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Cultivability of *Escherichia coli* and *Staphylococcus aureus* in the presence of hydroethanolic extracts of *Lantana camara* stems and leaves: Importance of bioactive compounds in the cellular inhibition process.

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ABSTRACT

The presence of pathogenic micro-organisms in drinking water is responsible for certain public health diseases. The use of plant extracts is an alternative to commonly used chemical and physical treatments. The aim of this study is to evaluate the hydroethanolic extracts of *Lantana camara* stems and leaves on the cultivability of planktonic bacteria *Escherichia coli* and *Staphylococcus aureus* in an aquatic microcosm, to determine the effect of extract concentration (0,5 g/L, 1 g/L, 1,5 g/L and 2 g /L), incubation temperature (7 and 37°C) and incubation time (3, 6 and 9 hours) on the cultivability of bacterial cells and also to evaluate the effect of some bioactive compounds isolated from these bacteria. The bioactive compounds identified are Stigmasterol (sterol), lantanilic acid and lantic acid (triterpenes) isolated in the leaf extract of *L. camara* and β-sistosterol (sterol) and oleanolic acid (triterpenes) isolated in the stem extract of *L. camara*. The cultivability of two bacterial strains studied is influenced by the presence of *Lantana camara* extract solution. A reduction in cell density was observed in the presence of the plant extract in question. With *Lantana camara* leaf extract, at an incubation temperature of 37°C, the percentage of inhibition ranged from 3-100% for *E. coli* with the highest value observed after 6 hours at extract concentrations of 1.5 g/L and 2 g/L. In general, the extract has both a bactericidal and bacteriostatic effect on each bacterium, depending on the type of organ, whereas the effect of the bleach is specific to each bacterium. It should be noted that in the presence of *E. coli*, the MICs were 0.00048 mg/ml with the leaf extract and 0.0078 mg/ml with the stem extract. For this bacterium, the MBCs were 0.0039 mg/ml and 0.0312 mg/ml with the leaf and stem extracts respectively. For S. *aureus*, the MICs were 0.0156 mg/ml with the leaf extract and 0.0078 mg/ml with the stem extract and the MBCs were 0.0019 mg/ml and 0.0156 mg/ml (leaf and stem, respectively). The CMB/MIC ratio shows a bactericidal effect at the level of β-sistosterol in *E. coli*. On the other hand, in *S. aureus*, this report shows a bacteriostatic effect and tolerance with the two isolated chemical compounds. The Kruskal-Wallis test shows that the abundance of cultivable cells of each bacterial species differed significantly from one incubation temperature to another at each extract concentration and at each incubation temperature for each part of the plant ($P<0.05$). The data obtained from this exploratory study makes it possible to consider the use of *Lantana camara* hydroethanolic extract as an alternative method in water disinfection.

Key words: Cultivability, *Escherichia coli*, *Staphylococcus aureus*, hydroethanolic extracts, *Lantana camara*, bioactive compounds, cellular inhibition process.

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Introduction

Water obviously plays a vital role in the world. In general, people use it for their daily needs, for economic activities and for leisure. However, the various uses of water can become sources of pollution. Changes in the physicochemical and microbiological quality of water are sometimes the result of poor human management of domestic, agricultural and industrial waste and wastewater **[1]**. The use of contaminated water exposes people to health risks. Contamination of drinking water generally leads to waterborne diseases due to the presence of pathogenic micro-organisms. This water must therefore be treated before consumption. The lack of drinking water is also due to the lack of adequate water treatment facilities. This situation can be resolved by promoting water treatment at the family level. The individual approach is to boil water or use chlorine products. But there are problems with this method of water disinfection. Boiling water requires a lot of energy, which rural people find in wood. This type of treatment is not appropriate as it can lead to deforestation. Current water treatment methods using chlorine and its derivatives, ozone and ultraviolet (UV) lamps are expensive and often inaccessible to underprivileged populations **[2]**. Solar water desinfection (SODIS), a simple, natural and inexpensive treatment technique, has also been proposed to destroy germs in water **[3]**. However, treated water should not be stored for later consumption, as dormant bacteria can resurface after 24 hours of storage **[4]**. Researchers are therefore already trying to find effective and accessible alternatives based on plant extracts, which are currently attracting renewed interest. Most of these plants are used in traditional medicine to treat infectious diseases. The use of plants for therapeutic purposes has been common practice for thousands of years. In Cameroon, as elsewhere, a number of plants used in traditional medicine to combat infectious diseases have been the subject of extensive research **[5-7]**. This work, which includes microbiological and chemical screening, is of great importance. It helps to draw people's attention to plants with real healing power and offers them inexpensive medicines. Traditionally, diarrhoea, microbial infections, rashes and dermatoses were treated with *Lantana camara*. However, despite their antibacterial (bactericidal or bacteriostatic) activity, plant extracts are still underused in the treatment of water intended for human consumption. *Lantana camara* belongs to the Verbenaceae family and is widely used in folk medicine in certain African, American and Asian countries **[8]**. Previous studies using extracts from *Lantana* species showed that they were able to inhibit the growth of gram-positive bacteria strains **[9]**. The antibacterial activity of different extracts from the flowers, leaves, stems and roots of *L. camara* was tested in vitro against different bacterial strains such as *Escherichia coli*, *Staphylococcus saprophiticus*, *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*

and *Vibrio cholerae*. The results obtained showed that these extracts have different spectra of sensitivity to these bacterial strains, with Gram-positive bacteria generally being more sensitive than Gram-negative bacteria. The essential oils of this plant have also shown antibacterial activity against various strains **[10]**. *Lantana camara* it is rich in secondary metabolites possessing beneficial biological activities. In India, these plants are used on folk and traditional medicine system like antimicrobial, fungicidal, insecticidal and nematicidal activity including hepatotoxic in animals tration, incubation temperature and period on this effect. Some authors have shown that aqueous extracts of *Lantana camara* have a bactericidal effect, as demonstrated by the inactivation of microorganisms (faecal coliforms) in the dark condition **[2]**. This bactericidal effect observed in aqueous extracts of *Lantana camara* has been attributed to the presence in these extracts of alkaloids, most of which are considered toxic by several authors **[32]**. However, there is still little information available on the synergistic effect of incubation temperature and hydroethanolic extract of *Lantana camara* on bacterial cells in aquatic environments, as well as the duration of their incubation. Little information is available on the concentration of this plant extract on *Escherichia coli* and *Staphylococcus aureus* bacterial cells. We also have little information on the effect of the bioactive compounds isolated from the leaves and stems of *L. camara* on the bacteria selected for this experiment. The main objective of the present study is to investigate the effect of hydroethanolic extract of *Lantana camara* on the cultivability of planktonic bacteria *Escherichia coli* and *Staphylococcus aureus* in an aquatic microcosm, also to evaluate the effect of extract concentration, incubation temperature and time on the cultivability of bacterial cells and finally to evaluate the effect of some bioactive compounds isolated from these bacteria.

