

Antimicrobial potential of *Erigeron floribundus* extracts against some aquatic bacteria of sanitary importance: influence of pH

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SUMMARY

Nowadays, humans suffer from many infectious water borne diseases such as gastro-enteritis, cholera and typhoid fever. Antibiotics that have been used for several decades to limit disease phenomena are increasingly losing their effectiveness due to their excessive and uncontrolled use. Hence, necessitates alternative treatments, including plant derived remedies. The aim of this study was to evaluate the aqueous and ethanolic leaves extract of *Erigeron floribundus* on three important sanitary bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*) with a variation of the pH medium. *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis* were isolated from well water in the city of Yaounde. The qualitative phytochemical screening was conducted using colorimetric precipitation methods. The pH of the medium was adjusted using a pH meter, HCl and NaOH solutions. The antimicrobial activities were

evaluated by the surface spreading on agar medium, membrane filter technique and microdilution methods. The phytochemical screening of both extracts revealed in common the presence of alkaloids, saponins, polyphenols and tannins. Cardiac glycosides, flavonoids, resins, quinones and mucilages were only present in the ethanolic extract. With the exception

of *Enterococcus faecalis*, the bacterial strains were more sensitive to the ethanolic extract compared with the aqueous extract; and the minimum inhibitory concentration (MIC) values ranges from 3.12 to 25 mg/mL. Statistical analyses showed no significant correlation between bacterial abundance and extract concentration at various pH range. Conclusively, the aqueous and ethanolic extracts of the leaves of *Erigeron floribundus*, have secondary metabolites with antibacterial activity; therefore, justifying the use of *Erigeron floribundus*, as disinfectant and for the treatment of some water borne diseases.

KEYWORDS: Water, phytochemical screening, *Erigeron floribundus* extracts, antibacterial activity.

INTRODUCTION

Water is a natural resource of capital importance for the maintenance of life on earth. It is essential for man-kinds need, including his agricultural and industrial activities.^[1] In spite of its importance, it can become a source of diseases when its quality is altered due to pollution.^[2] Today, many infectious diseases suffered by humans are of water origin (gastro-enteritis, cholera, typhoid fever, ect.). These diseases are caused by various germs that can belong to the genera *Salmonella*, *Shigella*, *Escherichia*, *Vibrio* and *Campylobacter* among others.^[3] Several studies have shown the preponderant effect of pH variation on bacterial growth. Indeed, bacteria only grow in well-defined pH ranges.^[4] The growing demand for pure water, compels humans to resort to water sources of "doubtful" quality to satisfy their daily needs.^[5] For instance, in Cameroon and other developing countries, contaminated water are known to inhabit microbiological organisms such as gastro-enteritis, cholera and typhoid fever. Though, there are antibiotics such as penicillin, erythromycin, ampicillin and amoxicillin that have been used for several decades to disinfect and treat water borne diseases. There are increasingly losing their effectiveness due to their excessive and uncontrolled use. Due to this ineffectiveness, other remedial measures are required, including therapeutics from medicinal plants that are effective and accessible to all.^[6-7]

Erigeron floribundus is a species of the Asteraceae family. In Cameroon, as in other African countries, it is found throughout the forest zone, preferably along waterways and in forest galleries in the savannah.^[8] Ethnobotanical data reveals that this plant is used in traditional medicine in the treatment of conjunctivitis, angina, dyspepsia, gastralgia, toothache, headache, snake bite^[9]; gout, cystitis, female infertility, AIDS and various diseases of microbial and non-microbial origin.^[10-11] Based on its folk medicinal use in treating microbial diseases, this

study, was design to evaluate the disinfecting potential and antibacterial activities of the aqueous and ethanolic leaves extract of *Erigeron floribundus* on three important sanitary bacteria (*Escherichia coli*, *Staphylococcus aureus* and *Enterococcus feacalis*) with a variation of the pH medium.

MATERIAL AND METHODS

Bacterial strains

The used bacterial species: *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus feacalis* were isolated from well water in the city of Yaounde (Cameroon).

Phytochemical screening

Preparation of the different extracts

After harvesting and identification at the Cameroon National Herbarium (CNH), the leaves of *Erigeron floribundus* were cleaned, cut, and air-dried in the shade for three weeks. The dried leaves were pulverized to fine powder using a mechanical grinder. The first extract was obtained by homogenizing 280g of leaf powder with 3 liters of distilled water for 48 hours; and the second by mixing the same mass of powder in 3 liters of ethanol for 48 hours as well. The operation was repeated two (02) times with renewal of solvent every 48 hours. The different fractions obtained after filtration with wattman paper number 1 were oven dried at 45°C. The extraction yields (Rd) were calculated using the following formula:

$$Rd = \frac{\text{Obtained Mass extract}}{\text{Initial Mass of powder}} \times 100$$

Research of the main classes of secondary metabolites

The major groups of secondary metabolites present in the different extracts were highlighted by colorimetric and precipitation tests using the combined methods of Harborne (1973), N'guessan *et al.* (2009), Alilou *et al.* (2014) and Mbayo *et al.* (2015).^[12–15]

Determination of the antibacterial activity of extracts

Bacteriological analyses covered both qualitative and quantitative aspects. Antibacterial activities were evaluated by the surface spreading on agar medium, membrane filter technique and microdilution methods, respectively.

