

## Original Research Article

### Comparison of the inhibition of commensally and enteropathogenic *E. coli* strains in the presence of *Eucalyptus microcorys* leaves extract in aquatic microcosm

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## ABSTRACT

### Keywords

*E.coli*;  
commensally  
strain;  
entero-  
pathogenic  
strain;  
inhibition;  
*E. microcorys*  
extract.

This study aimed to assess the inhibition of the commensally and enteropathogenic *E. coli* strains in aquatic microcosm under various concentrations of *Eucalyptus microcorys* leaves extract incubated under different temperatures. The extract concentrations were 1%, 1.5% and 2%. The incubation temperatures were 7°C, 23°C, 37° and 44°C. The abundance of the colony forming units (CFU) of each of the cell strains in the presence of *Eucalyptus* extract, decreased in most cases with respect to the increasing of the concentration of aqueous extract and the incubation temperature. The highest CAIRs were registered at 2% of aqueous extract for both cell strains. The highest CAIRs of the commensally *E. coli* strains were 4.176.h<sup>-1</sup>, 5.722.h<sup>-1</sup>, 5.865.h<sup>-1</sup> and 6.319.h<sup>-1</sup> registered under the incubation temperature 7°C, 23°C, 37°C and 44°C respectively. For the Enteropathogenic *E. coli* strains they were 3.547.h<sup>-1</sup>, 6.112.h<sup>-1</sup>, 8.365.h<sup>-1</sup> and 7.027.h<sup>-1</sup> under the same incubation temperatures respectively. The cell inhibition percentage varied from one strains to another and with respect to the extract concentration and temperature incubation. With each *E. coli* strains, the highest cell inhibition percentage was registered under 37°C at 1%, and under 23°C and 37°C at 1.5% of the extract concentration. At the extract concentration 2%, the highest values of the inhibition percentages were noted with most of the incubations temperatures. A significant difference was noted between the inhibition rate of commensally and enteropathogenic strains (P<0.05).

## Introduction

Most regions of the world are confronted with a serious water crisis. But the real tragedy is its effect on the daily life of the

water borne diseases (WWDR, 2003). These diseases are generally due to the presence of numerous pathogenic bacteria

in water such as *Escherichia coli*. Some strains of this bacterium are the commensally of the digestive tract of man and most warm-blooded animals that they colonizes as from the first hours of the birth (Le Minor and Véron, 1989). Frequently isolated from the digestive tract and stools of mammals, *E. coli* is a Gram negative bacillus. It belongs to the group of faecal coliforms and the family of *Enterobacteriaceae*. Other species of *Escherichia* genus such as *E. vulneris*, *E. fergusonii*, *E. hermanii* and *E. blattae* have been recently described (Baraduc *et al.*, 2000; Holt *et al.*, 2000).

Several strains of *E. coli* are frequently pathogenic (Holt *et al.*, 2000). Since its discovery, *E. coli* was naturally susceptible to antibiotics active against gram-negative bacilli (Jehl *et al.*, 2003). However, the rapid emergence of many bacterial strains insensitive to therapeutic antibiotics makes this a real problem of public health. Many studies have reported several cases of infections caused by contact or consumption of water contaminated by pathogenic strains of *E. coli* (Hörman *et al.*, 2004; Hörman and Hänninen, 2006). Angulo *et al.*, (1997) reported that in many parts of the world, several cases of infections due to consumption of contaminated water by pathogenic bacteria, sometimes causing epidemics which are often followed by loss of human life.

Since thousands of years, humanity has used various plants found in its environment, in order to treat and to take care of all kinds of illnesses. These plants represent an immense reservoir of potential compounds assigned to secondary metabolites that have the advantage of having a large chemical and structural diversity and they possess a very

large range of biological activities. The active principles of plants are the essential components of the majority of our drugs and products of care (Hans, 2007). In spite of the multiple progress of modern medicine, there is a net interest of phytotherapy. More than 80% of the world's populations have resorted to traditional pharmacopy to face the problems of health (Farnsworth *et al.*, 1986). In fact, of the 300 000 plant species counted on the planet more than 200 000 species found in tropical Africa have medicinal virtues (Millogo *et al.*, 2005). Medicinal and aromatic plants are widely used as medicine and constitute a major source of natural organic compounds (Seenivasan *et al.*, 2006). Research data has demonstrated that the extracts exhibit various biological effects, such as antibacterial, antihyperglycemic and antioxidant activities (Lee and Shibamoto, 2001; Gray and Flatt, 1998).

Recently, attention has been focused on disinfection of water with plants extracts (Sunda *et al.*, 2008). Aqueous extract of *Lantana camara*, *Cymbogon citratus* and *Hibiscus rosa-sinensis* have shown a bactericidal effect in aquatic milieu (Sunda *et al.*, 2008). The results obtained by Voss-Rech (2011) indicated that the vegetal extracts tested present potential antimicrobial activity with efficient properties in the inhibition of *Salmonella*, especially vegetal extracts from the Myrtaceae family.

*Eucalyptus* belongs to Myrtaceae family. It is an aromatic plant and native to Australia. The genus *Eucalyptus* contains about 700 species (Chaix, 2002). The essential oils of the kind *Eucalyptus* sp are especially indicated against the respiratory illnesses (De Billerbeck *et al.*, 2002).