2. MATERIALS AND METHODS

2.1 Harvesting of the leaf and stem of *Lantana camara*, crude extract preparation

Fresh leaves and stems of *Lantana camara* were collected in Limbe, a locality in the South West Region of Cameroon. The leaves and the stem were dried up at laboratory temperature (23±2°C) for 30 days. The leaves and stems were then ground into powder. 2.1 kg of stem powder and 1 kg of leaf powder were macerated in ethanol-water (7:3) for 3 days. Filtration and evaporation of each resulting solution under reduced pressure yielded a dark greenish leaf extract (20 g) and brown stem and twig extracts (30 g). Extraction yields (ratio of weight of extract to weight of powder used) were 3.3% (± 0.5%) for stem and 4.0% (± 0.5%) for leaves. The crystals obtained were used to prepare the crude extract. Four extract concentrations of 0.5 g/L, 1 g/L, 1.5 g/L and 2 g /L were thus prepared using sterile physiological water **[11]**. Each concentration was filtered with a nitrate

cellulose membrane with a porosity of 0.45 µm **[11]**. A qualitative phytochemical screening was done according to the protocols developed by some authors **[12, 13]**.

2.2 Isolated compound extraction, isolation, purification, and characterization

The various extracts were purified by a combination of chromatographic techniques using silica gel and Sephadex LH-20 as the stationary phase to afford 7 known compounds, which were subjected to 1H-NMR and 13C-NMR spectroscopy. And were identified by comparing their spectroscopic data with those reported in the literature as stigmasterol **[14]**, β-sistosterol **[15]**, lupéol **[16]**, betulinic acid **[17]**, oleanolic acid, lantic acid **[18]**, lantanilic acid **[19].** Only five of these compounds were used in this study.

2.3 Bacterial isolation and storage

The bacteria used were *Escherichia coli* and *Staphylococcus aureus*. These strains were chosen because of their importance in hygiene and sanitation. These bacteria were isolated from an urban stream in the equatorial region of Cameroon. The *E. coli* cells were isolated on Endo-agar medium (Bio-Rad, France) using the membrane filtration technique **[20, 21]**. *S. aureus* were isolated on Chapman Mannitol agar medium. Their identification was performed according to the standard method **[22]**.

2.4 Experimental setup to evaluate the effect of *L. camara* extract on planktonic bacteria at different incubation temperatures

The experimental tests consisted of introducing 1 mL of a specific bacterial density of 27.10^8 CFU/mL into four Erlenmeyer flasks each containing 200 mL of *L. camara* extract solution at different concentrations : 0.5 g/L, 1 g/L, 1.5 g/L and 2 g/L. For each replicate, a control was prepared using only 200 mL of physiological water (NaCl: 0.85%). Incubation times were 3, 6 and 9 hours. The test was performed at 7±1 °C and 37±1 °C. Bacteriological analysis was performed in triplicate for each incubation time.

2.5 Determination of the minimum inhibitory concentrations (MICs)

We used the microplate method. A volume of 100 µL of Mueller-Hinton broth was added into all 96 wells of the microplate as described by some authors. **[23-25]**. In the wells of columns 1, 2 and 3 corresponding to the replicates, we added a series of volumes of 100 µL of diluted stock extract solution, reason 2, to obtain a range of nine concentrations from (500 to 0.0488 mg/mL, for isolated metabolites. A microbial suspension of each germ was prepared in sterile physiological water (NaCl, 8.5%) from a 24 h early culture on slant cast agar by adjusting the turbidity to that of 0. 5 Mac Farland solution (adding 0.5 mL of 0.0048M $BaCl_2$ (1.17% w/v $BaCl_2 . 2H_2O$) to 99.5 mL of 0.18M H_2SO_4 (1% v/v), i.e. 1.5×10^8 CFU/mL for both bacteria **[26, 24, 27]**. A first dilution was then made to obtain intermediate solutions at 10^7 CFU/mL and 10^6 CFU/mL respectively. This

was followed by a second dilution to obtain inocula at 10^6 CFU/mL and 10^5 CFU/mL. 100 µL of each inoculum was then added to each well, resulting in a final volume of 200 µL per well. The positive control was included in columns 11 and 12. This positive control, consisting of ciprofloxacin for *Escherichia coli* and penicillin for *Staphylococcus aureus*, was prepared under the same conditions as the extract and tested at a concentration of 08 serial dilutions ranging from 500 to 0.976 mg/L **[28]**. The negative control consisted of a mixture (culture medium + bacterial inoculum in the proportions (1/1; V/V)). Wells containing only culture medium were reserved for sterility control. The microtitre plates were incubated at 37°C for 18 to 24 hours. After incubation, bacterial growth was detected using iodonitrotetrazolium chloride (INT), the principle of which is based on the capture of protons emitted by dehydrogenase enzymes present in the membrane of living bacteria **[29]**. 40 µL of INT solution was added to each well of the microtiter plate. After metabolism of the glucose, the medium turned pink after 30 min of re-incubation, indicating that the concentration of the metabolite was insufficient to inhibit bacterial growth. In the unstained well containing the lowest amount of secondary metabolite, the concentration of the extract corresponds to the MIC **[28]**. The MIC corresponds to the concentration of the unstained well containing the lowest amount of secondary metabolite. Assays were repeated in triplicate.

2.6 Determination of the Minimum Bactericidal Concentrations (MBCs)

The protocol used was that recommended by some authors **[30]**. A 150 µL volume of sterile medium (Mueller-Hinton broth) was introduced into the wells of another microplate, also sterile and different from the MIC microplate. We then added 50 µL of extract taken from the wells corresponding to the MICs obtained. We also took 50 µL of extract from the wells preceding the MIC wells (ISO standard 20766-1: 2006). The sterility control consisted of 200 µL of medium (Mueller Hinton broth). The positive control was ciprofloxacin/penicillin treated as the extracts. The negative control was the medium without extracts. The Minimum Bactericidal Concentration was represented by the well corresponding to the lowest extract.