Preparation of culture media

The different culture media Mueller Hinton Broth (MHB), Plate Count Agar (PCA), Endo, Mannitol Salt Agar (MSA) and Bile Esculin Azide (BEA) were prepared according to the manufacturer's recommendations (Sigma-Aldrich).

Preparation of bacterial inocula

A few colonies of the different bacterial species preserved in 5 °C glycerol were defrosted at room temperature (23 ± 2 °C) for 30 minutes. 100 µL was taken and plated on plain agar (PCA) cast in Petri dish. A colony isolated after 24 hours of incubation at 37 °C was picked up with a sterile platinum loop and plated on plain agar poured downhill in test tubes. After 18 hours of incubation at 37 °C, bacterial suspensions were prepared by taking colonies from this pure culture, which were introduced into sterile physiological water until a turbidity corresponding to point 0.5 of Mc Farland's scale corresponding to the concentration of 1.5×10^8 CFU/mL that constituted the parent suspension.^[16] The stock suspension was diluted to an appropriate titer for evaluation of antibacterial activities.

Preparation of extract solutions

The extract solutions were prepared at concentrations of 2 mg/mL, 1 mg/mL, 0.5 mg/mL and 0.1 mg/mL from sterile distilled water. The prepared solutions were first filtered on hydrophilic cotton, then on sterile WHATMAN paper and finally through a 0.45 µm porosity membrane filter under absolute sterility conditions.^[17]

Determination of cell abundances of microorganisms as a function of extract concentration, incubation time and medium pH

Glass vials of 500 mL, each containing 100 mL of sterile distilled water were arranged in 3 sets of 4 vials each. The pH of the sterile distilled water in each series was adjusted using a pH meter, hydrochloric acid (HCl, 0.1M) and sodium hydroxide (NaOH, 0.1M).

- Series 1 vials (pH adjusted to 5) were coded as f1A, f1B, f1C and f1D.
- Series 2 vials (pH adjusted to 7) were coded as f2A, f2B, f2C and f2D.
- Series 3 vials (pH adjusted to 9) coded as f3A, f3B, f3C and f3D.

All the vials were sterilized by autoclaving at 121°C for 15 minutes. Then 0.5 mL of the bacterial suspension was introduced accordingly to the tested bacteria in each vial. This time is the initial time t_0 . After 0, 6 and 12 hours of incubation, 100 µL of sample was taken from each vial and plated onto the surface of specific agar culture media poured into Petri dishes,

labeled according to extract concentrations. After 18 to 24 hours of incubation of the plates, the viable and cultivable bacteria present on the surface of the specific agar culture media were counted. Cell abundances expressed as CFU/100 mL were obtained using the following formula.^[18]

$$\text{Abundance (N)} = \frac{\text{cell colony number}}{\text{water volume (mL)}} \times 100$$

Determination of the minimum inhibitory concentration (MIC) of the different extracts

The minimum inhibitory concentration (MIC) of the different extracts were determined by the liquid microdilution method using the M07-A9 protocol described by the *Clinical and Laboratory Standard Institute* (CLSI) with some modifications. This is the lowest concentration that will inhibit any visible growth of a microorganism after incubation at 37 °C for 18 to 24 hours.^[16]

In each microplate well (96 wells), a volume of 100 µL of Mueller Hinton broth was introduced. In the first wells of the series of three columns including 1, 2, 3; 5, 6, 7 and 9, 10, 11 were introduced a volume of 100 µL of the stock solution at a concentration of 400 mg/mL of the aqueous and ethanolic extract respectively. Successive serial dilutions of reason 2 resulted in a concentration range from 1.56 mg/mL to 100 mg/mL. A volume of 100 µL of inoculum (2×10^6 CFU/mL) was subsequently introduced into each well, for a final volume of 200 µL per well. Plates were covered and sealed with parafilm paper and incubated at 37 °C for 18 to 24 hours. The positive control was a reference antibiotic (Ampicillin) with a concentration range of 4 µg/mL to 256 µg/mL.

After incubation, the revelation of bacterial growth was done using iodonitrotetrazolium chloride (INT). For this purpose, 40 µL of the INT solution was introduced into each well of the microplate.^[19] The MICs were defined as the smallest concentrations for which no pink coloration was observed. The tests were repeated in triplicates.

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

In order to determine the MBC, a volume of 150 µL of culture broth was introduced into new plates, and the volume was completed to 200 µL by adding a volume of 50 µL of the content of the wells with a concentration greater than or equal to the MIC. These plates were then incubated for 24 hours at 37 °C followed by revelation with INT. All concentrations at which

we did not observe pink staining were taken as bactericidal and the lowest of these was scored as the MBC. The tests were performed in triplicates.