It is used internally and externally as an expectorant, and to treat infections and fevers. It is also used topically to treat sore muscles and rheumatism (Armando *et al.*, 2009). Many studies showed that *Eucalyptus cinerea* has antimicrobial activity against many bacteria (Franco *et al.*, 2005). The antibacterial activity of essential oils from leaves of *Eucalyptus globulus* and *E. camaldulensis* indicated the potential usefulness as a microbiostatic, antiseptic or as a disinfecting agent against *Staphylococcus aureus* and *Escherichia coli* (Ghalem and Mohamed, 2008).

Although commensally *Escherichia coli* is widely found in the environment, pathogenic strains including enteropathogenic, enterotoxigenic, enteroinvasive and enteroaggregative are often found in water (Nougang *et al.*, 2011). Less information is available about the impact of the aqueous extract of medicinal plant on commensally and pathogenic strains of this bacterium. The main objective of this study is to investigate the effects of aqueous extract of *Eucalyptus microcorys* leaves on culturability of commensally and enteropathogenic *Escherichia coli* (EPEC) in aquatic system and then compare cell inhibition rate and the impact of incubation temperature and incubation duration.

## **Materials and Methods**

### **Qualitative phytochemical screening**

Phytochemical sifting has been done according to the usual protocols (Rizk, 1982; Trease and Evans, 1983; Parekh and Chanda, 2007). It ascertained the presence of polyphenol, triterpenoids, sterols, alkaloids, saponins, catechics and gallic

tannins, Flavonoids, Anthraquinones and Anthocyanines. This phytochemical screening revealed the absence of catechic tannins and lipids, and the presence of other compounds.

### **Bacterial isolation and storage**

The bacteria used were the commensally and enteropathogenic *Escherichia coli* (EPEC) strains. These strains were chosen because of their importance in hygiene and sanitation (Le Minor and Veron, 1989; WHO, 2003). 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 membrane filtration technique (Rodier, 1996; APHA, 1998). Their identification was performed according to standard method (Holt *et al.*, 2000). For the identification and characterization of the commensally and enteropathogenic strains, only metallic green sheen CFUs were considered and re-growth on the standard agar medium according to Holt *et al.*, (2000).

Ten minutes before the experiments, 10 mL of human blood (group A+ Rh blood) was collected in Falcon tubes. Human blood cells were then harvested by centrifugation at 2515,5 g for 5 minutes at 10 °C, and washed three times using 10 mL of sterilized phosphate buffer (pH = 7.4). The pellets were then resuspended in 1 mL of sterilized phosphate buffer. Characterization tests were done in two steps (Okeke *et al.*, 2000; Yasmeen *et al.*, 2009). Firstly, haemagglutination test was used to characterize the potential pathogenic strains. This characterization was done by agglutination of human red blood cells with strains of isolated *E. coli*. The test was done by emulsifying on a sterile slide one solution consisting of

washed (3 times) human red blood cells (group A+ Rh blood), phosphate buffer (pH = 7.4), a pinch of  $\alpha$ -D-mannose, and 2 to 3 colonies collected overnight from cultures of *E. coli* grown at 37°C on Mueller Hinton agar. The slide was rotated manually for 2 min and the result was macroscopically observed for haemagglutination. When the suspension remains consistent after 2 min, the test is negative and the strains is considered as the commensally. It is positive if the suspension agglutinates and the strains is considered as potentially pathogenic.

Secondly, antisera *E. coli* (Bio-Rad, France) were used to confirm the different potential pathogenic strains noted after haemagglutination tests. The antisera *E. coli* used were the Nonavalent, Trivalent I (O111+O55+O26), Trivalent II (O86+O119+O127), Trivalent III (O125+O126+O128), and the Trivalent IV (O114+O124+O142) (Bio-Rad, France).

For the preparation of bacterial stocks, a colony forming unit (CFU) of each strain from standard agar medium was inoculated into 100 ml of nutriment broth (Oxford) and incubated for 24h at 44°C. After these cells were harvested by centrifugation at 8000 rev/min for 10 min at 10°C and washed twice with NaCl (8.5 g/l) solution. Each pellet was re-suspended in 50 ml of NaCl solution. After homogenization, 1ml of this was then transferred into 500 ml of sterile NaCl solution (0.85%) contained in an Erlenmeyer flask and stocked.

### **Sampling of *E. microcorys* and crude extract obtainment**

Fresh leaves of *E. microcorys* were harvested in Yaoundé, center region (Cameroon) and dried up at room

temperature (23±2 °C) in the laboratory for 30 days. Thereafter, leaves were ground into powder. Fifty grams of the obtained powder were mixed with 100 ml of warm distilled water and heated immediately up to boiling point; thirty minutes later, the mixture was left to settle, the supernatant was removed by filtration. The filtrate obtained constituted the decoction. The latter was dried in an oven at 45°C (Bella et al., 2012). The method used in this experiment was selected for its closeness to that of herbalists. The obtained crystals were used to prepare the crude extract. The output of the extraction was 22.43% (±0.5%). The output of the extraction was the ratio of the mass of crystals on the mass of the plant powder (50g) used. Three ranges of extract concentration 1%, 1.5%, 2% were prepared while using sterile physiological water. Every range was first filtered with the aid of a sterile cotton, then on Whatman membrane, and finally on membrane in nitrate of cellulose of 0.45µm porosity (De Souza *et al.*, 1994).

