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# Synergistic effect of light, pH and *Artemisia annua* extract on *Enterococcus faecalis* in aquatic microcosms

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## ABSTRACT

**Synergistic effect of abiotic and biotic factors is not completely established in the process of disinfecting water by plant extracts. The present study aims to evaluate the synergistic effect of different pH values (4, 5, 6, 7, 8 and 9) and different infusion concentrations (10, 20 and 30%) of *Artemisia annua* on *Enterococcus faecalis* growth in aquatic microcosm under dark and lighting conditions. The results showed that under dark condition, there was a relative increase in abundance of *E. faecalis* cells in the presence of *A. annua* extract. Under lighting, there is a decrease in the abundance of *E. faecalis* at different infusion concentrations of *A. annua*. Extract of *A. annua* seems to favour the growth of *E. faecalis* cells in the dark, especially at slightly acidic pH. This growth was relatively significant at pH 6. In the presence of light, *A. annua* extract inhibited the growth of *E. faecalis* cells and this varied from one extract sample to another; and was significant at slightly basic pH. Under the lighting, different pH values do not appear to have significant influence on the inhibition of bacterial growth except for pH 8. Monoterpenes, sesquiterpenes and triterpenes molecules present in infusions could be involved in chemical reactions responsible for cellular inhibition under light condition. Research on photosensitizing compounds and a comparative study with conventional photosensitizers might attest for the involvement of photosensitivity reaction with the *A. annua* extracts.**

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## INTRODUCTION

*Enterococci*, which are commensal bacteria of the gastro intestinal and genital urinary tracts of man and warm blooded animals are found in the environment, including

land and water, where they are regarded as the cause of faecal contamination (Holt et al., 2000). They can become pathogenic in immuno-depressed individuals, in whom they cause opportunistic bacteremia, endocarditis, infections of the urinary tract and surgical wounds (Gilmore, 2002). They are generally opportunistic pathogens. The incidence of nosocomial enterococci-

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linked infections has increased rapidly over the last 20 years in many parts of the world, due to their resistance to antibiotics (Oteo et al., 2007; Top et al., 2008).

The evolution of virulence and antibiotic resistance in enterococci are sometimes the result of the massive and uncontrolled use of antibiotics both in medicine and in the food industry on one hand, and the ability of enterococci to exchange and integrate the genetic material on the other hand (Aguilar-Galvez et al., 2012). These micro-organisms have relatively high potentials to stand with various environmental conditions and can survive in almost all environments, including soil, plants, water and food (Van den Bogaard and Stobberingh, 2000; Teixeira et al., 2007). They are used as indicators of faecal contamination of aquatic environments and as key indicators to assess the effectiveness of water treatment (OMS, 2000).

Statistics show that over 80% of African and Asian households use medicinal plants to treat themselves. Hundreds of plant species shall be used for therapeutic purposes by the indigenous population (Weathers et al., 2014). In recent years, many studies focused on disinfection of water with plants extracts (Sunda Makuba et al., 2008; Sunda Makuba, 2012; Tamsa Arfao et al., 2013). These studies revealed that aqueous extract of *Lantana camara*, *Cymbogon citratus* and *Hibiscus rosa-sinensis* have a bactericidal effect in aquatic medium (Sunda Makuba et al., 2008). In addition, *Eucalyptus microcorys* aqueous leaves extract acts against planktonic bacterial metabolism (Tamsa Arfao et al., 2013). Other studies assessed antimicrobial potential of several plant species extracts including plants of the genus *Artemisia*.

*Artemisia* is one of the diverse genera of Asteraceae family with many important medicinally valuable essential oils and secondary metabolites (Bhakuni et al., 2001; Lopes-Lutz et al., 2008). The results of clinical trials conducted *in vitro* or in laboratory on animals, and epidemiological studies carried out, among others, in Cameroon, Senegal, Uganda, using infusions of *Artemisia annua* seem to confirm the curative potential of this mode of treatment (Weathers et al., 2014). When we add an infusion of *A. annua* to well water soiled on the bacteriological plan, we observe a very strong reduction of the bacterial load to the faecal coliforms. However, the faecal streptococci, after a significant reduction, proliferate again after 24 h of contact time (Allahdin et al., 2008).

The aquatic environments in which bacteria live consist of a set of parameters that characterize them. They consist of both physicochemical and biological factors. Factors such as nutrient concentration, temperature and pH are among those whose fluctuations have a significant impact on bacterial growth in aquatic medium (Wiebe et al., 1992). Although many studies have been focused to the exposure of bacteria to plant extracts, and

light condition often leads to inactivation of the latter at varying degrees, there is little information on the combined effect of these factors on the survival of *Enterococcus faecalis* in aquatic environments. The present study aims at evaluating under dark and light conditions, the synergistic effect of light, pH and *A. annua* extract on *E. faecalis* cells growth in aquatic microcosms.