2.7 Determination of the MBC/MIC ratios

The ratio obtained should indicate whether the extracts tested are bacteriostatic or bactericidal. If this ratio is greater than 4, the substance is considered bacteriostatic. If this ratio is less than 4, the substance is considered bactericidal **[31, 30]**.

3. RESULTS AND DISCUSSION

3.1 Phytochemical constituents and bioactive *Lantana camara* compounds

Phytochemical screening showed that phenolic compounds, tannins, flavonoids and polysaccharides are the main constituents of the ethanolic extracts of each of the parts of *L. camara* considered. Sterols and triterpenes are also present, according to the colourings observed (Table 1).

Table 1. Phytochemical constituents of *Lantana camara* leaves and stems extract

Chemical compounds tested	**Appreciation of relative abundance**	
	Hydroethanolic extracts from the leaves	**Hydroethanolic extracts from the stems**
Phenolic compounds	++++	++++
Flavonoids	++++	++++
Alkaloids	++--	++--
Anthocyanins	+---	+---
Coumarin compounds	----	----
Saponins	++--	++--
Tannins	++++	++++
Sterol and Triterpenoids	+++-	+++-
Quinons	+---	+---
Polysaccharides	++++	++++

Legend:++++: more abundant; +++-: abundant; ++--: scanty; -: Non detected

The compounds identified after purification and spectroscopic studies belong to two different classes. They are sterols and triterpenes. These two classes were found in the two parts of the plants studied. Stigmasterol (sterol), lantanilic acid and lantic acid (triterpenes) were isolated in the leaf extract of *L camara* and β-sistosterol (sterol) and oleanolic acid (triterpenes) in the stem extract of *L camara* (Table 2).

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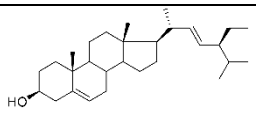
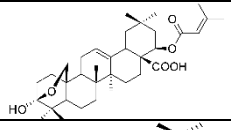
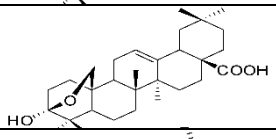
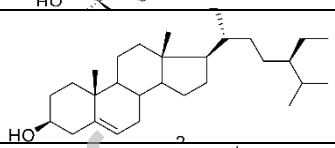
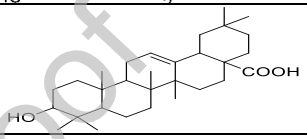
Table 2. Bioactive compounds profile of the ethanolic extracts of each of the parts of *L. camara* considered

Part of the plant	Compound name	Class of molecule	Compound formula	Structural formula	Molecular mass (g/mol)
Leaves	**Stigmasterol**	Sterol	$C_{29}H_{48}O$		412,37
	Lantic acid	Triterpen	$C_{30}H_{46}O_4$		470,34
	Lantanilic acid	Triterpen	$C_{35}H_{52}O_6$		568,38
Stems	**β-sistosterol**	Sterol	$C_{29}H_{50}O$		414,39
	Oleanolic acid	Triterpen	$C_{30}H_{48}O_3$		456,36

3.2 Temporal variation of the cell abundance

In the absence of *Lantana camara* stems extract (0 g/L), the abundance of *Escherichia coli* varied between 193.10^3 and 215.10^3 CFU/100 mL and between 233.10^3 and 460.10^3 CFU/100 mL at the incubation temperatures of 7°C and 37°C respectively (**Figure 1**). In the presence of Extract from the stems of *Lantana camara*, the abundance of *Escherichia coli* ranged from 37.10^3 to 203.10^3 CFU/100 mL at 7°C. After 3 hours of contact with an extract concentration of 1.5 g/L, the lowest cell densities were observed (**Figure 1A**). At 37°C, the abundance of *Escherichia coli* ranged from 174.10^3 to 315.10^3 CFU/100 mL with the lowest cell densities observed after 3 hours of contact with an extract concentration of 1.5 g/L (**Figure 1B**). In the same condition, the cell concentration of *Staphylococcus aureus* varied between 67.10^3 and 104.10^3 CFU/100 mL and between 42.10^3 and 81.10^3 CFU/100 mL at the incubation temperatures of 7°C and 37°C, respectively (**Figure 2**). In the presence of *Lantana camara* stem extract, we observed a reduction, in the cell density of *Staphylococcus aureus*, from 86.10^3 CFU/100 mL to 1.10^3 CFU/100 mL after 3 hours of contact with an extract concentration of 2 g/L, from 104.10^3 CFU/100 mL to 2.10^3 CFU/100 mL and from 67.10^3 CFU/100 mL to 2.10^3 CFU/100 mL after 3 hours of contact with an extract concentration of 1 g/L when the incubation temperature is 7°C. At an incubation temperature of 37°C, we observed a reduction, in the cell density of *Staphylococcus aureus*, from 81.10^3 CFU/100 mL to 8.10^3 CFU/100 mL after 3 hours of contact with an extract concentration of 1 g/L, from 42.10^3 CFU/100 mL to 2.10^3 CFU/100 mL after 6 hours of contact with an extract

concentration of 1 g/L and from 53.10^3 CFU/100 mL to 1.10^3 CFU/100 mL after 9 hours of contact with an extract concentration of 0.5 g/L (**Figure 2**).