### **Experimental setup**

80 flasks of 250ml were used for this study. They were organized in four series: A, B, C and D; each series of flask was divided into four subgroups of 5 flasks each. All 20 flasks of series A contained 200ml of physiological water (NaCl: 0.85%) each and were used as control. The three other series contained 200ml of the extract at different concentrations 1%, 1.5% and 2% for series' B, C and D respectively. One ml of the bacterial stock was then transferred into each flask.  $T_0$ , the initial time, corresponded to the point of time when the transfer of stock was done. At  $T_0$ , the cell concentration was  $27 \times 10^8$  CFU/ml. The first subgroup of each group of A, B, C and D were

incubated at  $7\pm 1$  °C. The second subgroups were incubated at  $23\pm 1$  °C and the third and final subgroups of each group were respectively incubated at  $37\pm 1$  °C and  $44\pm 1$  °C. The incubations lasted 3, 6, 9, 12 and 24 hours. The counting of bacteria was done in each flask through the surface spreading method on agar medium, this at the end of each incubation period. The Endo agar (Bio-Rad) was used for EPEC and commensally *E. coli*. Results were expressed in the number of units that constituted a colony in 100 ml water sampled.

### Data analysis

Data were first described using histogram to show the evolution of cell abundance of each bacteria strains in the presence of different extract concentrations of *Eucalyptus microcorys* at different temperatures. In the same time, the percentage of inhibition was calculated and also illustrated with histogram. The percentage inhibition (PI) of the bacteria was calculated using the following formula as described by Garcia-Ripoll *et al.*, (2009) and Edima *et al.*, (2010):

$$PI = \left( \frac{N_0 - N_n}{N_0} \right) \cdot 100$$

Where  $N_0$  = initial number of bacteria;  $N_n$  = remaining bacteria after the action of *Eucalyptus* extract.

To estimate speed of inhibition, a straight regression line was calculated. A linear 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 cell apparent inhibition rate (CAIR). Statistical analysis was performed using SPSS version 12.0. The correlation coefficients among considered parameters were assessed using Spearman correlation test. The comparison of the abundances of cells of the both strains considered was carried out using Kruskal-Wallis, Mann Witney and ANOVA tests.

## Result and Discussion

### Temporal variation of the cell abundance

In the absence of *Eucalyptus microcorys* extract (Control), the abundance of commensally *Escherichia coli* varied between 22.20 and 23.24 Ln(CFU/100 ml); 22.22 and 23.13 Ln(CFU/100 ml); 22.35 and 23.32 Ln(CFU/100 ml) and between 22.17 and 22.93 Ln(CFU/100 ml) at the incubation temperatures 7°C, 23°C, 37°C and 44°C respectively. In the presence of extract concentration of *Eucalyptus microcorys*, the abundance of commensally *Escherichia coli* ranged from 18.23 to 21.46 Ln(CFU/100 ml) at the extract concentration 1%. The highest and lowest cell densities were observed at 7°C and 44°C respectively (Figure 1). The extract concentrations 1.5% and 2% induced a variation of cell abundance from 15.52 to 21.37 Ln(CFU/100 ml) and from 13.91 to 20.36 Ln(CFU/100 ml) respectively. A relative reduction of planktonic cells was noted in most cases in the presence of *Eucalyptus microcorys* extract. The importance of this reduction depends on concentration of aqueous extract and the incubation at 37°C. When the extract concentration increased, the cell abundance greatly decreased (Figure 1).

In the same condition, the culturability of EPEC was influenced by aqueous extract of *Eucalyptus microcorys*. In the control, the concentration of cells fluctuated between 20.76 and 21.71 Ln(CFU/100 ml) (Figure 2). The extract concentrations 1% and 1.5% induced respectively a variation of cell abundance from 12.44 to 21.55 Ln(CFU/100 ml) and 12.44 to 21.48 Ln(CFU/100 ml). In the presence of extract concentration 2%, the cell count ranged from 12.44 to 21.08 Ln(CFU/100 ml). The lowest cell abundance was observed in most cases at 44°C. The same observation was made as for commensally *Escherichia coli*.

### Cell's inhibition assessment

The percentage of cell inhibition was calculated to assess the influence of each parameter considered (aqueous extract concentration, incubation temperature and duration) on the culturability of each of the two bacteria strains. Figures 3 and 4 show that the culturability of commensally *E. coli* was rapidly inhibited by aqueous extract. This inhibition speed was highest with extract concentration 2% after 1 day. With the 3 extract concentrations, the inhibition percentages of commensally *E. coli* at 7° and 23°C were higher than 92 and 95% respectively. The inhibition of culturability was 100% after 6hours of incubation at 37 and 44°C (Figure 3).