## MATERIALS AND METHODS

### Collection and identification of the bacterial strain

*E. faecalis* was provided by the Centre Pasteur of Cameroon (Central Africa). These cells are anaerobic facultative, Gram-positive and generally have white colonies on Plate Count Agar medium (PCA). They are oxidase positive, generally catalase negative and quickly reduce triphenyl tetrazolium chloride (TTC). *E. faecalis* cells were highlighted on Bile-Esculin Azide (BEA). On this medium, colonies are black and surrounded by a clear halo; the black color of the colonies reflects the production of H<sub>2</sub>S and the hydrolysis of esculin that binds with iron (Holt et al., 2000). After biochemical identification, cells were collected by centrifugation (3600 rpm for 15 min at 10°C) and washed twice with sterile NaCl (8.5 g/l) solution. *E. faecalis* cells were then stored in glycerol at -70°C before use to avoid excessive sub-culture.

### Collection and preparation of extracts

Leaves of *A. annua* were harvested from Bangangté (Western Region, Cameroon). This town lies between longitudes 10° and 11° west meridian and latitudes 5° and 16° north. Its climate is tropical-humid with two main seasons: A dry season from October to March and a rainy season that runs from end of March to October. The topography of the area is mountainous, with plains and plateau with height between 1000 and 1500 meters. Temperatures range from 15 to 27°C with peaks in some areas up to 37°C. The nights are generally cool, especially between July and October. The soils of this Region of Cameroon are mostly lateritic, clay and volcanic in certain parts (PNUD, 2010).

The leaves were harvested in July 2013 and then dried in the laboratory at room temperature (23±2°C) for 1 month. After drying, the leaves were ground into powder and used to prepare different infusions (Mobili et al., 2013; Tamsa et al., 2013). The extraction of natural products can be made by sophisticated methods or by conventional methods such as maceration, infusion, decoction among others (Chougouo Kengne, 2010). The infusion method has been used for this study. Preparation of *A. annua* leaves as an infusion or tisane

being secular use in Chinese traditional medicine (Onimus et al., 2011). Thus 10, 20 and 30 g powder of *A. annua* were used to prepare infusions  $I_1$ ,  $I_2$  and  $I_3$ , respectively. For preparation, 1 liter of boiling distilled water was added to the plant material. The mixture was stirred briefly and the container covered for 15 min. The plant material was removed by filtration and the infusion was cooled at room temperature (Mueller et al., 2004; R  th et al., 2004).

### Experimental protocol

One set of 14 Erlenmeyer flasks, each containing 250 ml of NaCl solution (8.5 g/l), were used. They were first grouped into two subsets, A and B with each subset containing 7 Erlenmeyer flasks labeled  $F_1$ ,  $F_2$ ,  $F_3$ ,  $F_4$ ,  $F_5$ ,  $F_6$  and  $F_7$ . For each subset, values of pH in flasks  $F_1$ ,  $F_2$ ,  $F_3$ ,  $F_4$ ,  $F_5$  and  $F_6$  were adjusted to 4, 5, 6, 7, 8 and 9, respectively with HCl (0.1 M) and NaOH (0.1 M). The Erlenmeyer flask labeled  $F_7$  containing only NaCl solution (8.5 g/l) was used as Controls. The whole was then autoclaved.

Prior to the experiment, a frozen vial containing *E. faecalis* strains was defrosted at room temperature. The culture (100  $\mu$ l) was then transferred into 10 ml of nutrient broth (Oxford) and incubated at 37°C for 24 h and the cells latter collected by centrifugation at 3600 rev/min for 15 min at 10°C, then washed twice with sterile NaCl solution. After dilution, 100  $\mu$ l of sediment was added to 100 ml of physiological solution and then placed in each flask containing 250 ml of sterile NaCl solution. Initially, infusion  $I_2$  (20 g of plant extract) was chosen because of its similarity with the infusion dosage of *A. annua* commonly used (Allahdin et al., 2008; Onimus et al., 2011). In each contaminated solution, was added 20 ml of infusion  $I_2$  that is a mixture of 8%. After mixing, the flasks of subset A were each covered with aluminum foil and placed in the dark while those of subset B were placed under lighting. The electrical device used consisted of three incandescent bulbs of 100 Watts power. The bulbs were connected in series and placed under each solution (Nola et al., 2010). The measured light intensity reaching the solutions was 5900 lx.