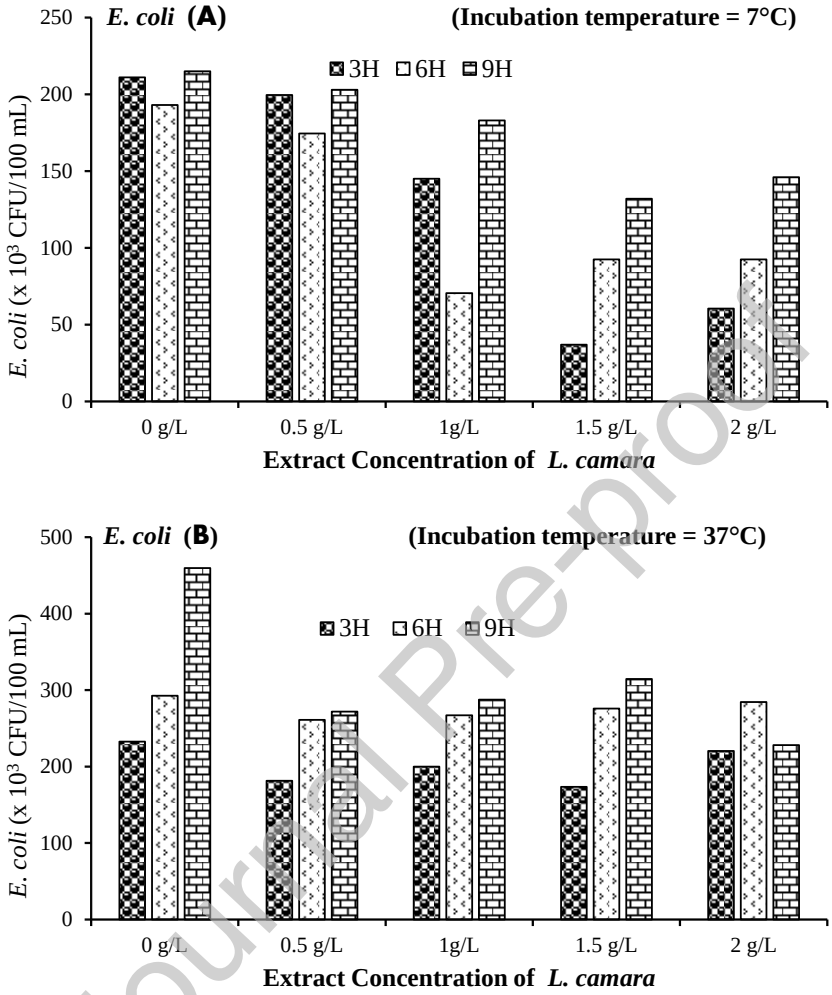


Figure 1. Temporal variation of the abundance of planktonic *E. coli* cells in the presence of *Lantana camara* extract stems at different extract concentrations (**A**: 7°C and **B**: 37°C).

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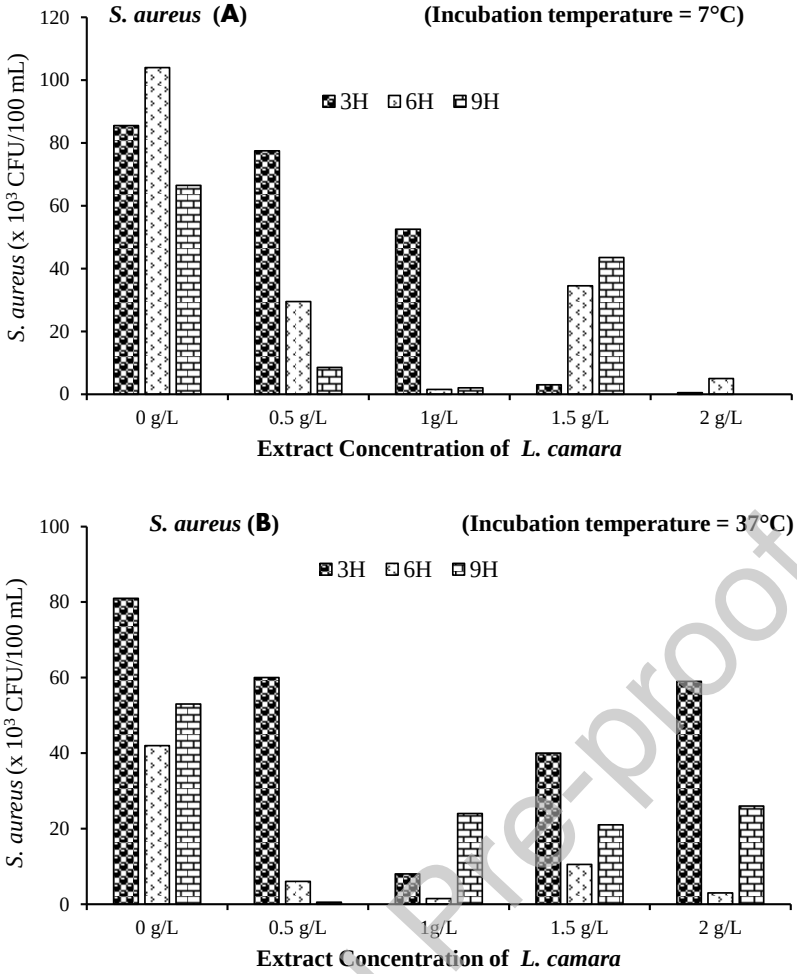

Figure 2. Temporal variation of the abundance of planktonic *S. aureus* cells in the presence of *Lantana camara* extract stems at different extract concentrations (**A**: 7°C and **B**: 37°C).

In the absence of *Lantana camara* leaves extract (0 g/L), the abundance of *Escherichia coli* varied between 410.10^3 and 651.10^3 CFU/100 mL and between 452.10^3 and 520.10^3 CFU/100 mL at the incubation temperatures of 7°C and 37°C respectively (**Figure 3**). At 7°C and in the presence of *Lantana camara* leaf extract, we observed a reduction, in the cell density of *Escherichia coli*, from 455.10^3 CFU/100 mL to 199.10^3 CFU/100 mL after 3 hours of contact with an extract concentration of 1.5 g/L, from 410.10^3 CFU/100 mL to 301.10^3 CFU/100 mL after 6 hours of contact with an extract concentration of 2 g/L and from 651.10^3 CFU/100 mL to 311.10^3 CFU/100 mL after 9 hours of contact with an extract concentration of 2 g/L. At 37°C in the same condition, we observed a reduction, in the cell density of *Escherichia coli* , 452.10^3 CFU/100 mL to 0.10^3

CFU/100 mL after 6 hours of contact with an extract concentration of 1.5 g/L and 2g/L (**Figure 3**).