Concerning EPEC with the extract concentration 1%, the percentage of inhibition ranged from 3 to 24%, 8 to 91% and 3 to 100% at 7, 23 and 37°C respectively (Figure 4). At 44°C, the speed of inhibition was higher than 98%. With 1.5% of extract concentration, 100% of cell inhibition at 37°C after 6h and at 44°C for the whole incubation duration was observed. The highest rate of inhibition was observed at 44°C in all incubation

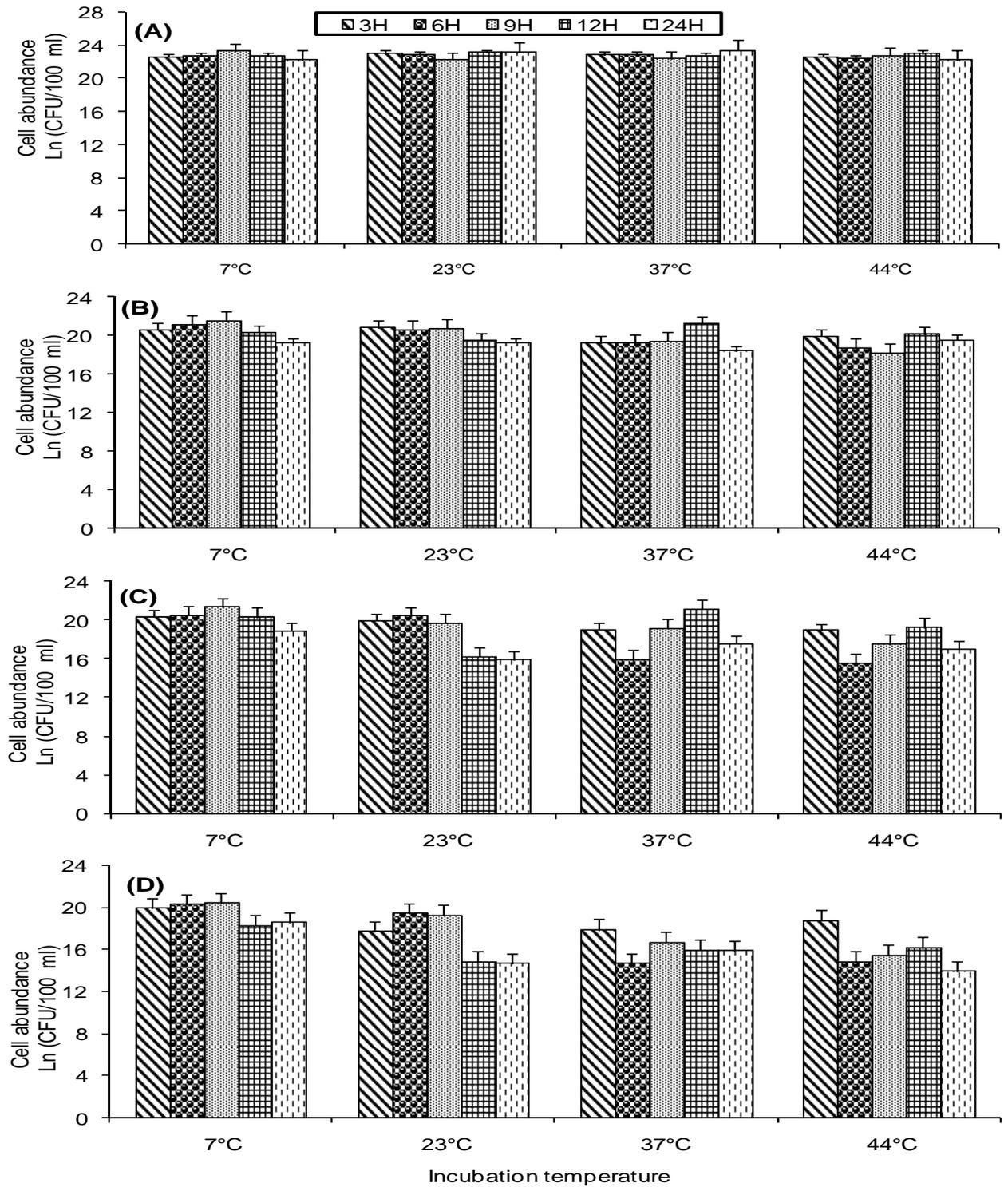
periods. The same observation was made with 2% extract concentration. On the whole, aqueous extract of *Eucalyptus microcorys* influenced the culturability of both bacteria studied with 100% of inhibition.

Considering the first 12 hours incubation period, the straight Ln (number of CFUs) lines against exposure duration were plotted. The slope of each regression line was then considered as the hourly cell apparent inhibition rate (CAIR). It appeared that in the presence of extract solution of *Eucalyptus microcorys*, the CAIRs of commensally *E. coli* was respectively 2.962 h<sup>-1</sup>, 2.951 h<sup>-1</sup>, and 4.176 h<sup>-1</sup> with 1%, 1.5% and 2% of extract concentration at 7°C. At 23°C and 37°C of incubation, the CAIRs ranged respectively from 3.642 h<sup>-1</sup> to 5.722 h<sup>-1</sup> and from 2.661 h<sup>-1</sup> to 5.865 h<sup>-1</sup>. The speed value was 6.319 h<sup>-1</sup> with the extract concentration 2% at 44°C (Table 1). Considering EPEC and all extract concentrations, the CAIR ranged respectively from 2.785 h<sup>-1</sup> to 3.547 h<sup>-1</sup>, 3.861 h<sup>-1</sup> to 6.112 h<sup>-1</sup>, 6.465 h<sup>-1</sup> to 8.537 h<sup>-1</sup> and from 6.226 h<sup>-1</sup> to 7.027 h<sup>-1</sup> at 7°C, 23°C, 37°C and 44°C respectively. In addition, a relative increase of the hourly CAIR with increasing extract concentration was noted in most cases (Table 1).