After determination of MICs and MBCs, MBC/MIC ratios were calculated.

Data analysis

Data from this study were represented by histograms in semi-logarithmic coordinates using Excel 2007 software. The degrees of relationship between variables were evaluated by Spearman's "r" correlation tests. Comparisons of abundance means were performed using the Kruskal-Wallis H test. All these analyses were performed using SPSS version 16.0 software.

RESULTS

Extraction yields

For the extractions, 280 g of powdered leaves in each case was extracted in 3L of either aqueous or ethanolic solvent. The different yields obtained are presented in Table I. It was observed that, the aqueous extraction yielded better than the ethanol extraction with a difference of 0.47%.

Table I: Extraction yields of the plant extracts.

Type of extract	Powder mass of the leaves of the plant (g)	Mass of the extract Dry (g)	Yield obtained (%)
Aqueous extract	280	35,9	12,82
Ethanolic extract	280	34,6	12,35

Phytochemical screening of aqueous and ethanolic extracts of *Erigeron floribundus* leaves

Table II shows the major groups of secondary metabolites present in the aqueous and ethanolic extracts of *Erigeron floribundus* leaves. Alkaloids, polyphenols, tannins and saponins are present in both extracts. Cardiac glycosides, resins, quinones and mucilages were identified in the ethanolic extract only.

Table II: Qualitative phytochemical composition of aqueous and ethanolic extracts of *Erigeron floribundus* leaves.

Phytochemical classes	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Polyphenols	+	+
Flavonoids	-	+
Cardiac glycosides	-	+
Resin	-	+

Tannins	+	+
Quinones	-	+
Mucilage	-	+
Saponins	+	+

Legend: (-) absent; (+) present

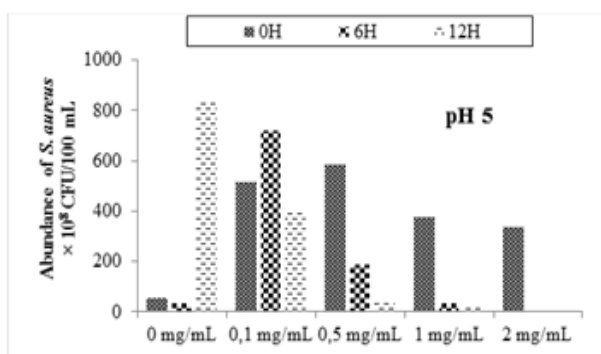
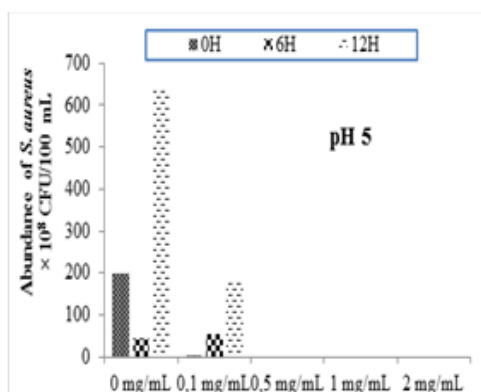
Temporal variation of bacterial densities as a function of different concentrations of *Erigeron floribundus* extracts and pH.

Effect of *Erigeron floribundus* on the survival of *S. aureus* as a function of different contact times at each pH.

In the presence of the different plant leaf extracts, bacterial abundances varied across the pH range (figure 1).

The abundances of *S. aureus* reached 176, 83, and 41 ($\times 10^8$ CFU/100mL) in the solutions containing the ethanolic extract of *Erigeron floribundus* leaves at pH 5, 7, 9, respectively. The lowest numbers were noted at pH 5 and concentrations 0.5 mg/mL, 1 mg/mL, and 2 mg/mL after 0, 6, and 12 hours of incubation. The highest value was recorded at pH 7 at concentration 0.1 mg/mL after 6 hours of incubation. In control solutions, *S. aureus* abundances ranged from 45 to 637 ($\times 10^8$ CFU/100mL).

In the presence of the aqueous extract of *Erigeron floribundus* leaves, the abundances of *S. aureus* reached 827, 662, and 837 ($\times 10^8$ CFU/100mL) at pH 5, 7, and 9, respectively. The lowest densities were observed at pH 5 and 9, at the concentration 2 mg/mL after 12 hours of incubation. The highest value was recorded at pH 9, at the concentration 0.1 mg/mL after 6 hours of incubation. In control solutions, *S. aureus* abundances ranged from 35 to 827 ($\times 10^8$ CFU/100mL).