Concerning *Staphylococcus aureus*, the cell density ranged from 44.10^3 CFU/100 mL to 262.10^3 CFU/100 mL in the absence of *Lantana camara* leaf extract (0 g/L) at 7°C incubation temperature. The variation was from 41.10^3 CFU/100 mL to 69.10^3 CFU/100 mL at 7°C incubation temperature without contact with *Lantana camara* leaf extract. In the presence of *Lantana camara* leaf extract at 7°C, the lowest abundance was observed after 9 hours of contact with an extract solution at a concentration 1 g/L. At 37°C, we observed a reduction, in the cell density of *Staphylococcus aureus*, from 57.10^3 CFU/100 mL to 15.10^3 CFU/100 mL after 3 hours of contact with an extract concentration of 0.5 g/L, from 41.10^3 CFU/100 mL to 23.10^3 CFU/100 mL after 6 hours of contact with an extract concentration of 0.5 g/L and from 69.10^3 CFU/100 mL to 20.10^3 CFU/100 mL after 9 hours of contact with an extract concentration of 2 g/L (**Figure 4**).

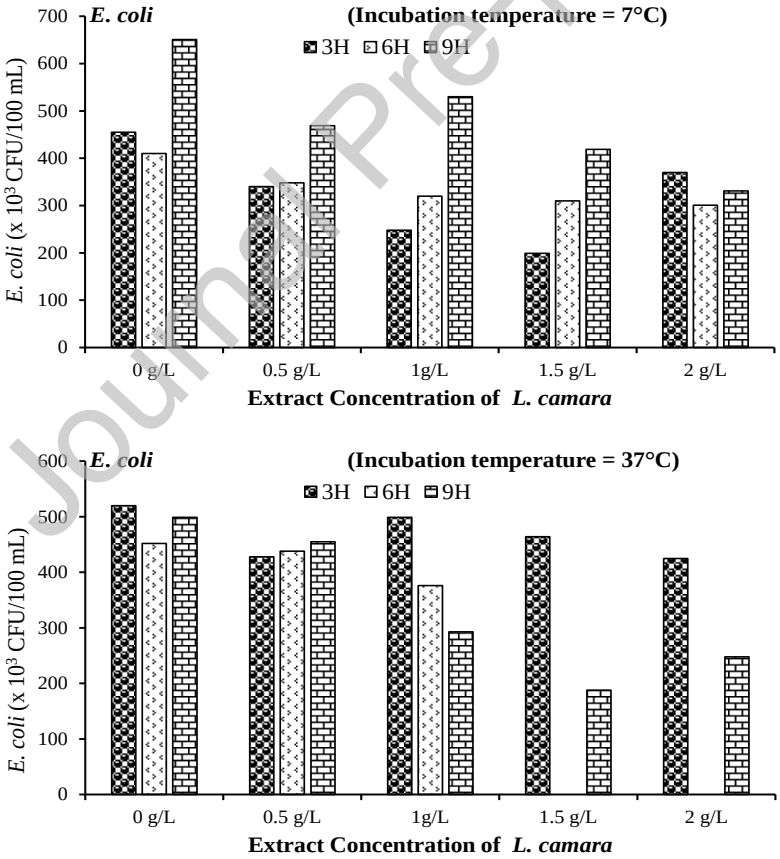

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Figure 3. Temporal variation of the abundance of planktonic *E. coli* cells in the presence of *Lantana camara* extract leaves at different extract concentrations (**A**: 7°C and **B**: 37°C).

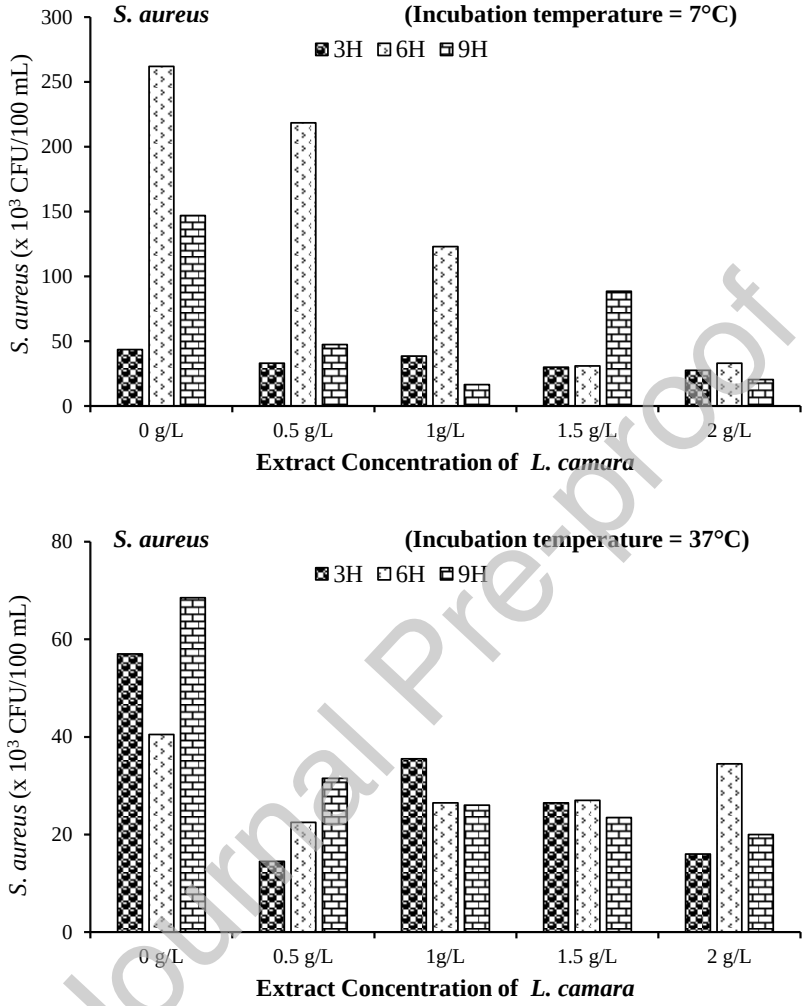

Figure 4. Temporal variation of the abundance of planktonic *S. aureus* cells in the presence of *Lantana camara* extract leaves at different extract concentration (**A**: 7°C and **B**: 37°C).

