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Time Kill Kinetic, Antibiofilm Activity and Effect on Biomolecules Release of *Drypetes Gosswelleri* and *Echinops Giganteus* Essential Oils on Bacteria

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ABSTRACT

Finding alternative strategies to confront bacterial resistance is an urgent need. Biofilm-forming bacteria have become a serious problem in medicine. Bacteria can use biofilm as a mechanism of resistance against antibacterial drugs. The aim of this study was to study times kill kinetic of *Drypetes gosswelleri* and *Echinops giganteus* essential oils, the antibiofilm formation activity and their effect on cell release compounds. Times kill kinetic was study by quantification of cellular growth over time in Petri dish after her inoculation by the cells treated with antibacteria at MBC and incubation at different times during 24h. The antibiofilm activity carried out by microdilution using 96 wells microplate. The production of biofilms by cells treated was observed and quantified after coloration by Crystal Violet dye and spectrophotometric dosage at 630nm. The effect on cell release compounds are study by determination of absorbent material in extracellular medium at 260nm after exposure at the antibacterial during 24h. The results obtained showed that, these EOs have an ability to kill bacterial cells over time, *Drypetes gossweileri* EOs like Ciprofloxacin, caused the death of all the cells in the inoculum treated before 14h of exposure of *Staphylococcus aureus* strain. Against *Salmonella enteritidis* strain, *Drypetes gossweileri* and *Echnops giganteus* EOs kill all the cells after 24h like the two antibiotics. One effect of the action these EOs on the strains elucidated in this study was the leakage of intracellular absorbent materials (DNA and RNA) this materialized a diminution of membrane permeability and cell wall integrity.

KEYWORDS: Antibacterials, time kill, biofilm, cell release, Minimal Biofilm inhibitory concentration.

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INTRODUCTION

The development of antibiotic resistance mechanisms in bacteria is one of the most important public health problems in the world because the emergence of infections due to multiresistant bacteria compromises the activity of antibiotics and this situation is the more alarming when these bacterial strains produce biofilms [1, 2]. It has been estimated that 99% of all bacteria in a natural environment are able to form biofilm [3]. it is a self-enclosed population of bacterial cells[4].

Many problems are associated with the development of biofilms, the most important is their extremely high resistance to antibacterial agents (antibiotics and disinfectants) and to the immune system of the host compared to simple bacteria [5, 6, 7, 8]. There are many mechanisms involved in this resistance bacteria in biofilm for example: poor penetration or inactivation of antimicrobials in the extracellular polymeric matrix, an altered (dormant) bacterial metabolic state; the presence of persisted cells, resistance induced by the antimicrobial itself following the use of sub-lethal concentrations and the upregulation of efflux pumps [6, 9, 10]. In the field of the food industry, biofilms constitute a serious problem since they affect the food chain and consequently the health of consumers [11].

Biofilms are responsible of chronic infections that mainly affect people with a weak immune system. Bacteria

frequently implicated are commensal bacteria such as *Staphylococcus epidermidis, Staphylococcus aureus* and *Pseudomonas aeruginosa* [12]. *Staphyloccus aureus* and *Salmonella enteritidis* are known to form biofilm causing several and chronic infections that are difficult to treat [13, 14]. Moreover, Biofilm formation is important for the spread of *Salmonella* because bacteria in the biofilm resulting in a chronic infection and the development of these bacteria carrier state [15].

The formation of biofilms therefore becomes an important problem to be solved by the search for treatment alternatives based on new molecules [4]. EOs known for their strong antimicrobial power therefore represent a good prospect for solving this problem [16]. EOs have been shown to affect bacterial proliferation and contain many compounds with different mechanisms like damaging cell membrane, increasing its permeability, damaging cytoplasmic membrane, cell lysis, leakage of intracellular material, inhibiting efflux pump mechanism of antibiotics rendering them more efficient [17]. The effects of plant products to prevent biofilm formation and adherence have been shown in earlier studies [18, 19]. For this reason, the present work aimed to study the time kill kinetic, antibiofilm activity of two essential oils of the Cameroonian pharmacopoeia *Drypetes gosswelleri* (*D. gosswelleri*) and *Echinops giganteus* (*E. giganteus*) and their effect on cell release against *Staphyloccus aureus* and *Salmonella enteritidis* strains.