Secondly, one set of 12 Erlenmeyer flasks were divided into 4 subsets A, B, C and D. In each flask, the pH of the solution was adjusted to 7 with HCl and NaOH. A bacterial suspension of known concentration was added to each solution. Then infusions  $I_1$ ,  $I_2$  and  $I_3$  of *A. annua* extract were used for the subsets A, B and C respectively. These infusions were added in volumes of 100, 200 and 300 ml for 1 liter of bacteriological contaminated water. This provided infusion proportions of 10, 20 or 30% in the mixture. No infusion was added to the D subset, which was regarded as the control solution. The preparations were placed under lighting, with the

same device as described above.

### Incubation of bacterial suspensions and analysis

The flasks were incubated for 6 h with the different pH values and 24 h after with the different infusions concentrations of *A. annua*, bacteriological analysis for each flask was carried out every 2 h. The content of each flask was first homogenized and then 1 ml was collected and analyzed. Petri dishes were incubated for 24 h at 37°C (Diagnostic Pasteur, 1987). The number of colony forming units (CFU) were then determined.

### Qualitative phytochemical screening

To identify the phytochemical derivatives in the extracts, standard phytochemical screening was performed (Harborne, 1973; Trease and Evans, 1983). Alkaloids test was performed by Dragendorff's and Meyer's tests, amino acids by ninhydrin, carbohydrates by Barfoed's and Fehling tests, flavonoids by  $FeCl_3$ , saponin by frothing test, tannins by  $FeCl_3$  and lead acetate and terpenoids by Salkowski test (Sofowora, 1993; Krishnaiah et al., 2009).