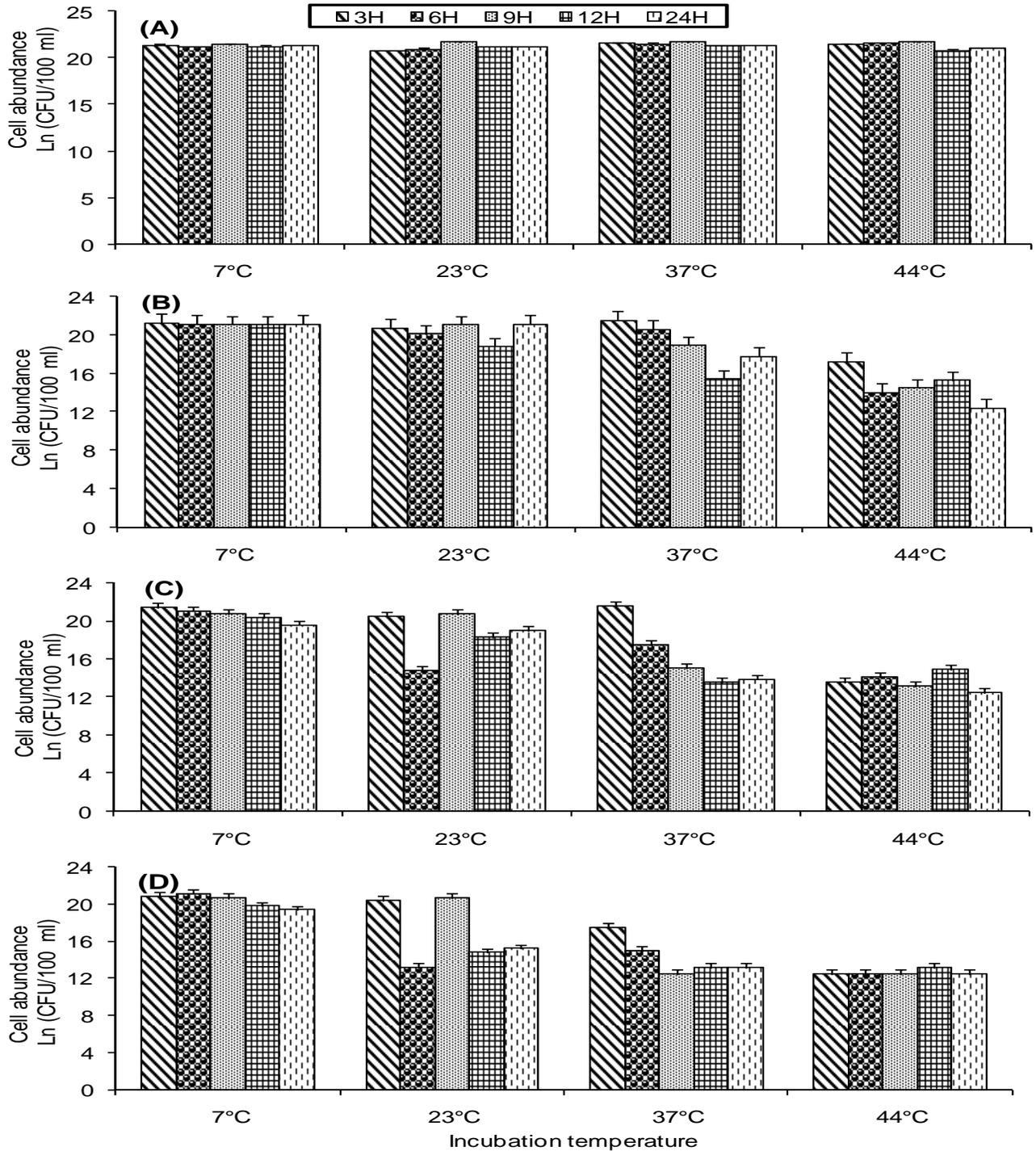
### Relationship and comparison between the considered parameters

The relationships were assessed between cell abundances and extract concentrations (1%, 1.5% and 2%) of *Eucalyptus microcorys* at each incubation temperature when considering the whole incubation periods. With the both *E. coli* strains a negative and significant correlation was noted between the incubation duration and cell abundance (P<0.05) (Table 2). The same results was registered between the