MATERIAL AND METHODS

Collection of plants and extraction of essential oils

The plants were collected in Cameroon. The bark of *Drypetes gosswelleri (D. gosswelleri)* harvested in Littoral Region, especially in Gwei near Edea. The roots of *Echinops giganteus (E. giganteus)* were bought at Mokolo market in the Center Region (Yaoundé-Cameroon). EOs were obtained by hydrodistyllation methods using Clevenger apparatus. The volatile compounds (EO) present in the plant material initially crushed and immersed in the water are trained by the water vapor during 6 to 8 hours and condensed in the refrigerant. The oily phase and the aqueous phase are separated by decantation. EO was collected, and dried with anhydrous sodium sulfate and stored [20].

Antimicrobials agents

Two standards antimicrobials drugs were used: injectable Ciprofloxacin (Ciprofloxacin) N°RA/DRUGS/RAJ/1594 (BONCIPRO$^{(R)}$) and injectable Ceftriaxone (Ceftriaxone) N°GUJ/DRUGS/G/198 (XONE).

Bacterials strains.

The study was performed on two bacterial strains have been used in this study. One Gram positive: *Staphylococcus aureus* ATCC 25923 (*S. aureus)* and one Gram negative: *Salmonella enteritidis* 155A (*S. enteritidis)*. The strains was stored at -80°C at the Pharmacology and Drugs Discovery Laboratory (at the Institute of Medical Research and Medicinal Plants Studies). Before starting, sub-cultured on solid medium Mueller Hinton Agar (MHA) followed by Mueller Hinton Broth (MHB) was done.

Time kill kinetic of Essential oils.

The time kill study were performed according to M26-A document of CLSI with a few adaptations [21]. The minimum bactericidal concentration (MBC) (table 1) of *D. gosswelleri* and *E. giganteus* EOs and antibiotics (Ciprofloxacin and Ceftriaxone) against *S. aureus* and *S. enteritidis* were determined by microdilution method in our previous work [22].

Table 1: Minimal Bactericidal Concentration according to Feudjieu *et al.*, 2023 [22] (µg/mL).

		D. gosswelleri	*E. giganteus*	Ciprofloxacin	Ceftriaxone
MBC	*S. aureus*	5.88	23.43	0.39	1.56
	S. enteritidis	2.92	46.87	0.39	1.56

EOs and antibiotics were prepared at concentrations equal to 4 times the MBC and the same volume of culture medium was added in sterile tubes. Bacterial inoculum at 6 LOG CFU/ml was added. The concentration of EO or antibiotics in the solution equal to the MBC. The mixture was incubated at 37°C. At the following times: 0h (representing the contact time between the inoculum and EOs or antibiotics), 30min, 2h, 6h, 10h, 14h and 24h for *S. aureus* and 0h, 30min, 1h30min, 2h30min, 4h, 10 and 24 hours for *S. enteritidis*, the inoculum was inoculated by spreading in the Petri dishes contained MHA medium in triplicate, this was occurred after a series of dilutions in a sterile saline solution (100 µL of inoculum in 900 µL of saline solution). After incubation the count of the colonies in the Petri dishes was done. The data was subjected to statistical analysis using one way ANNOVA followed by Dunnett's post hoc test. The difference were considered significant when $p<0.0001$.

Biofilm formation assay.

The ability bacteria to form biofilms were assayed as described by O'toole and Kolter, 1998 [23] with a few modifications previously described by Stiefel *et al.*, 2016 [24]. The strains were previously cultured in an MHB medium supplemented with 2.5% glucose. In each well of a sterile microplate (96 wells), 100µL of MHB medium supplemented with 2.5% glucose, was introduced into two columns of wells from A to H. 100 µL of inoculum at 6 Log CFU/mL was added to the wells. A negative control containing only 200 µL of culture medium was made. The

microplate was sealed with its lid, covered with film paper and then incubated at 37°C for 24 hours. After incubation, the wells of microplates were washed with sterile distilled water 3 times to remove the supernatant, and was dried at 60°C for 45 min. After drying, 200 µL of Crystal Violet (0.1%) was added to the wells of the microplates and they were incubated at room temperature for 30min. Subsequently, the microplates were washed 3 times with sterile distilled water, then 200 µL of 95° alcohol were added to each well. The anti-biofilm activity was demonstrated firsty visually, by observation of the blue coloration by Crystal Violet, a presence of coloration therefore indicated a formation of biofilm. After observation, the Optical Densities (OD) were read using a microplate reader at 630 nm to quantify biofilm formation. Biofilm formation were classified as follows: OD ≤ ODc no biofilm production; OD > ODc biofilm production, ODc represents the mean of the O.D of the negative control (culture medium) [8].

Study of antibiofilm activity of the essential oils.