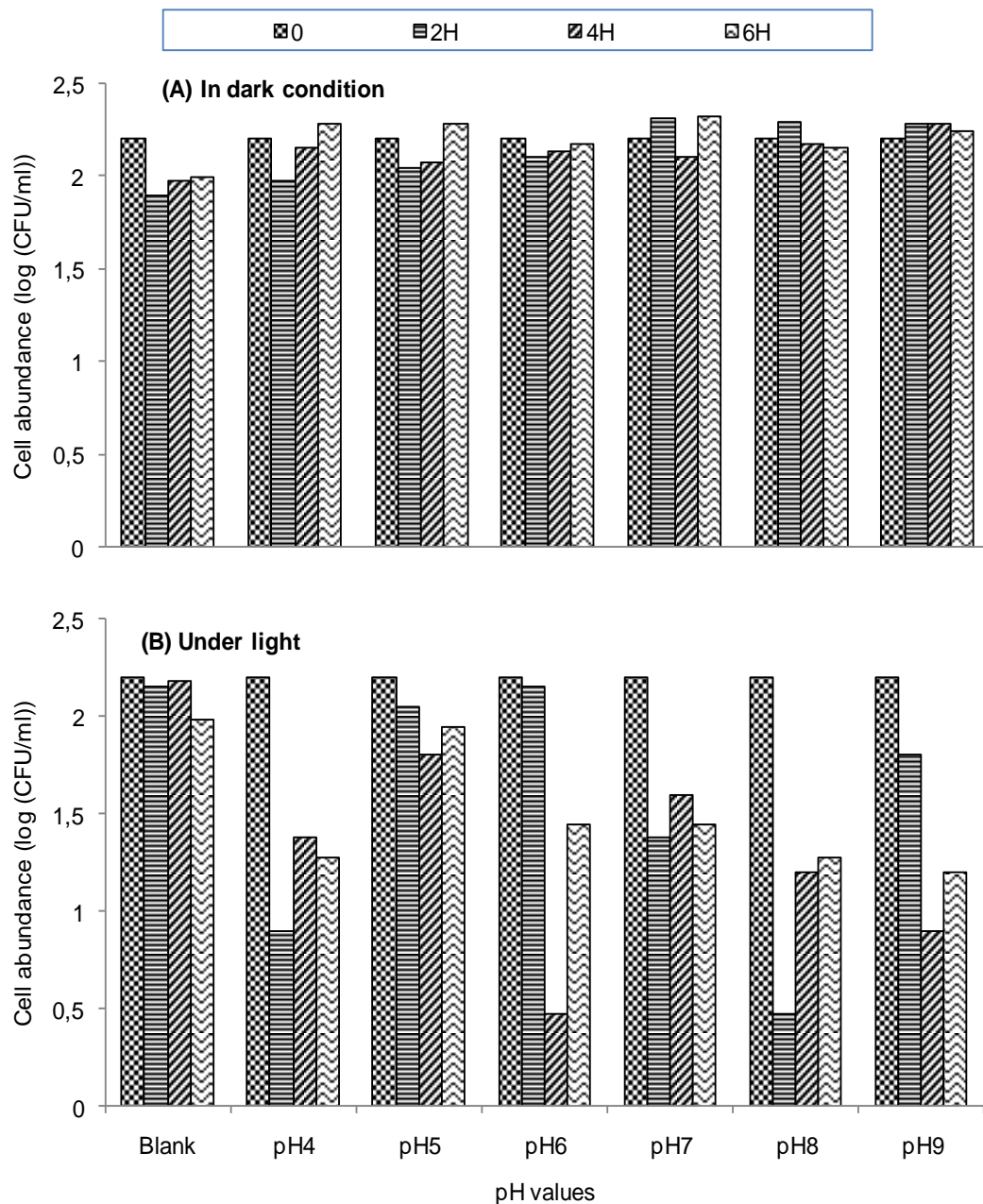
### Data analysis

The mean abundance of *E. faecalis* cells observed in each experimental condition was counted and illustrated by histograms. To estimate hourly evolution rate, a straight regression line was calculated for each pH depending on the duration of exposure to the extract of *A. annua*. A regression line has an equation of the form:

$$y = ax + b$$

where,  $x$  is the explanatory variable and  $y$  is the dependent variable,  $a$  is the slope of the regression line, and  $b$  is the intercept point of the regression line on the  $y$  axis (the value of  $y$  when  $x = 0$ ) (Bailey, 1981; Tofallis, 2009).

The slope of each regression line was considered as the hourly evolution rate. When this value was positive, it indicated cellular growth and when negative, it indicated inhibition of cell growth. The degrees of relations between the abundance of *E. faecalis* and incubation durations for each experimental condition were determined using Spearman " $r$ " correlation test. Data processing was performed using the statistical software package SPSS 12.0. A  $p$  value of 0.05 was assumed to be statistically significant.



**Figure 1.** Temporal variation of *E. faecalis* cells abundance in the presence of *A. annua* extract at different pH under dark (A) and light conditions (B).

## RESULTS

### Temporal evolution of bacterial abundance

The cells abundance of bacteria varied considerably depending with the presence and absence of light on one hand, and on the other hand with the concentration of *A. annua* extract in the aquatic medium.

In the dark condition, there was a slight increase in the

number of *E. faecalis* cells in solutions of pH 4 to 9 in the presence of *A. annua* extract. The highest cell density was obtained at pH 7 after 2 h incubation with cells abundance fluctuated between 2.20 and 2.32 units (log (CFU/ml)). However, in the Control solutions, there was a progressive decrease of the *E. faecalis* cells abundance (Figure 1).

In the presence of light and the extract of *A. annua*, cells abundance were reduced in all the solutions of

different pH values, compared to the initial cell abundance {2.20 units [log (CFU/ml)] after 2 h incubation. The lowest cells abundance recorded were 0.48 unit [log (CFU/ml)] with pH 6 and 8 after 2 h. However, after 6 h of contact between the extract of *A. annua* and *E. faecalis* cells, there was a slight increase of cell abundance in solutions with pH 5, 6, 8 and 9 (Figure 1).

Considering the various infusions, there was a decrease in the number of *E. faecalis* cells, oscillating from one sample to another in the presence of light. The inoculation of 10, 20 and 30% infusion I<sub>1</sub> concentrations lead to a decrease of bacterial abundance from 2.20 to 1.68 units [log (CFU/ml)] after 2 h incubation. After 4 h, there was a slight proliferation of *E. faecalis* cells (Figure 2). For infusion I<sub>2</sub>, there was a decrease in the number of cells after 2 h. This decrease fluctuated with the concentration of *A. annua* introduced in different solutions. The lowest cell abundance was obtained after 4 h. These values ranged from 1.45 to 0.95 unit [log (UFC/ml)] for extract proportions of 20 and 30%, respectively (Figure 2). In the case of infusion I<sub>3</sub>, there was a relative decrease in bacterial abundances after 6 and 24 h exposure. The lowest values were recorded after 24 h for extract proportions of 10 and 20% respectively (Figure 2). It was found that to have a significant inactivation of *E. faecalis* increased with the mass of *A. annua* leaves introduced in the various infusions in general.

### Kinetic evolution of cell abundance

Hourly *E. faecalis* cells rates were evaluated in each experimental condition. Under dark condition, the cells growth speeds observed were 3, 4, 5 and 7 cells/h. These speeds were recorded respectively in solutions of pH 9, 7, 5 and 4. However, inhibition speeds of 1, 5 and 8 cell/h were registered respectively with pH 6, 8 and in the control solution (Table 1).