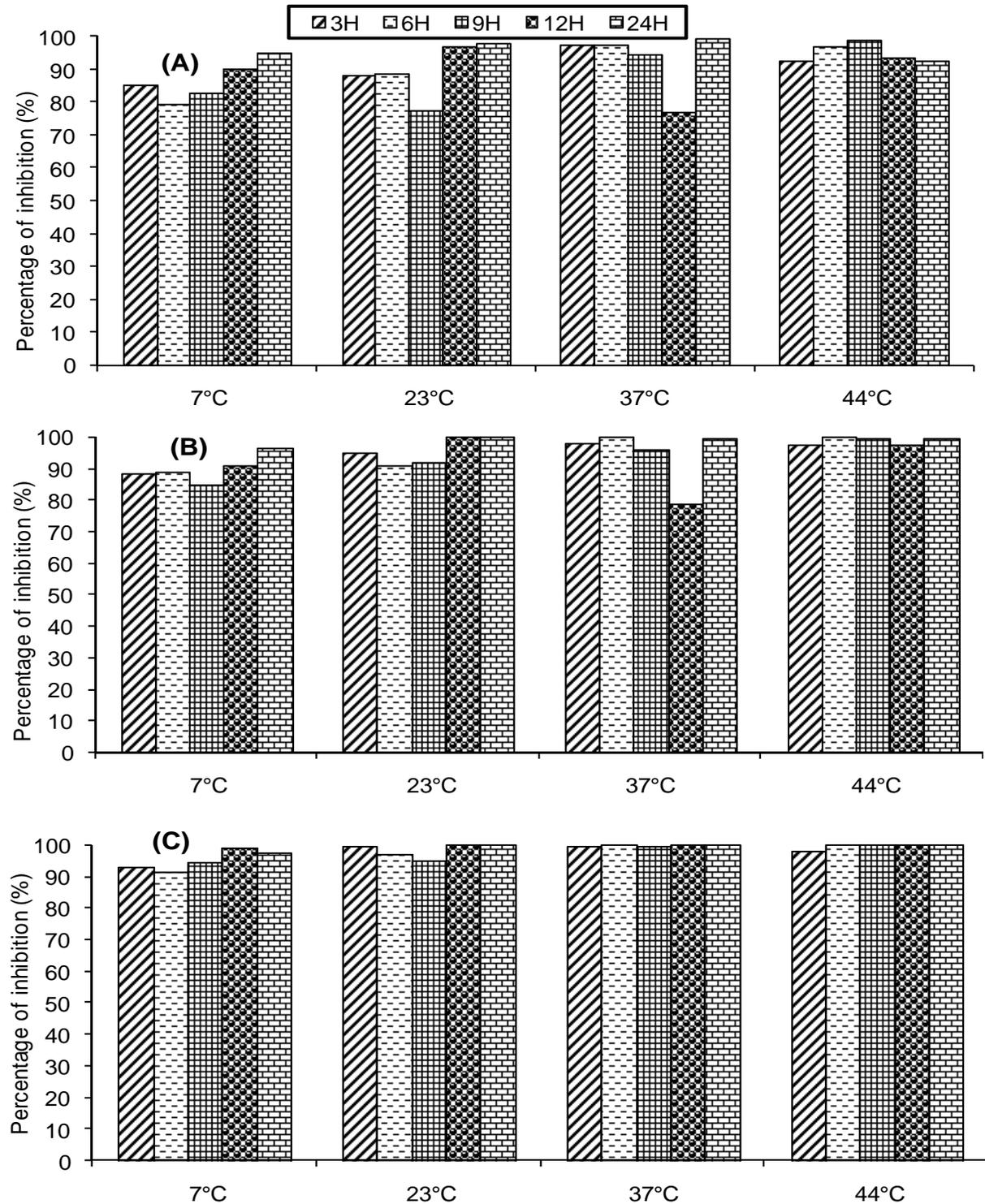
**Figure .1** Temporal variation of the abundance of planktonic commensally *E. coli* strains in the presence of *Eucalyptus microcorys* extract at different temperatures (**A**: Control; **B**: Extract concentration 1% ; **C**: Extract concentration 1.5% and **D**: Extract concentration 2%).



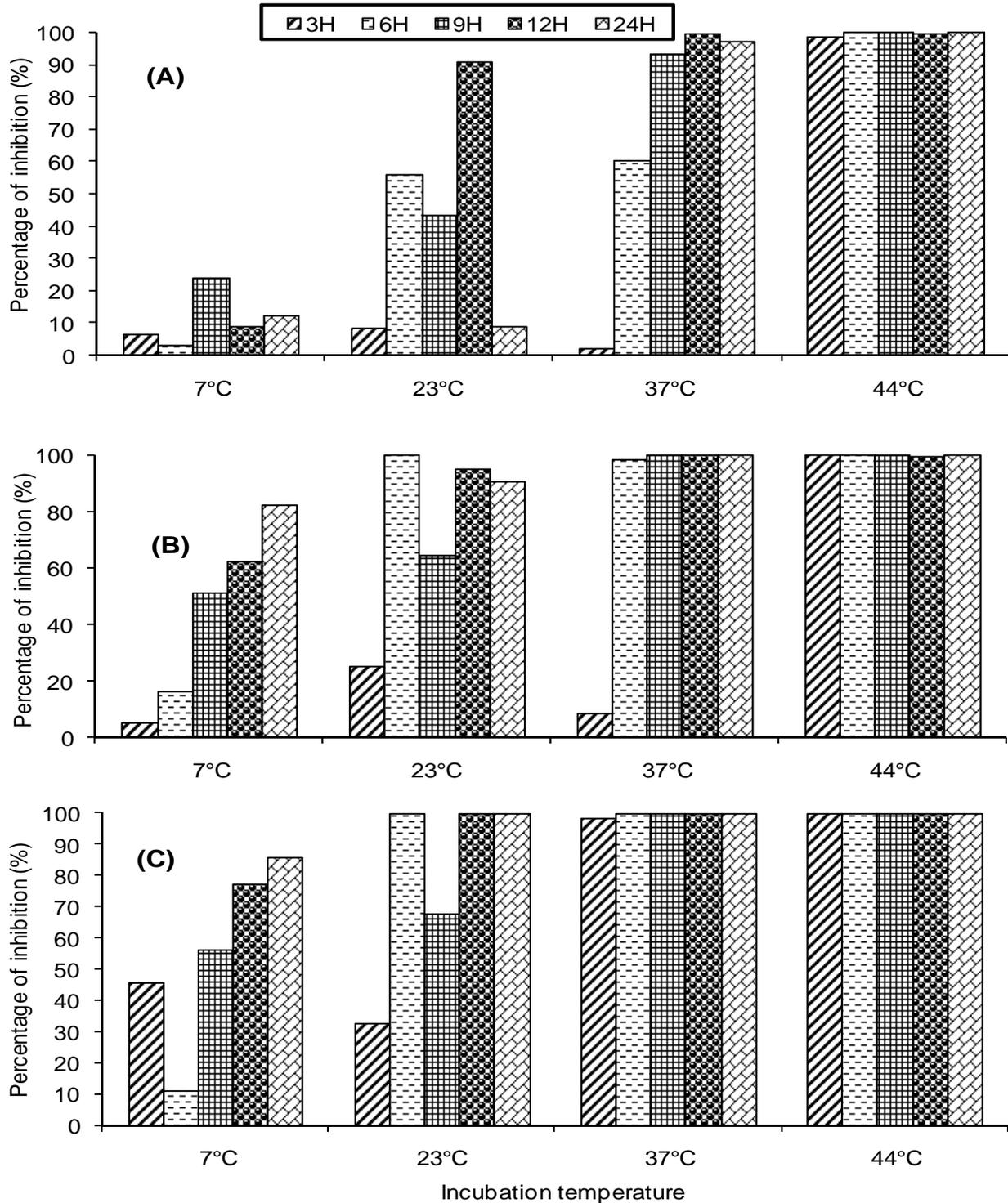
**Figure .2** Temporal variation of the abundance of planktonic Enteropathogenic *E. coli* (EPEC) strains in presence of *Eucalyptus microcorys* extract at different temperatures (A: Control; B: Extract concentration 1% ; C: Extract concentration 1.5% and D: Extract concentration 2%).