The solutions were prepared at 6000 µg/mL by dilution of 12 µL EOs with a mixture of tween (11%), DMSO (5%) and sterile distilled water for a total volume of 2 mL. Antibiotics tested solutions were prepared at 100 µg/mL for Ciprofloxacin. Ceftriaxone was prepared at 400 µg/mL. This concentration was chosen for weighing reasons, because to avoid denaturation of the solution, it was necessary to prepare a few volume necessary for each manipulation.

Antibiofilm activity was studied using the method describe by O'Toole, 2011 [23] with a few modifications previously described by Stiefel *et al.,* 2016 [24]. Cells were cultured in MHB medium supplemented with 2.5% glucose. A stock of EOs solution initially prepared at 6000 µg/mL was added in the three wells (row A to H) containing culture medium MHB supplemented with 2.5% glucose. Serial dilution was occurred (from the wells of row 1 to wells of row 11) to reach final samples concentrations ranging from 3000 µg/mL to 2.92 µg/mL for EOs, from 50 µg/mL to 0.05 µg/mL for Ciprofloxacin and from 200 µg/mL to 0.2 µg/mL for Ceftriaxone. The wells were inoculated with bacteria inoculums (6log UFC/mL prepared from the MHB medium) to obtain concentration ranging from 1500 µg/mL to 1.46 µg/mL for EOs, from 25 µg/mL to 0.024 µg/mL for Ciprofloxacin and from 100 µg/mL to 0.1 µg/mL for Ceftriaxone. The wells of last two columns were used as a blank, they contained only the MHB culture medium and the EOs at decreasing concentrations. Wells in row 12 were used as a positive control for biofilm formation and contained MHB medium and inoculum. The microplate was sealed with its lid, covered with film paper and then incubated at 37°C for 24 hours. After incubation, the wells of the microplates were washed three times with sterile distilled water, then the microplates were dried at 60°C for 45 min, after drying, 200 µL of Crystal violet (0.1%) were added in the wells and the microplates were incubated at room temperature for 30 min; The microplates were then washed three times with sterile distilled water, then 200 µL of 95° alcohol were added to each well.

The antibiofilm activity was demonstrated firstly by observation of the blue coloration by Crystal Violet, a presence of coloration therefore indicated a formation of biofilm and the absence of coloration an inhibition. The Minimal Biofilm Inhibitory Concentration (MBIC) being defined as the smallest concentration at which no biofilm formation. Secondary, by quantification, the ODs were read using a microplate reader at 630 nm. The absorbance in blank well was subtracted from absorbance reading and percentage inhibition and efficiency was determined. The percentage of inhibition was then compared with the positive control [19]:

$$\text{Percentage of inhibition} = \frac{(OD_{Negativecontrol} - OD_{Experimental})}{OD_{Negative\ control}} \times 100$$

The equation on the right was generated by Excel software and the $MBIC_{50}$ (which represent the concentration where 50% inhibition of biofilm formation is observed) was calculated.

Effect of the Essential oils on biomolecules release.

The leakage of biomolecules was determined using spectrophotometric dosage of absorbents materials at 260 nm (nucleic acids release: DNA and RNA) release in extracellular medium according to the method describe by [25]. The dosage of the biomolecules released by *S. aureus* and *S. enteritidis* after treatment by EOs or antibiotics was study over time, during 24 hours using spectrophotometer. The EOs or the antibiotics was added to the bacterial inoculum (6log UFC/mL) in sterilized MHB medium. The concentration of antibacterial in the solution equal to MBC. The solution was incubated at 37 °C. At the same times like the study of time kill kinetic, the Optical Density (OD) at 260 nm was measured. Statistical analyzes consisted of one way ANOVA and Duncan's multiple range tests, with $p < 0.005$ considered to indicate significance difference.

RESULTS

Time kill kinetic of essentials oils

The time kill kinetics of *S. aureus* and *S. enteritidis* strains by *D. gossweileri* and *E. giganteus* EOs and antibiotics (Ciprofloxacin and Ceftriaxone) was present in Figure 1. The result showed that, at the concentration equal to MBC, all antibacterial of this study are able to disable the cells of the inoculum of *S. aureus* and *S. enteritidis* strains at 24h exposure time.

For *S. aureus* (Figure 1-A), all the antibacterials of these study kill all the bacteria cells in the inoculum after 24h of exposure. These effect was observed for *D. gossweileri* and Ciprofloxacin after 14h of exposure. For *S. enteritidis* (Figure 1-B), *D. gossweileri* and Ciprofloxacin are the two

antibacterials that kill all bacteria in the inoculum after 24h exposure. This data demonstrated that, *D. gossweileri* EO have the same potency action as the Ciprofloxacin and *E. giganteus* EO as the Ceftriaxone which are the standard antibacterial drugs used against *S. aureus* and *S. enteritidis* infections.