In the presence of light, the metabolism of *E. faecalis* cells was inhibited in all solutions for pH considered. Inhibition speeds ranging from 13 to 27 cells/h were recorded in solutions containing the extract of *A. annua*. The cell growth speed in the control solution was 9 cells/h (Table 1).

In the case of infusion I<sub>1</sub>, relatively slow growth of bacterial cells oscillating between 1 and 2 cells/h was recorded. In the solutions containing infusions I<sub>2</sub> and I<sub>3</sub>, at various concentrations, inhibition speeds fluctuated between 20 and 21 cells/h in the first case, and between 3 and 15 cells/h in the second case. In the Control solutions, these values ranged from 2 to 6 cells/h (Table 2). It was generally found that the highest inhibition speeds were obtained with infusion I<sub>2</sub>.

### Correlation between cells abundance and incubation duration at each pH in the presence of *A. annua* extract in each experimental condition

Spearman "r" correlation coefficients between the abundance of *E. faecalis* and incubation duration, at each pH value in the presence of *A. annua* in the dark and the light conditions were assessed and presented in Table 3. Under dark condition, it was noted that only pH 6 was significantly correlated ( $P < 0.01$ ) to the abundance of *E. faecalis* during the incubation duration (Table 3). Under the lighting, different pH values do not appear to have any significant influence on the inhibition of bacterial growth ( $P > 0.05$ ) except for pH 8 ( $P \leq 0.01$ ). The combined action of *A. annua* extract and light was influenced by slightly basic pH.

### Correlation between cells abundance and incubation durations at each concentration of *A. annua* for different infusions and under lighting

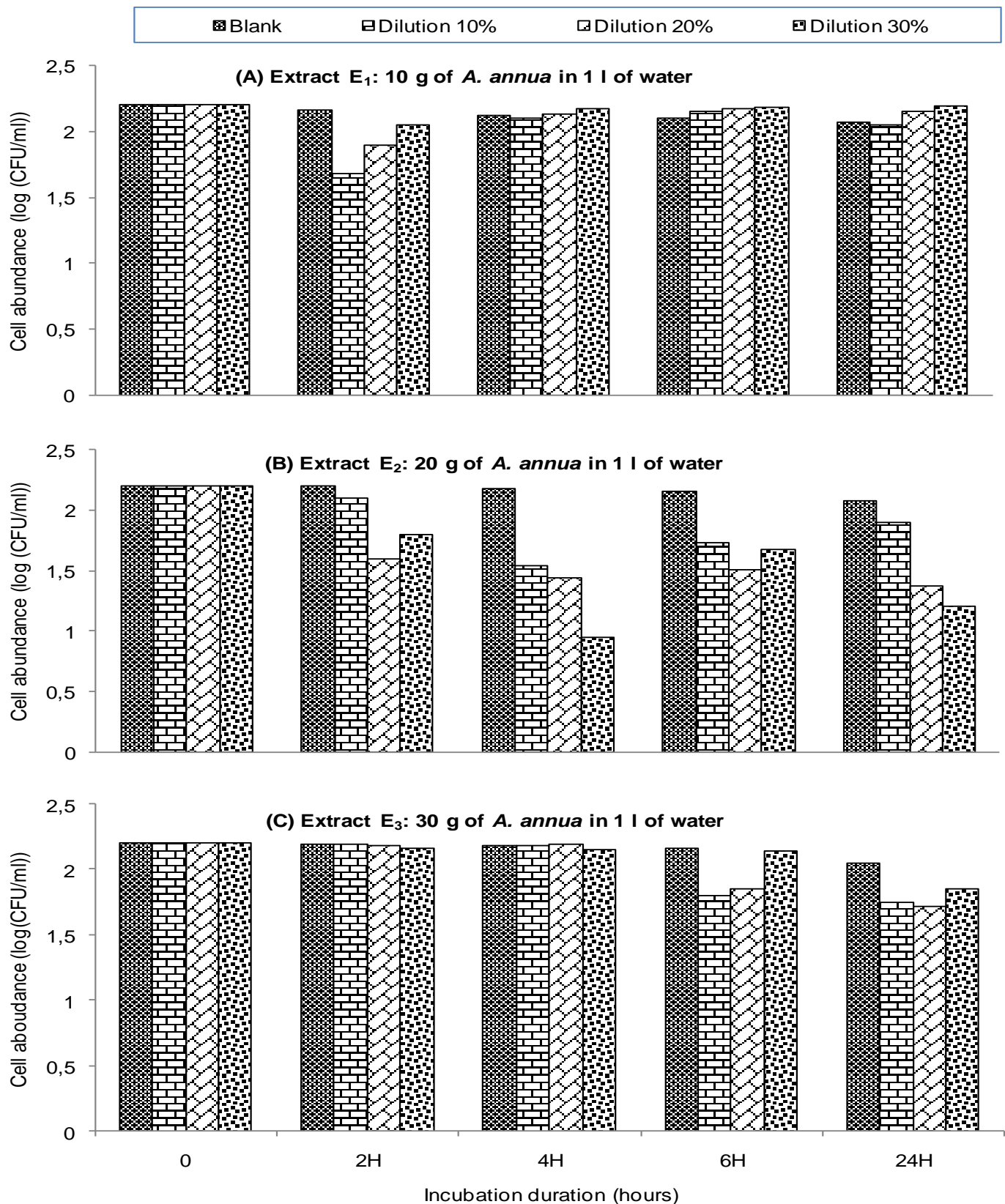
Spearman "r" correlation coefficients between the concentration of the extract and the abundance of *E. faecalis* cells, were also assessed (Table 4). It was observed that addition of infusion I<sub>1</sub> caused a significant reduction ( $P \leq 0.01$ ) in the number of *E. faecalis* cultivable cells for a volume of 10% after 2 h incubation. A significant inhibition ( $P \leq 0.01$ ) was also observed with infusion I<sub>2</sub> for mixtures with 10% and 20% of extract, after 2 h incubation. As for infusion I<sub>3</sub>, the inhibitory effect observed was not significant ( $P > 0.05$ ).