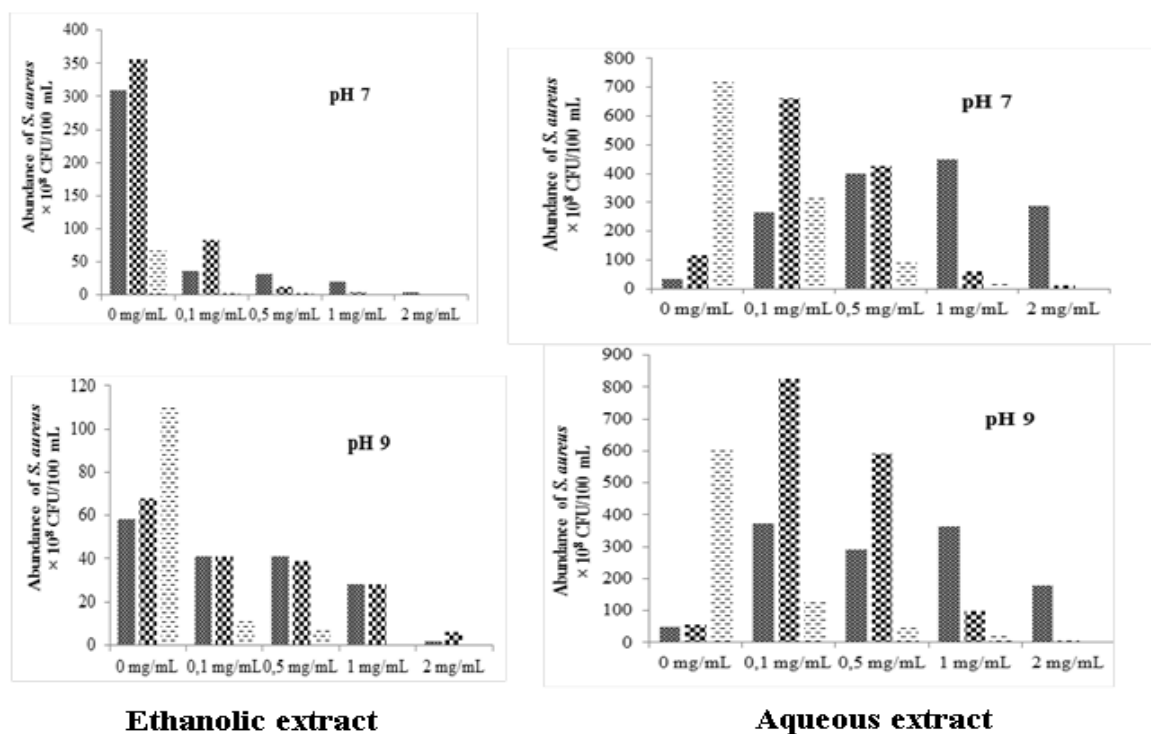


Figure 1: Temporal variation of *S. aureus* abundances in the presence of aqueous and ethanolic extracts at different pH levels.

Effect of *Erigeron floribundus* on *E. coli* survival as a function of different contact times at each pH

The abundances of *E. coli* reached 386, 268, and 517 ($\times 10^8$ CFU/100mL) in the solutions containing the ethanolic extract of *Erigeron floribundus* at pH 5, 7, 9, respectively. The lowest count was observed at pH 7, at the concentration 2 mg/mL after 12 hours of incubation. The highest value (517×10^8 CFU/100mL) was recorded at pH 9, at the concentration 0.1 mg/mL after 12 hours of incubation. In control solutions, *E. coli* abundances ranged from 35 to 836 ($\times 10^8$ CFU/100mL).

In solutions containing the ethanolic extract of *Erigeron floribundus*, the abundances of *E. coli* reached 662, 720, and 827 ($\times 10^8$ CFU/100mL) at pH 5, 7, 9, respectively. The lowest numbers were noted at pH 7 and 9, at the concentration 2 mg/mL after 12 hours of incubation. The highest value (827×10^8 CFU/100mL) was recorded at pH 9, at the concentration 0.1 mg/mL after 12 hours of incubation. In control solutions, *E. coli* abundances ranged from 35 to 837 ($\times 10^8$ CFU/100mL).