The growth of the negatives control observed confirmed the viability of the *S. aureus* and *S. enteritidis* strains under study. The curve of treatments obtained revealed that, the number of cells was significantly reduced when compared to the curve of control (p<0.0001).

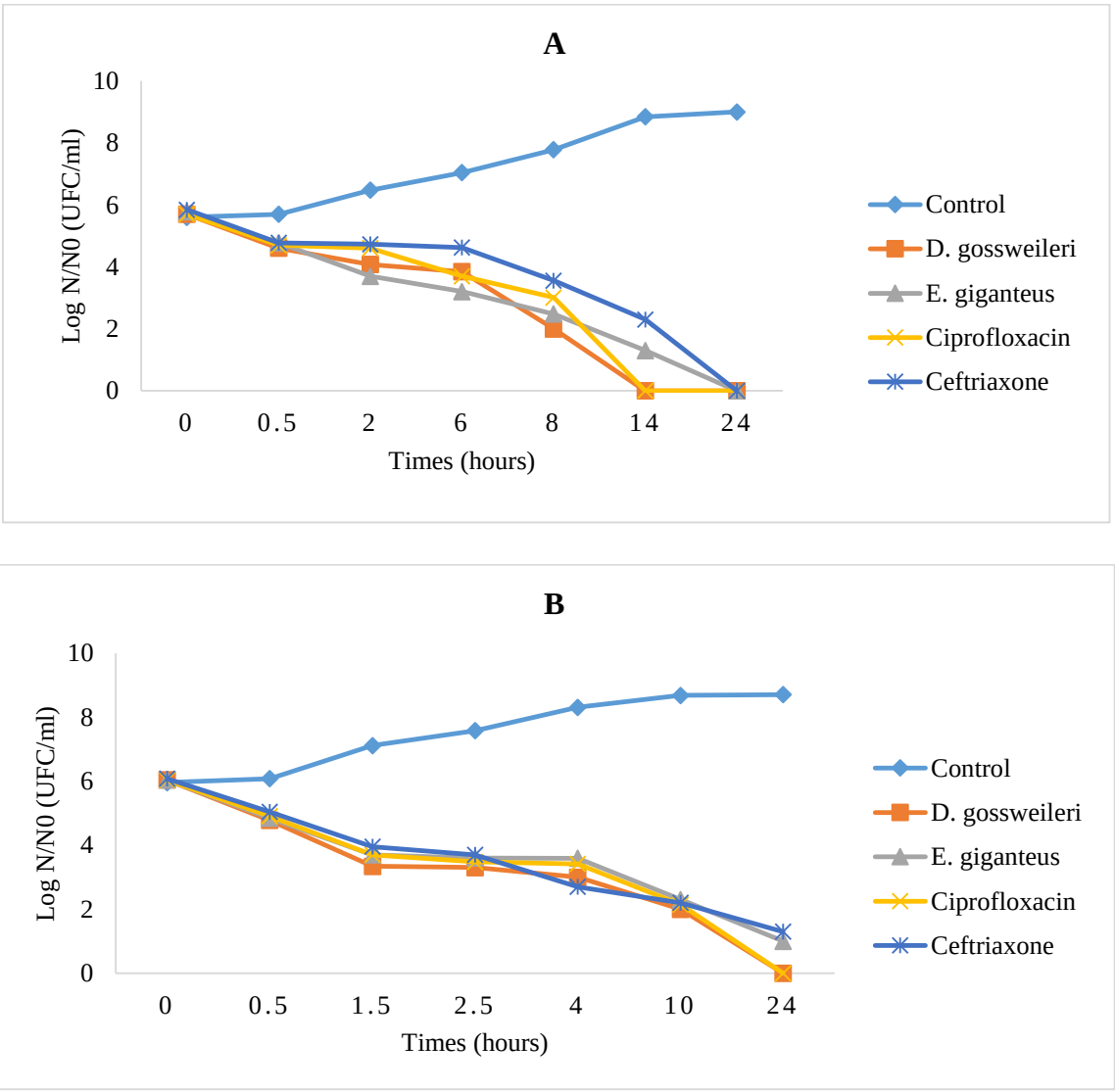


Figure 1 : Time kill kinetic of *S. aureus* (Figure 1-A) and *S. enteritidis* (Figure 1-B) treated with *D. gossweileri* and *E. giganteus* EOs and antibiotics (Ciprofloxacin and Ceftriaxone). Legend: N: number of colonies count in plate dish at each time, N0 : number of colonies at 0h.