### Comparison of the cells abundance amongst different infusions

For each infusion used on the cells metabolism, the mean abundance of *E. faecalis* cells was calculated for each incubation period. The mean abundances noted from the three infusions concentrations used were compared using the H test of Kruskal-Wallis (Table 5). A significant difference ( $P < 0.05$ ) amongst the mean abundance of *E. faecalis* cells exposed to different infusions of *A. annua*, for the proportions 20 and 30% were observed. However, there was no significant difference amongst the effects of different infusions of 10%.

## DISCUSSION

In this study, the synergistic effect of infusion of *A. annua* and different pH values on *E. faecalis* cells growth under dark and light conditions was evaluated. The main results showed that bacterial abundance increased under



**Figure 2.** Temporal variation under light of the *E. faecalis* cell abundance at different extract (infusion) dilutions obtained from 10 g (A), 20 g (B) and 30 g (C) of *A. annua*.

**Table 1.** Hourly evolution rate of *E. faecalis* abundance (and regression coefficient) in the presence of *A. annua* extract, under light and dark conditions, for each pH value.

Experimental condition	Hourly evolution rate of cell abundance (cells/h) at each pH value						
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	Blank
Dark condition	7(0.216)	5(0.130)	-1(0.020)	4(0.055)	-5(0.250)	3(0.163)	-8(0.375)
Light condition	-20(0.535)	-13(0.691)	-27(0.756)	-19(0.569)	-21(0.511)	-24(0.813)	-9(0.682)

**Table 2.** Hourly evolution rate of *E. faecalis* abundance (and regression coefficient) under light condition, in different dilution solutions of each infusion considered at pH 7.

<i>Artemisia annua</i> extracts	Hourly evolution rate of cell abundance (cells/h)			
	10% dilution	20% dilution	30% dilution	Blank
Infusion I <sub>1</sub>	2(0.006)	2(0.013)	1(0.021)	-6(0.973)
Infusion I <sub>2</sub>	-21(0.797)	-20(0.647)	-20(0.619)	-3(0.940)
Infusion I <sub>3</sub>	-15(0.659)	-13(0.634)	-3(0.883)	-2(0.996)

**Table 3.** Spearman «r» correlation coefficient between abundance of *E. faecalis* cells and incubation duration at each pH value, in the *A. annua* extract solution and under light and dark conditions.

Experimental condition	Different pH values					
	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
Dark condition	0.800	0.800	1.000**	0.800	-0.105	-0.258
Light condition	0.800	-0.400	-0.200	0.800	1.000**	0.400

\*\*: P≤0.01 df=3

**Table 4.** Spearman «r» correlation coefficient between incubation duration and abundance of *E. faecalis* cells in different dilution solutions of each infusion considered under light conditions.