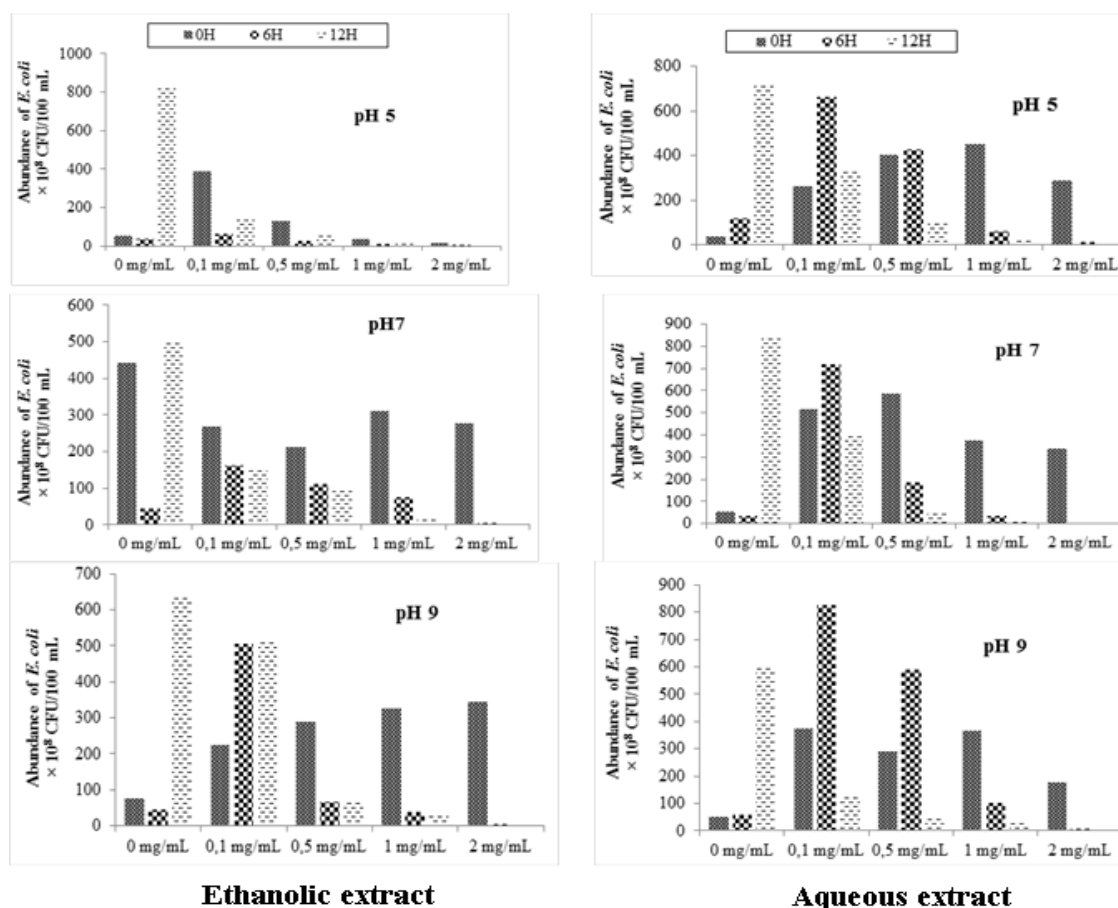


Figure 2: Temporal variation of *E. coli* abundances in the presence of aqueous and ethanolic extracts at different pH.

Effect of *Erigeron floribundus* on the survival of *E. faecalis* according to different contact times at each pH

In the solutions containing the ethanolic extract of *Erigeron floribundus*, the abundances of *E. faecalis* reached 1125, 594, and 781 ($\times 10^8$ CFU/100mL) at pH 5, 7, and 9, respectively. The lowest bacterial density was observed in the solution at pH7, at the concentration 2mg/mL after 12 hours of incubation. The highest bacterial density was recorded at pH 5, at the concentration 0.1 mg/mL after 6 hours of incubation. In control solutions, *E. faecalis* abundances ranged from 45 to 1697 ($\times 10^8$ CFU/100mL).

In solutions containing the aqueous extract, the abundances of *E. faecalis* reached 1124, 594, and 720 ($\times 10^8$ CFU/100mL) at pH 5, 7, 9, respectively. The lowest abundances were observed at pH 7 and 9, at the 2mg/mL concentration after 12 hours of incubation. The highest abundance was recorded at pH 5, at the concentration 0.1 mg/mL at the beginning of

the experiment. In control solutions, *E. faecalis* abundances ranged from 35 to 1245 ($\times 10^8$ CFU/100mL).

