the constitution of their cell envelope and the mode of action of antibacterial agents [37].

Conclusion

The aqueous extract of the leaves of *C. arborea* showed remarkable antibacterial activity (100% inhibition) on all strains tested with a strong bactericidal effect. The extract also exhibited variable activities with MICs ranging from 12.5 to 25 mg/mL. The amoxicillin/extract combinations were synergistic on the two bacterial species tested for the different concentrations of the extract. However, antagonistic effects were observed for *E. coli* observed at baseline and after 6h incubation. These results show that the use of *C. arborea* leaves in reducing the flow of bacteriopollutants from the waters could be an alternative. However, it would be relevant to take into account the role of its association with amoxicillin in the spread of microorganisms resistant to antibacterial agents.

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Data Availability Statement

All relevant data are included in the paper.

Disclosure Statement

The authors declare no conflict of interest.

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