**Figure.3** Temporal variation of the percentage of inhibition of commensally *E. coli* strains at each incubation temperature and after each incubation period (A: Extract concentration 1%; B: Extract concentration 1.5% and C: Extract concentration 2%)



**Figure .4** Temporal variation of the percentage of inhibition of Enteropathogenic *E. coli* (EPEC) strains at each incubation temperature and after each incubation period (**A**: Extract concentration 1%; **B**: Extract concentration 1.5% and **C**: Extract concentration 2%).



**Table.1** Values of Cell Apparent Inhibition Rate (CAIR) (and regression coefficient) of cells at each experimental condition.

Bacterial strains and incubation temperatures		Hourly values of CAIR			
		NaCl (0.85%)	Extract (1%)	Extract (1.5%)	Extract (2%)
Commensally <i>E. coli</i>	7°C	1.758 (0.424)	2.962 (0.507)	2.951 (0.463)	4.176 (0.643)
	23°C	1.936 (0.499)	3.642 (0.645)	5.450 (0.786)	5.722 (0.642)
	37°C	2.054 (0.551)	2.661 (0.266)	2.700 (0.174)	5.865 (0.559)
	44°C	1.769 (0.400)	3.727 (0.463)	4.141 (0.363)	6.319 (0.627)
Enteropathogenic <i>E. coli</i> (EPEC)	7°C	2.690 (0.506)	2.785 (0.522)	3.394 (0.654)	3.547 (0.641)
	23°C	2.465 (0.392)	3.861 (0.637)	4.264 (0.359)	6.112 (0.476)
	37°C	2.625 (0.528)	6.465 (0.942)	8.537 (0.955)	8.365 (0.776)
	44°C	2.843 (0.574)	6.619 (0.596)	6.226 (0.436)	7.027 (0.462)

**Table.2** Correlation coefficients between cell abundances and extract concentrations at each incubation temperature when considering the whole incubation periods

Bacterial strains	Correlation coefficients			
	7°C	23°C	37°C	44°C
Commensally <i>E. coli</i>	-0.548*	-0.680**	-0.738**	-0.756**
Enteropathogenic <i>E. coli</i> (EPEC)	-0.662**	-0.520*	-0.719**	-0.655**

Number of observations: 15; \* :P<0,05 ; \*\* : P<0,01

**Table.3** Correlation coefficient between the cell abundances and the incubation durations at each of the incubation temperature when considering the whole extract concentrations

Bacterial strains	Correlation coefficient			
	7°C	23°C	37°C	44°C
Commensally <i>E. coli</i>	-0.546*	-0.633*	-0.055	-0.131
Enteropathogenic <i>E. coli</i> (EPEC)	-0.590*	-0.087	-0.584*	-0.271

Number of observations: 15; \*: P<0,05 ; \*\*: P<0,01

**Table.4** Correlation coefficient between cell abundances and incubation temperatures at each extract concentration when considering the whole incubation periods

Bacterial strains	Correlation coefficient		
	1%	1.5%	2%
Commensally <i>E. coli</i>	-0.496*	-0.516*	-0.609**
Enteropathogenic <i>E. coli</i> (EPEC)	-0.791**	-0.753**	-0.859**

Number of observations: 20 ; \*: P<0,05 ; \*\*: P<0,01

cell abundances and the incubation duration at each of the incubation temperature when considering the whole extract concentrations, with the commensally strains at 7°C and 23°C, and with enteropathogenic strains at 7°C and 37°C (Table 3). For the both *Escherichia coli* strains studied, a negative correlation was noted between cell abundance and incubation temperature at each extract concentration when considering the whole incubation periods (Table 4).