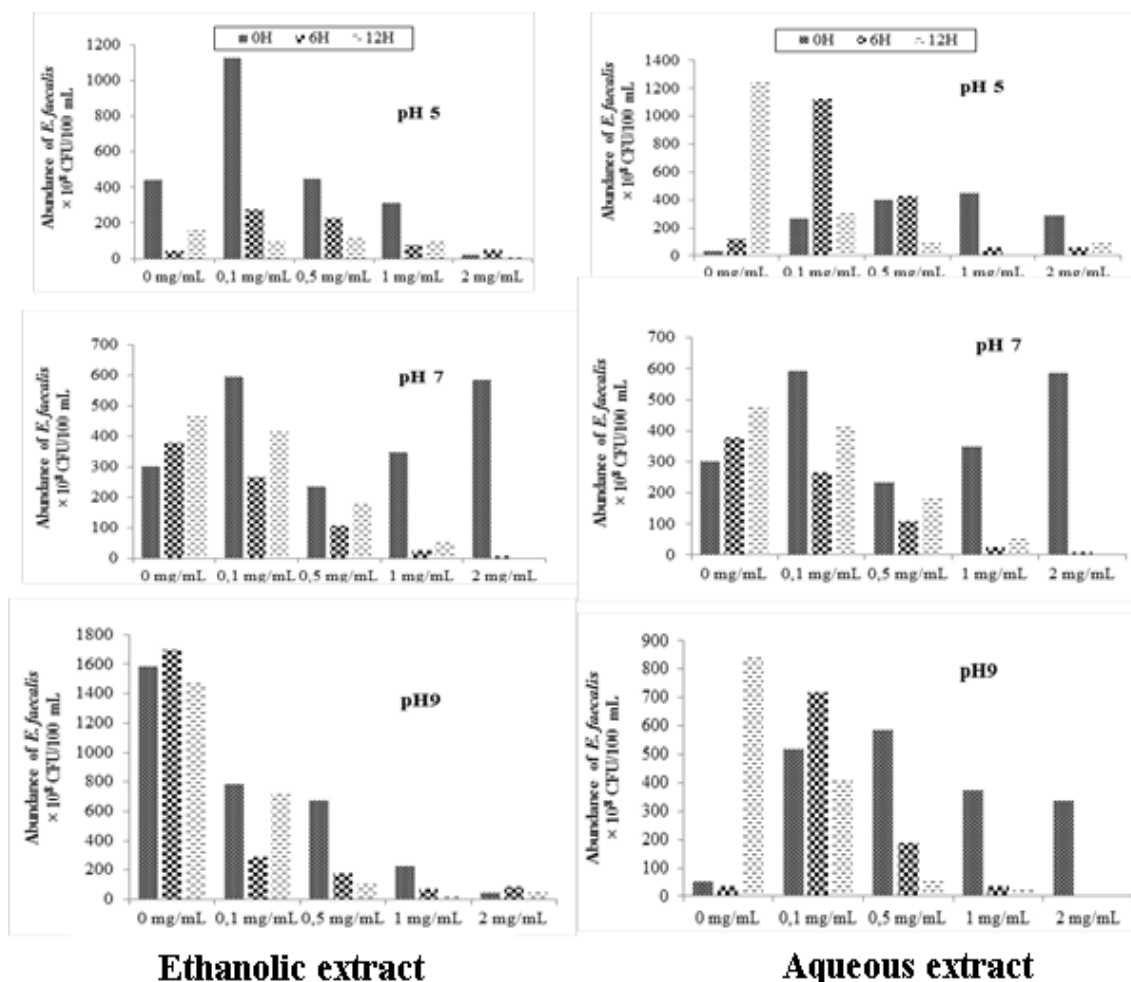


Figure 3: Temporal variation of *E. faecalis* abundances in the presence of aqueous and ethanolic extracts at different Ph.

Minimum Inhibitory Concentrations (MIC) of extracts

The two extracts considered showed antibacterial activity on the germs tested with MICs varying between 3.12 and 25 mg/mL. The ethanolic extract showed a better activity on *E. coli* with a MIC of 3.12 mg/mL. *Enterococcus faecalis* was insensitive to the different extracts tested. The minimum inhibitory concentrations of the aqueous and ethanolic extracts of *Erigeron floribundus* on the tested strains are presented in Table III.

Table III: Determination of Minimum Inhibitory Concentrations (MIC)

Bacteria tested	CMI		
	Aqueous extract (mg/mL)	Ethanollic extract (mg/mL)	Ampicillin (mg/mL)
<i>S. aureus</i>	25	12,5	- //-
<i>E. coli</i>	12,5	3,12	- //-
<i>E. feacalis</i>	- //-	- //-	- //-

- //- : no inhibition

Minimum Bactericidal Concentrations of Extracts (MBC)

The MBC of the extracts was determined and the MBC/MIC ratios were calculated. The MBC/MIC ratios ranged from 4 to 8 on the tested strains. Thus, the aqueous extract showed a bacteriostatic effect on *S. aureus*. The ethanollic extract showed the same effect on *S. aureus* and *E. coli*.

The results of the obtained MBCs and the calculated MBC/MIC ratios are presented in Table IV.

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

Bacteria tested	Aqueous extract (MBC)	MBC/MIC Report	Ethanollic extract (MBC)	MBC/MIC Report
<i>S. aureus</i>	ND	8	100 mg/mL	4
<i>E. coli</i>	100 mg/mL	ND	12.5 mg/mL	8
<i>E. feacalis</i>	ND	ND	ND	ND

ND : Not determined

Spearman's r correlation between bacterial abundances and the different variables

Spearman correlations were performed between bacterial abundances and extract concentrations at each incubation period. A negative and significant correlation ($P \leq 0.05$) was observed between *S. aureus* densities and ethanollic extract after 0, 6, and 12 hours of incubation. Similarly, negative and highly significant correlations at the $P < 0.001$ threshold were observed between ethanollic extract and *E. coli* abundances at all incubation times. However, no significant correlations were recorded between *Enterococcus feacalis* densities and the different extracts (Table V).

The variation of pH did not significantly influence the abundances of bacterial cells in the different extracts regardless of the incubation time.

Table V: Spearman correlations between bacterial abundances and extract concentrations at each incubation period.