<i>Artemisia annua</i> extracts	Different dilutions of each infusion		
	10% dilution	20% dilution	30% dilution
Infusion I <sub>1</sub>	1.000**	-0.200	0.800
Infusion I <sub>2</sub>	1.000**	1.000**	0.800
Infusion I <sub>3</sub>	-0.600	0.800	1.000

\*\*: P≤0.01 df=3

**Table 5.** Comparison of the mean abundance of *E. faecalis* amongst the different dilutions when considering all the *A. annua* infusions and under light condition.

Bacterium strain	Different dilutions of each infusion		
	10% dilution	20% dilution	30% dilution
<i>E. faecalis</i>	P= 0.402	P= 0.024*	P= 0.015*

\*P≤0.05; Total number of replicates considered = 12; df=10



dark condition and decreased under light condition. Under dark condition, there was a relative increase in abundance of *E. faecalis* cells in the presence of *A. annua* extract. This increase was significant ( $P < 0.01$ ) in solutions at pH 6. The pH value favorable for the maximum growth of *E. faecalis* under adequate culture conditions is around 7.6. However, like all enterococci, it can grow in a pH range from 4.4 to 9.6 (Meyer et al., 1991). To ensure their growth, bacteria are bound to find in their environment the essential nutrients necessary for their energy and cellular synthesis (Rubio, 2002). The energy needs of enterococci are provided by the breakdown of glucose by the Embden-Meyerhof homo-fermentative route with the final product being lactic acid. They are able to metabolize a variety of sugars (Schleifer and Klipper-Balz, 1984; Le Blanc, 2006). Cell growth requires nutrient intake. This contribution depends on the availability of these in the environment and their ingestion by cells (Lontsi Djimeli et al., 2013). It has been shown that the extract of *A. annua* can serve as a source of carbon and energy for the growth of bacteria in aquatic environment (Mobili et al., 2013). It is known that the transport of nutrients across the plasma membrane is done either by simple or facilitated diffusion or by active transport. In the case of the simple diffusion, the transport mechanism is influenced by temperature and pH, because the movement is due to a concentration gradient. Levels of hydroxide and hydronium ions influence the assimilation of the different minerals or organic substrates by microorganisms. Enzyme activity canal was greatly influenced by the pH of the medium (Lacasse, 2004).

Under light condition, there is a decrease in the abundance of *E. faecalis* for different infusions of *A. annua*. At high concentration of the extracts leading to relatively high inhibition rates (Table 2). A gradual decrease in the abundance of cultivable cells observed during the exposure period, under each experimental condition, indicating a gradual increase in cell inhibition. It is known that light can directly act on bacteria via endogenous photosensitizers, localized in the cell or through photosensitive substances present in the medium (Fujioka et al., 1981).

In general, bacteria have photosensitive sites ( $P$ ) which are converted to reactive forms ( $P^*$ ) under light condition. These activated forms ( $P^*$ ) convert an oxygen molecule to an oxygen singlet ( $^1O_2$ ), which is a powerful oxidant capable of destroying cells. This toxicity is due to superoxide radicals such as hydrogen peroxide and hydroxyl radicals which are produced during oxidation (Dahl et al., 1987; Cooper et al., 1988). However, it was proven that the luminous irradiation of *E. faecalis* only does not have an effect and that its exposure to classical photosensitizers after exposure to the light significantly reduced its viability (Pileggi, 2013). This inhibition can be explained by the effect of photosensitive compounds

present in the medium.

These reactions could be explained by the effect of photosensitive compounds present in the medium. The experiments were performed with bacteria in sea waters and of river behind this hypothesis. In fact, Barcina et al. (1990) obtained an evolution of *E. coli* and *E. faecalis* towards a viable non-cultivable state during their exposure to visible light in seawater or natural fresh waters. Visible light would act on bacteria via photosensitizers present in the medium (Cooper and Zita, 1983). Inactivation of Fecal Coliforms and Enterococci in a river is the most important in day as well as the night (Sinton et al., 2002).

For the substances in the test medium from extracts of *A. annua*, chemical screening performed revealed the presence of alkaloids, triterpenes and sterols, tannins, anthocyanins and reducing compounds in extracts of *A. annua*. These results are consistent with those of some authors who have shown that the major components of *A. annua* are terpenes, flavones, sterols and coumarins (Sanner, 2008). The most of these bioactive molecules have antimicrobial potential. Flavonoids are attributed antibacterial, antiparasitic-properties, etc. (Sohn et al., 2004; Ganapaty et al., 2008). Terpenes that derived from *Artemisia* are mostly monoterpenes, sesquiterpenes and triterpenes (Verdian-rizi et al., 2008). Several studies have revealed the presence of artemisinin in *A. annua* infusions for varied concentration with respect to the amount of leaves used (Mueller et al., 2004; R  th et al., 2004). Artemisinin is a sesquiterpene lactone with an unstable endoperoxide bridge (Jansen and Soomro, 2007). The effectiveness of artemisinin against *E. coli*, *Streptococcus faecalis* and *Salmonella abony* was demonstrated by Vikas et al. (2000) and Chougouo Kengne et al. (2013). These compounds may induce a photosensitive reaction capable of inhibiting cell growth of *E. faecalis* because in the absence of light, no significant inhibition was observed at the course of the experiments with extracts of *A. annua*.