3.3 Evaluation of the cell inhibition

The percentage of cell inhibition was calculated to evaluate the effect of *Lantana camara* extract concentration, incubation temperature and contact time on the cultivability of *E. coli* and

S. aureus. The **Table 3** shows that the cultivability of two bacterial strains studied is influenced by the presence of *Lantana camara* extract solution. With the extract from the stem of *Lantana camara*, the percentage of inhibition reached the maximun values at an extract concentration of 1 g/L after 6 hours and at 2 g/L after 9 hours at an incubation temperature of 7°C for *S. aureus*. For *E . coli*, the percentage of inhibition varied between 5 and 85% with this maximum value obtained after 6 hours at an extract concentration of 1.5 g/L. The lower percentages of 5% and 9% for *E. coli* and *S. aureus* respectively were observed after 3 h at 7°C at 0.5g/L extract. In the majority of cases, the percentage of inhibition was higher than 80%. At 37°C, the percentage of inhibition ranged from 3-50 % for all incubation contacts for *E. coli* and ranged from 26-99% for all incubation contacts for *S. aureus*.

With *Lantana camara* leaf extract, the percentage of inhibition ranged from 12-56% for *E. coli* and 11-89% for *S. aureus* for all incubation contacts at an incubation temperature of 7°C. At an incubation temperature of 37°C, the percentage of inhibition ranged from 3-100% for *E. coli* with the highest value observed after 6 hours at extract concentrations of 1.5 g/L and 2 g/L. At the same incubation temperature, inhibition of *S. aureus* ranged from 15-75% for all incubated contacts.

Table 3. Percentage of inhibition (PI) of *Escherichia coli* and *Staphylococcus aureus* after each incubation contact with *Lantana camara* extract solution at different concentrations.

	Incubation temperature and different extract concentration		Value of the percentage of inhibition (%) after each incubation period					
			Escherichia coli			*Staphylococcus aureus*		
			3 h	6 h	9 h	3 h	6 h	9 h
Stems	7 °C	0.5 g/L	5	10	6	9	72	87
		1 g/L	31	63	15	39	99	97
		1.5 g/L	82	85	39	96	67	35
		2 g/L	71	52	32	99	95	100
	37 °C	0.5 g/L	22	11	41	26	86	99
		1 g/L	14	9	37	90	96	55
		1.5 g/L	25	6	32	51	75	60
		2 g/L	5	3	50	27	93	51
Leaves	7 °C	0.5 g/L	25	15	28	24	17	68
		1 g/L	45	12	19	11	53	89
		1.5 g/L	56	24	36	31	88	40
		2 g/L	19	27	49	37	87	86
	37 °C	0.5 g/L	18	3	9	75	44	54
		1 g/L	4	17	41	38	35	62
		1.5 g/L	11	100	62	54	33	66
		2 g/L	18	100	50	72	15	71

3.4 Extract's minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of leaf and stem plant on bacteria studied

Table 4 shows the minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) of *Lantana camara* extracts on some bacteria of health importance. The minimum bactericidal concentrations and the minimum inhibitory concentrations on the inactivation of bacteria, their MBC/MIC ratio was calculated. This ratio is bacteriostatic when it is ≥ 4 and bactericidal when it is < 4. When organ extracts were used to treat leaves, the lowest MIC was found for *E. coli*. This MIC value is also lower than that of the three controls: sodium hypochlorite (1.25 mg/mL for *E. coli* and 0.019 mg/mL for *S. aureus*), ciprofloxacin for *E. coli* (0.125 mg/mL) and penicillin for *S. aureus* (0.0312 mg/mL). The CMB/MIC ratio shows a bacteriostatic effect at the leaf level for *E. coli*. On the other hand, this report shows a bactericidal effect on *S. aureus* in both organs. In general, the extract has both a bactericidal and bacteriostatic effect on each bacterium, depending on the type of organ, whereas the effect of the bleach is specific to each bacterium. On the other hand, the two reference antibiotics only have a bactericidal effect.

Table 5 shows the minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) of some bioactive compounds isolated from *Lantana camara* extracts (leaves) on some bacterial strains. Extracts of the compounds isolated from the leaves showed the lowest MIC value against *S. aureus*. This MIC value is also very high compared to the three controls: sodium hypochlorite, ciprofloxacin for *E. coli* and penicillin for *S. aureus*. The CMB/MIC ratio shows a bacteriostatic effect at the level of lantanic acid in *E. coli*. On the other hand, in *S. aureus* this report shows a bactericidal effect with the three compounds. In general, the isolated compounds have both a bactericidal and bacteriostatic effect on each bacterium, whereas the effect of the bleach is specific to each bacterium. On the other hand, the two reference antibiotics only have a bacteriostatic effect.

Using the extracts of the compounds isolated from the stems, we noted the lowest MIC value in *E. coli*, we also noted the absence of CMB with oleanolic acid with respect to the same bacteria (Table 6). This MIC value is also low for *S. aureus*. Compared with the three controls: sodium hypochlorite, ciprofloxacin for *E. coli* and penicillin for *S. aureus*. The CMB/MIC ratio shows a bactericidal effect at the level of β-sistosterol in *E. coli*. On the other hand, in *S. aureus* this report shows a bacteriostatic effect and tolerance with the two chemical compounds isolated (Table 6).

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Table 4. Minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) of *Lantana camara* extracts on some bacteria of health importance

Bacteria strains	Part of the plant	CMI			CMB			CMI/CMB	Effect
		Extract plant (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)	Extract plant (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)		
E. coli	Leaves	0.00048	0.125	1.25	0.0039	0.125	0.078	8.125	Bacteriostatic
	Stems	0.0078	0.125	1.25	0.0312	0.125	0.078	4	Bactericidal
S. aureus	Leaves	0.0156	0.0312	0.019	0.0019	0.0625	0.312	0.12	Bactericidal
	Stems	0.0078	0.0312	0.019	0.0156	0.0625	0.312	0.5	Bactericidal

ATB* = ciprofloxacin for *E. coli* and penicillin for *S. aureus*
NaOCl** = sodium hypochlorite

Table 5. Minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) of some bioactive compounds isolated of *Lantana camara* extracts (leaves) on some bacteria strains.

Bacteria strains	Compound name isolated	CMI			CMB			CMI/CMB	Effect
		Compound isolated (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)	Compound isolated (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)		
E. coli	Stigmasterol	12	0.976	1.25	3	15.625	0.078	0.25	Bactericidal
	Lantic Acid	7.813	0.976	1.25	62.5	15.625	0.078	8	Bacteriostatic
	Lantanilic acid	62.5	0.976	1.25	15.625	15.625	0.078	0.25	Bactericidal
S. aureus	Stigmasterol	0.75	3.906	0.019	1.5	31.25	0.312	2	Bactericidal
	Lantic Acid	0.976	3.906	0.019	0.976	31.25	0.312	1	Bactericidal
	Lantanilic acid	1.953	3.906	0.019	3.906	31.25	0.312	2	Bactericidal

ATB* = ciprofloxacin for *E. coli* and penicillin for *S. aureus*
NaOCl** = sodium hypochlorite

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Table 6. Minimum inhibitory concentrations (MICs) and bactericidal concentrations (MBCs) of some bioactive compounds isolated of *Lantana camara* extracts (stems) on some bacteria strains.