Bacterial species and types of extracts		Incubation times (hours)		
		0	6	12
<i>S. aureus</i>	Aqueous	-0,239	0,215	-0,249
	Ethanollic	-0,439*	-0,430*	-0,439*
<i>E. coli</i>	Aqueous	-0,015	0,025	-0,567**
	Ethanollic	-0,560**	-0,570**	-0,540**
<i>E. feacalis</i>	Aqueous	-0,128	0,049	0,172
	Ethanollic	0,172	0,170	0,107

** : $P < 0,001$ * : $P \leq 0,05$ ddl: 29

Correlation between bacterial abundances and incubation times for each extract concentration

Correlations were recorded between cell abundances and incubation times in the presence of different concentrations of *Erigeron floribundus* extracts (Table VI).

Table VI: Correlation between bacterial abundances and extract concentrations.

Bacterial species and types of extracts		Plant extract concentrations (mg/mL)				
		0	0,1	0,5	1	2
<i>S. aureus</i>	Aqueous	-0,552*	0,001	-0,567*	-0,230	-0,269
	Ethanollic	0,767**	0,695**	0,771**	0,459	0,762**
<i>E. coli</i>	Aqueous	-0,342	0,512*	0,210	0,092	-0,184
	Ethanollic	-0,717**	0,717**	0,717**	0,718**	0,720**
<i>E. feacalis</i>	Aqueous	0,072	-0,211	-0,112	0,244	-0,079
	Ethanollic	-0,137	-0,274	-0,274	-0,594**	-0,360

** : $P < 0,001$ * : $P \leq 0,05$ ddl: 17

Comparison of the average bacterial densities according to the different parameters studied

Comparison of average bacterial densities in different extract concentrations at each incubation time

Comparison of the means of bacterial abundances in the different concentrations of *Erigeron floribundus* extracts at each incubation time using the Kruskal-Wallis H-test reveals a significant difference ($P \leq 0,05$) between the abundances of *S. aureus* in the control solutions at concentrations of 0.1, 0.5, and 2 mg/mL of the ethanollic extract. A significant difference ($P \leq 0,05$) was also observed between the mean densities of *E. coli* immersed in the 0.1 and 0.5 mg/mL aqueous extract solutions (Table VII).

Table VII: Comparison of the average bacterial abundances in the different extract concentrations at each incubation time.

Bacterial species and types of extracts		Extract concentrations (mg/L)				
		0	0,1	0,5	1	2
<i>S. aureus</i>	Aqueous	P=0,010*	P=0,822	P=0,070	P=0,235	P=0,097
	Ethanollic	P=0,003*	P=0,003*	P=0,003*	P=0,310	P=0,003*
<i>E. coli</i>	Aqueous	P=0,239	P=0,040*	P=0,008*	P=0,926	P=0,641
	Ethanollic	P=0,121	P=0,674	P=0,117	P=0,117	P=0,117
<i>E. feacalis</i>	Aqueous	P=0,906	P=0,674	P=0,653	P=0,501	P=0,502
	Ethanollic	P=0,332	P=0,526	P=0,344	P=0,048*	P=0,291

* :P≤0, 05

ddl: 17

Comparison of the average bacterial densities in the different types of *Erigeron floribundus* extract at each pH range

Considering each pH range, the mean abundances of bacterial cells exposed to aqueous extracts were compared with the cell abundances exposed to ethanollic extracts of *Erigeron floribundus* leaves using the Kruskal-Wallis H-test. The values of the coefficients of significance are presented in Table VIII. It is shown that the abundances of *S. aureus* exposed in the ethanollic extract at pH 7 and pH 9 differed significantly ($P \leq 0.05$) from one extract to the other. In contrast, no significant difference ($P > 0.05$) was recorded between the mean densities of *E. coli* and *E. feacalis* in the different types of extract regardless of pH (Table VIII).

Table VIII: Comparison of average bacterial abundances in different extract concentrations at each pH range.

Bacterial species and types of extracts		PH range		
		5	7	9
<i>S. aureus</i>	Aqueous	P=0,143	P=0,189	P=0,023*
	Ethanollic	P=0,708	P=0,019*	P=0,004*
<i>E. coli</i>	Aqueous	P=0,687	P=0,571	P=0,268
	Ethanollic	P=0,454	P=0,553	P=0,454
<i>E. feacalis</i>	Aqueous	P=0,678	P=0,222	P=0,107
	Ethanollic	P=0,644	P=0,914	P=0,914

*: P≤0, 05

ddl: 17

Comparison of the average bacterial abundances between the different types of extracts

The mean abundances of the considered bacterial cells exposed to aqueous and/or ethanollic extracts of *Erigeron floribundus* leaves were compared using the Kruskal-Wallis H-test. The values of the coefficients of significance are presented in Table IX. It was found that these

bacterial abundances differed significantly ($P \leq 0.05$) when immersed in the ethanolic extract compared to the aqueous extract.

Table IX: Comparison of the average bacterial abundances between the different types of extracts.