Similar results have been obtained by several authors. Some plants used in traditional medicine to treat microbial infections and comprising photosensitive molecules have been reported (Towers, 1985; Ongoka et al., 2007). In the presence of light, these molecules can initiate a photosensitive reaction. The photodynamic activity established was attributed to the presence of psoralen or furocoumarins for some plants and others to quinones and anthraquinones (Naganuna et al., 1985; Sunda Makuba et al., 2008).

As photosensitizers, furocoumarines acts in two ways according to the abundance of oxygen. In a medium low in oxygen, these molecules under the action of light, will be inserted between the bits of DNA and block its replication (Cain et al., 2006). In a medium saturated with oxygen, these compounds absorb energy and promote the production of oxygen singlet. The generated oxygen

singlet damages the molecules rich in electrons, including DNA and inhibits the microorganisms contained in the medium (Valduga et al., 1993; Luksiene, 2005; Serrano et al., 2008).

Several authors cited quinones and anthraquinones as excellent generators of oxygen singlet (Taba and Luwenga, 1999; Fufezan et al., 2007). In the presence of light, these compounds are capable of generating oxygen singlet which attacks and damages most microorganisms in the medium (Sabbahi et al., 2000; Cavalcante et al., 2002; Bilia et al., 2006).

In the case of *A. annua* the likely photodynamic action may be attributed to the presence of terpene molecules, among other artemisinins. It was noticed that the infusion of *A. annua* became highly fluorescent under UV light at 365 nm. This fluorescence was attributed to the presence of artemisinin and/or a complex of artemisinin and essential oils present in the infusion (Lutgen and Michels, 2008). Artemisinin is a high-energy molecule, quick to react and lose energy as it is unstable, in the presence of light (Onimus et al., 2011). Bacterial inhibition observed in this study could be due to a synergistic action of different components of *A. annua* present in the medium and under light condition.

In this study, a low concentration of *A. annua* extract, showed a rapid reactivation of *E. faecalis* cells in the presence of light, then a massive proliferation in the dark. Rapid reactivation of *E. faecalis* cells in some cases could be the result of a photo adaptation. This phenomenon is able to increase tolerance to germicidal radiation by reducing the accumulation of photo products generated at the genetic support and maintain there after cell survival (Llyod et al., 1990; Ben Sai et al., 2011). Allahdin et al. (2008) indicated that the addition of *A. annua* infusion to bacteriological polluted water reduced the abundance of coliforms and cultivable faecal streptococci after 1 to 5 h, but the proliferation of faecal streptococci may occur after 24 h. In other cases, this reactivation occurs after a period of rest in the dark. The cells of *E. faecalis* exposed to light were reactivated in the dark by an obscure repair mechanism (Ben Sai et al., 2011), overcoming the damage induced by the combined action of light and extract of *A. annua*.

## Conclusion

This study showed that in an aquatic environment, under dark condition, there was a relative increase of *E. faecalis* cells growth in the presence of *A. annua* extract. Under lighting, there is a decrease of *E. faecalis* cells growth at different infusions of *A. annua*. Extract of *A. annua* favors growth of *E. faecalis* in the dark, especially at slightly acidic pH. It also showed that in the presence of light, *A. annua* extract inhibits growth of *E. faecalis* cells. This inhibition was significant at slightly basic pH. It depended

on the concentration of extract, up to a certain threshold. Spearman "r" correlation revealed that under dark condition only pH 6 was significantly correlated to the abundance of *E. faecalis* during the incubation duration. Furthermore, under the lighting, different pH values do not appear to have any significant influence on the inhibition of bacterial growth except for pH 8. Terpene molecules present in infusions especially monoterpenes, sesquiterpenes and triterpenes could be involved in chemical reactions responsible for cellular inhibition in the presence of light. Research on photosensitizing compounds and a comparative study with conventional photosensitizers might attest the involvement of photosensitivity reaction with extracts of *A. annua*.

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