Bacteria strains	Compound name isolated	CMI			CMB			CMI/CMB	Effect
		Compound isolated (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)	Compound isolated (mg/mL)	ATB* (mg/mL)	NaOCl** (mg/mL)		
E. coli	β-sistosterol	0.001	0.125	1.25	0.004	0.25	0.078	4	Bactericidal
	Oleanolic acid	0.00053	0.125	1.25	/	0.25	0.078	/	/
S. aureus	β-sistosterol	0.002	0.0312	0.019	0.25	0.0625	0.312	125	Tolerance
	Oleanolic acid	0.001	0.0312	0.019	0.25	0.0625	0.312	250	Bacteriostatic

ATB* = ciprofloxacin for *E. coli* and penicillin for *S. aureus*
NaOCl** = sodium hypochlorite

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3.5 Comparison between the concentration of the extract, the incubation temperature and the mean abundance of the cells bacteria

The Kruskal-Wallis test was used to determine the abundance of *E. coli* and *S. aureus* at each extract concentration and incubation temperature for each plant part, taking into account all incubation times. The probability risk values are shown in (Table 7). It shows that, in the majority of cases, the abundance of cultivable cells of each bacterial species differed significantly from one incubation temperature to another at each extract concentration and at each incubation temperature for each part of the plant ($P<0.05$).

Table 7. Comparisons of *E. coli* and *S. aureus* abundances at each extract concentration and incubation temperature for each plant part, taking into account all incubation times.

Bacterial species, incubation temperature, Part of plant and extracts concentration				P value
Bacterial species	**Incubation temperature**	**Part of plant**	**Extract concentration (g/L)**	
E. coli	7°C	Stem	0.5	P = 0.043*
			1	P = 0.024*
			1.5	P = 0.027*
			2	P = 0.039*
		Leaf	0.5	P = 0.066*
			1	P = 0.027*
			1.5	P = 0.027*
			2	P = 0.148^{ns}
	37°C	Stem	0.5	P = 0.027*
			1	P = 0.027*
			1.5	P = 0.027*
			2	P = 0.044*
		Leaf	0.5	P = 0.252^{ns}
			1	P = 0.027*
			1.5	P = 0.024*
			2	P = 0.024*
S. aureus	7°C	Stem	0.5	P = 0.027*
			1	P = 0.053^{ns}
			1.5	P = 0.027*
			2	P = 0.029*
		Leaf	0.5	P = 0.051^{ns}
			1	P = 0.027*
			1.5	P = 0.063^{ns}
			2	P = 0.528^{ns}
	37°C	Stem	0.5	P = 0.024*
			1	P = 0.024*
			1.5	P = 0.027*
			2	P = 0.024*
		Leaf	0.5	P = 0.129^{ns}
			1	P = 0.105^{ns}
			1.5	P = 0.698^{ns}
			2	P = 0.055^{ns}

*: Significant difference ($P<0.05$) ns : no Significant difference

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Discussion

This study showed that the level of activity of the extract varied according to the part of the plant used and the germ present. This activity would result from various interactions between the chemical compounds contained in the extract and the microorganisms present. Phytochemical screening of the hydroethanolic extract of *Lantana camara* revealed the presence of certain major secondary metabolites such as phenolic compounds, flavonoids, polysaccharides and tannins. Other phytocompounds present were alkaloids, sterols and triterpenes. Some authors have already reported the high presence of these compounds in several whole plant and leaf extracts of *Lantana camara* **[8]**. The alkaloids in aqueous extracts of *Lantana camara* are cited by several authors as having remarkable bactericidal activity **[32]**. New triterpenic compounds such as lantanilic acid and lantic acid have been identified in the leaves. Some studies have already demonstrated the activity of these two compounds against microorganisms **[33]**.

A temporal variation in the abundance of planktonic cells was observed in the presence of *Lantana camara* extract. This variation depends not only on the concentration of the plant extract, but also on related factors such as the incubation temperature and the duration of contact between the plant extract and the microorganism studied. According to some authors, the observed variation could be due to the variation of the antibacterial activities of bioactive compounds present in the extract plant **[34]**. 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 variation in the percentage of inhibition observed during the incubation period **[34]**. The direct effect of the hydroethanolic extract of *Lantana Camara* on the survival of the cell species considered was assessed by calculating the percentage of cell inhibition under each experimental condition. The observed variations in abundance and the high percentages of inhibition recorded during the experiment were probably due to the secondary metabolites present in the plant extract. Combining the results of cell culture and phytochemical screening, we can suggest that the antimicrobial activities are related to the presence of phenolic compounds, flavonoids, polysaccharides, tannins, alkaloids, sterols and triterpenes. Flavonoids and tannins are known to be toxic to microorganisms and tannins have antibacterial properties **[35-38]**. Some authors have shown that aqueous and hydroalcoholic extracts of leaves rich in phenolic compounds inhibit the growth of *B. cereus* and *S. aureus* **[39]**. Phenolic compounds exert their antimicrobial power through a mechanism similar to that of aldehydes, with inhibitory efficacy proportional to the degree of hydrophobicity **[40, 11]**. Aldehyde inhibition is essentially due to the reactivity of the aldehyde function with the thiol group

of the amino acids involved in cell division **[41]**. It has also been suggested that a complex is formed between the electron donor and the aldehyde, leading to a change in the ionic state of the membrane, resulting in an imbalance in exchange with the external environment **[41, 40]**. This state leads to the certain death of the fungal and bacterial cell. Other studies have also shown that the extract from the leaves of *L. camara* contains bioactive compounds, alkaloids, flavonoids, tannins and saponins, which is why this plant is used for various medicinal purposes and has antibacterial activity against *S. aureus* **[42]**. At low concentrations, phenolic compounds have a reversible effect, whereas at high doses they cause general coagulation and microbial cell death **[43]**.