Types of extracts	Bacterial species		
	<i>S. aureus</i>	<i>E. coli</i>	<i>E. feacalis</i>
Ethanolic extract	0,001*	0,03*	0,05*

*: $P \leq 0, 05$

ddl: 17

DISCUSSION

The phytochemical screening of both extracts revealed in common the presence of alkaloids, saponins, polyphenols and tannins. Cardiac glycosides, flavonoids, resins, quinones and mucilages were only present in the ethanolic extract. These results are similar to those of Obonga *et al.* (2017)^[20] and Ganiyat *et al.* (2013).^[21] In previous studies, the methanolic and hexanolic extract of *Erigeron floribundus* leaves indicated the presence of quinones (Obonga *et al.*, 2017).^[20] The observed variation of secondary metabolites could be related to the diversity of soils, climate, solvent, extraction method and the degree of ripening of the plant at harvest. In this regard, Falleh *et al* (2008)^[22] and Podsdek (2007)^[23] noted that high temperature, solar exposure, maturity at harvest and storage conditions affect the biosynthesis of secondary metabolites.

A temporal variation of cell densities as a function of pH of aqueous and ethanolic extracts of *Erigeron floribundus* was observed. This variation is dependent not only on the concentration of the plant extract, but also on the pH and incubation time of the bacterial cells in the different extract solutions. The quantitative analysis of the bacterial densities in the control solutions, after each incubation duration and that of the bacterial cell densities after exposure in the extract solutions indicated that the variation of the cell densities would be related to the action of the extracts of *Erigeron floribundus* on the cultivability of these cells.

The present studies proved that, the aqueous extract at pH 7, and concentration of 0.1 mg/m, effectively inhibited the density of *E. coli* after 12 hours of incubation. In contrast, the abundance of *S. aureus* was significantly inhibited in the presence of 0.5 mg/mL of the aqueous extract at pH 5 after 12 hours of incubation. Both types of extract at the concentration of 2mg/mL effectively reduced *Enterococcus feacalis* abundances at pH 7 after

12 hours of incubation. The Kruskal Wallis comparison H-test shows that a significant difference ($P \leq 0.05$) exists between the means of *S. aureus* abundances in the ethanolic extract solutions at pH 7 and pH 9. On one hand, no significant difference ($P \leq 0.05$) was recorded between the mean densities of *E. coli* and *E. feacalis* with the different types of *Erigeron floribundus* extract. Indeed, the pH of the medium modifies the surface charge of the microorganisms by shifting the ionization equilibrium of mineral or organic nutrient compounds. The importance of which will depend on the tolerance of the bacteria to the acidity of the medium.^[24] On the other hand, the activity of membrane proteins and enzymes involved in the mechanisms of assimilation and cellular synthesis is influenced by the pH of the medium. Most proteins are active only in a narrow pH range, usually between 5 and 9.^[25] However, microorganisms can themselves cause changes in the pH of a medium by producing acidic or basic metabolic waste products such as organic acids produced by organisms fermenting from carbohydrates.^[15] Alkalinization of the medium is often due to the production of ammonia after the degradation of amino acids.^[13] In general, acidic products are metabolized in alkaline media whereas, in acidic media, neutral products are secreted by bacteria.^[26]

The different extracts from the leaves of the tested plant showed variable antibacterial activities on the different bacterial isolates studied. The microdilution method on Muller Hinton medium shows us that *E. feacalis* was insensitive to the different extracts. The ethanolic extract and the aqueous extract inhibited the majority of *E.coli*. However, with a MIC value of 3.12 mg/ml, the ethanolic extract inhibited the visible growth of *S. Aureus*. This could be explained by the differences due to the constitution of their cell envelope and the mode of action of the antibacterial agents.^[27]

The results indicate that the aqueous and ethanolic extracts of *Erigeron floribundus* have antibacterial effect in the aquatic environment. Similar data were reported by Sunda *et al* (2008)^[28] and Tamsa Arfao *et al* (2018)^[29] who showed that aqueous extracts of *Lantana camara*, *Cymbogoncitratus*, *Hibiscus rosasinensis* and *Eucalyptus microcorys* have bactericidal effect in the aquatic environment. Also, Lutgen and Michels (2008)^[30] showed that by adding *Artemisia annua* tea to water contaminated with bacterial cells, a considerable reduction of the bacterio-contaminant load was observed at levels lower than those obtained after boiling water.

CONCLUSION AND PERSPECTIVES

This study showed a significant effect of bacterial inhibition of aqueous and ethanolic extracts of *Erigeron floribundus* in aquatic microcosm. The different extracts showed variable activities on the tested bacterial strains. The cell abundances decreased progressively with pH change, and increased in the concentration of the extracts and the bacterial-extract contact time. In all, the ethanolic extract was found to be more effective than the aqueous extract. This study therefore indicates that, plant extracts could be used to disinfect water.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are included in the paper.

Competing interests

The authors declare that they have no competing interest.

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Authors' information (optional)

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