The comparison between the average abundances of commensally *E. coli* and that of Enteropathogenic *E. coli* showed a significant difference when considering all the extract concentrations and incubation temperatures (P<0.05). The Mann Whitney test confirmed this significant difference of cell abundances between 7 and 23°C, 7 and 37°C, 7 and 44°C and 23 and 44°C; and between 1 and 2% of

extract concentrations (P>0.01). A comparison between the values of CAIRs of commensally and enteropathogenic *E. coli* was performed using ANOVA test. A significant difference (P<0.05) was noted under different temperatures.

The present study aimed at assessing the antimicrobial activity of aqueous extract of *Eucalyptus microcorys* leaves on cultivability of *Escherichia coli* (commensally and enteropathogenic strains) in water. The relative inhibition of the cell cultivability was observed under our experimental conditions. The investigations showed that the cell abundance varied according to the incubation temperature and the increase of *Eucalyptus* extract concentrations (Figures 1 and 2). Temperature seems a factor of a great importance. It acts on chemical reactions of microbial metabolism although this is with respect of bacterial enzyme properties (Regnault, 2002).

In psychrophilic conditions as under 7°C and even 23°C, the EPEC strains showed a resistance at the extract concentrations 1% and 1.5%. Under this range of temperatures, the toxic products are slowly metabolized by mesophilic bacteria (Lessard and Sieburth, 1983) and their metabolism would be relatively low.

In the other respect, the increase of the temperature is generally correlated to the increase of the speed of metabolic and biochemical reactions and hence the toxic products are metabolized, thus the high percentages of inhibition is observed at 44°C with these two bacterial strains with all extract concentrations (Figures 3 and 4). It has been noted that at 7° C, 23° C and 37°C, the enteropathogenic *E. coli* strains seems to resist the effects of the extract concentrations 1% and 1.5% in contrary to the commensally strains with which the relatively higher percentages were observed in the same conditions. Enteropathogenic *E. coli* strains resistance observed may be due to natural or acquired resistance to antibiotics. Different susceptibility of the serovars may be probably related to defense mechanisms, since it is well known that bacteria can develop protecting mechanisms such as changes in the permeability and structure of the cell wall, production of inhibitory enzymes and alteration of the antibacterial molecules (Trabulsi and Altherthum, 2005). This could explain the difference observed between CAIRs of both bacteria strains studied.

A negative and significant correlation ( $P<0.05$ ) between incubation duration and cell abundance was observed, while a high significant and negative correlation ( $P<0.01$ ) was noted with the incubation temperature and extract concentration. The increase of hourly CAIRs was correlated

with the increasing of the extract concentration in most cases. The phytochemical screening revealed the presence of many compounds like polyphenols, flavonoids, tannins, sterols, terpenes, alkaloids and saponins in *Eucalyptus microcorys* aqueous extract. The inhibition observed on the culturability of both strains may be due to the presence of flavonoids and tannins.

Polyphenols including flavonoids and tannins are recognized due to their antimicrobial properties although this could vary from bacterial species to another (Santos and Mello, 2003; Loguercio *et al.*, 2005; Voravuthikunchai, 2005; Raven *et al.*, 2007). The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrase) or other interactions leading to inactivate microbial adhesins, transport proteins and to change cell membrane properties (Cowan, 1999). Cowan (1999) assumed that flavonoids lacking the free hydroxyl groups have antimicrobial activity more than those that are filled, which leads to an increase of the chemical affinity for membrane lipids.

The phenolic compounds also entail the inhibition of the extracellular enzymes, the substrate sequestration needed for the microbial growth or the chelation of metals such as iron (Milane, 2004). The tannins also have potential to form complexes with proteins thus causing the inactivation of the enzymes, either directly by fixing to the active sites, or indirectly by steric clutter created by the fixing of tannins molecules on the enzyme (Zimmer and Coordesse, 1996). While taking into account the structural and molecular constitution, the polyphenols also play a role in the degradation of the cell wall, the disruption of the cytoplasmic membrane,

thus causing a loss in the cell composition. They also influence the synthesis of DNA and RNA (Zhang et al., 2009), proteins, lipids and the mitochondrial function (Balentine et al., 2006), as well as the formation of complexes with the cell wall (Gangoué Piéboji, 2007).

The Student "t" test showed a significant difference between extract concentration 1% and 2% for commensally *E. coli* strains and between 7° and 37°C, 7° and 44°C, and 23° and 37°C for the EPEC strains (P<0,05). The pathogenic strains differ from the commensally with the virulence genes that are most often located on plasmids. These virulence factors are antibiotics inactivating enzymes which confer a mechanism of bacterial resistance.

The results obtained during this study show a real force of inhibition of the aqueous extract of *E. microcorys* towards the *E. coli* strains studied. The culturability of these germs could not only be influenced by the concentration of the extract, but also by the temperature and the period of incubation. The high percentages of inhibition observed during the study could be due to the presence of the secondary metabolites in the plants extracts. Besides, the effectiveness of these extracts could be due to the combined action of all the secondary metabolites which act together. The results obtained lead to check how the *E. microcorys* aqueous leaves extracts can be used in the microbial treatment of water as *Lantana camara*, *Cymbopogon citratus* and *Hibiscus rosa-sinensis* which has been recommended in the disinfection of aquatic environment by Sunda et al., (2008).

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