Given their structural and molecular make-up, polyphenols also play a role in the degradation of the cell wall and disruption of the cytoplasmic membrane, resulting in the loss of cellular components. They also affect the synthesis of DNA and RNA **[44]**, proteins and lipids **[45]**, and the formation of complexes with the cell wall **[46]**. Tannins also have the ability to form complexes with proteins, leading to the inactivation of enzymes, either directly by binding to active sites or indirectly through steric hindrance created by the binding of tannin molecules to the enzyme **[47,48]**.

The antibacterial activity of flavonoids can be explained by the mechanism of toxicity to microorganisms. This mechanism involves non-specific interactions such as hydrogen bonding with cell wall proteins or enzymes, chelation of metal ions, inhibition of bacterial metabolism and sequestration of substances necessary for bacterial growth **[49]**. The antibacterial parameters, i.e. the MIC, the MBC and the MBC/MIC ratio, were determined between the hydroethanol extracts of the leaves and stems of *Lantana camara*. The lowest value at which the inhibitory effect of the hydroethanolic extract begins to take effect is 0.00048 mg/mL. This value was obtained using *Escherichia coli* on leaves. For *Staphylococcus aureus*, the lowest MIC value was obtained in the stems and was 0.0078 mg/mL. It can therefore be said that the hydroethanoic extract is more effective against these strains. Most of the MICs obtained in this study were below 0.1 mg/mL. Thus, according to a classification of the activity of plant extracts according to the value of their MIC revealed by Kuete (2010). Indeed, for this author, the activity of a medicinal plant extract is significant when the MIC < 0.1 mg/mL, moderate when $0.1 \leq$ MIC ≤ 0.625 mg/mL and not significant when the MIC > 0.625 mg/mL. For both strains tested, the BMC/MIC ratios were less than or equal to 4, indicating bactericidal activity.

Except for the CMB/CMI ratio obtained with leaf extracts on *E. coli*, which was greater than 4, hence the bacteriostatic effect observed. This observed bacteriostatic effect implies that *E. coli* develops a resistance strategy against hydroethanoic extracts of *Lantana camara* leaves. This

bacteriostatic effect was also observed with lantic acid on *E. coli*. Lantic acid, a natural product found in *Lantana camara*, is a bioactive triterpenoid. Lantic acid was found to have strong antibacterial activity against *E. coli* and *Bacillus cereus* **[50]**. In fact, *E. coli* has developed several mechanisms of resistance to antimicrobial compounds. These include (a) inactivation of antimicrobial compounds of the beta-lactam class through the production of beta-lactamases; (b) decreased penetration of antimicrobial compounds to the target site; (c) alteration of the penicillin-binding proteins at the target site; and (d) efflux from the periplasmic space through a specific pumping mechanism by the production of a group of enzymes referred to as "β-lactamases" **[51, 52]**.

The other compounds isolated from the leaf extracts, such as stigmasterol and lantanilic acid, had a bactericidal effect on the two strains of bacteria studied. Stigmasterol has an antioxidant role. Stigmasterol a plant sterol (phytosterol) is one of the most abundant plant sterols and has an important function in maintaining the structure and physiology of cell membranes. Stigmasterol is an unsaturated phytosterol found in the plant fats or oils of many plants Stigmasterol is a constituent of various vegetables, legumes, nuts, seeds **[53-55]**. Stigmasterol hydroperoxides are the major primary oxidation products, while hydroxystigmasterols, ketostigmasterols and stigmasterol epoxides are the major secondary oxidation products **[56]**. Lantanilic acid is a triterpene acid. Antibacterial and antifungal activity was tested using the agar diffusion method by determining the area of bacterial and fungal growth inhibition. Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and the fungus *Candida albicans* were used in this study **[57]**.

Bioactive compounds such as β-sistosterol and oleanolic acid have been isolated from the stems. The bactericidal effect was observed with β-sitosterol with a MIC of 0.001 mg/mL on *E. coli*. β-Sitosterol has been shown to have antibacterial activity against *S. aureus* and *E. coli* in previous reports. [58]. Indeed, β-sitosterol reduces the membrane permeability of phospholipid bilayers more efficiently than other group members and also acts in membranes to limit the movement of fatty acyl chains [59, 60]. β-Sitosterol is usually the most abundant membrane constituent in plant membranes. However, tolerance of *S. aureus* to β-sitosterol has been observed. This tolerance can be explained by several mechanisms developed by *S. aureus*. *S. aureus* resistance can develop either by horizontal transfer of resistance determinants encoded by mobile genetic elements via plasmids, transposons and the staphylococcal cassette chromosome, or by mutations in chromosomal genes **[61, 62]**.

Conclusion

In the aquatic environment containing Lantana camara extract, the ability of cells to grow is affected. The observed reduction in abundance may be due to the presence of some chemical compounds in *Lantana camara*, such as phenolic compounds, flavonoids, polysaccharides and tannins. Bioactive compounds like stigmasterol (sterol), lantanilic acid and lantic acid (triterpenes) were isolated in the leaf extract of *L camara* and β-sistosterol (sterol) and oleanolic acid (triterpenes) in the stem extract of *L camara*. These molecules have a bactericidal effect on the bacteria studied. This depends on the experimental conditions. With respect to extract concentration, incubation temperature and contact time between cell bacteria and extract, the magnitude of the effect was relatively variable. The abundance of cultivable cells differed significantly from one incubation temperature to another at each extract concentration and at each incubation temperature for each part of the plant ($P<0.05$). The data obtained from this preliminary study could be *Lantana camara* hydroethanolic extract as an alternative method of water disinfection.

CRediT authorship contribution statement

Carlain Emar Tchapo Djappa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **fils Mamert Onana:** Data curation, Investigation, review. **Antoine Tamsa Arfao:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing. **Claire Stéphane Metsopkeng:** Data curation, Methodology, Writing. **Maka Mouande Arnold Alex:** Data curation, Formal analysis, Methodology,. **Sophie Laurent:** Data curation, Methodology, Writing. **Céline Henoumont:** Data curation, Methodology, Writing. **Hugues Fouotsa:** Data curation, Methodology, Writing . **Pierre Mkounga[a]:** Data curation, Methodology, Writing, Supervision. **Ephrem Augustin Nkengfack:** Data curation, Methodology, Writing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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