Biofilm formation assay

The O.D values obtained are recorded in Table 2. The formation of biofilms is almost effective and similar for the two strains (Figure 2).

Table 2 : Optical density Legend: ODs: Optical density of strains, ODc : Optical density of the control

	ODs	ODc
S. aureus	0.26 +-0.03832424	0.005+- 0.00021
S. enteritidis	0.25+-0.08326831	0.0078+-0.00102

Figure 2 : biofilm formation assay. SA : *S. aureus,* SE : *S. enteritidis*

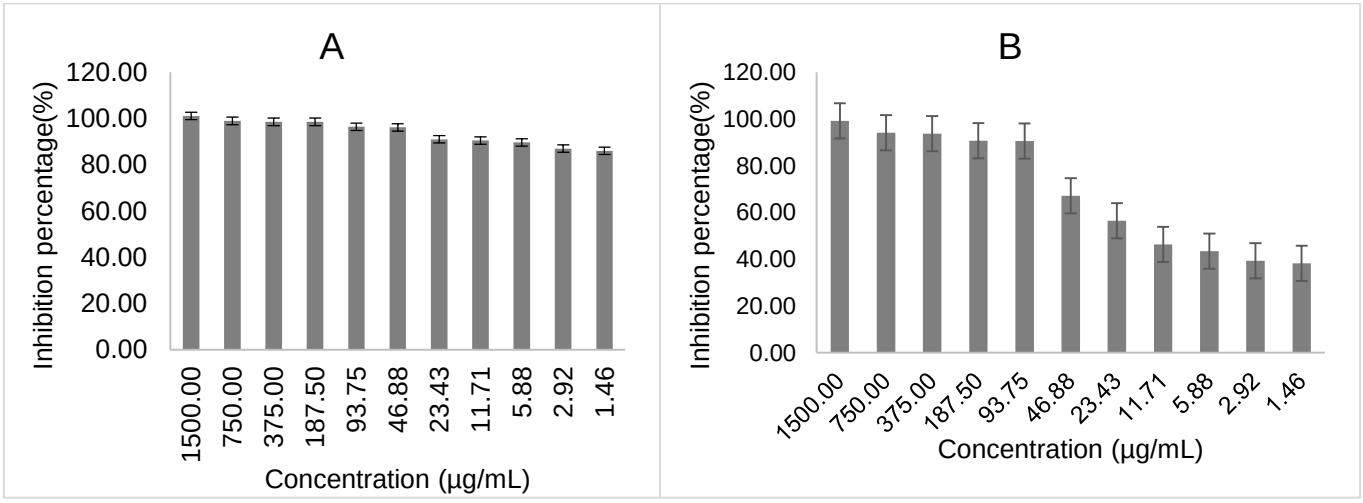
Antibiofilm activity of the essential oils.

The results of the Minimal Biofilm Inhibitory Concentration (MBIC) are recorded in Table 3. Among the four antibacterials tested, the essential oil of *D. gossweileri* is the one that showed the strongest anti-biofilm activity against *S. aureus* strain with an MBIC equal to 1.46 µg/mL, followed by Ciprofloxacin with an MBIC equal to 6.25 µg/mL. For *S. enteritidis* strain, Ciprofloxacin and Ceftriaxone showed the strongest activity with the same MBICs equal to 3.13 µg/mL and on this strain, the essential oil of *D. gossweileri* had a high activity(1.46 µg/mL) compared to that of *E. giganteus* (23, 43 µg/mL).

Table 3 : Minimal Biofilm Inhibitory Concentration (µg/mL)

	D. gossweileri	*E. giganteus*	Ciprofloxacin	Ceftriaxone
S. aureus	1.46	93.75	6.25	12.5
S. enteritidis	23.43	187.5	3.13	3.13

The percentages of inhibition of the biofilm formation of *S. aureus* by the antibacterials of this study are presented by figure 3. For *S. aureus* strain, it was between 100% at 86% for *D. gossweileri* EO for the concentrations ranging from 1500 µg/ mL at 1.46 µg/mL (Figure 3-A). Explicitly, for the concentrations equal to 1.46 µg/mL this EO was able to inhibit up to 86% of the formation of biofilms. For *E. giganteus* EO, the percentage of inhibition ranging from 99.23% to 38.27% for the same concentrations range like *D. gossweileri* EO (Figure 3-B). With the concentrations range from 25 µg/mL to 0.024 µg/mL of Ciprofloxacin on the microplate, it was between 99.6% and 16.06% (Figure 3-C), and between 99.77% and 4.2% for Ceftriaxone for the concentrations range from 100 µg/mL to 0.09 µg/mL (Figure 3-D).



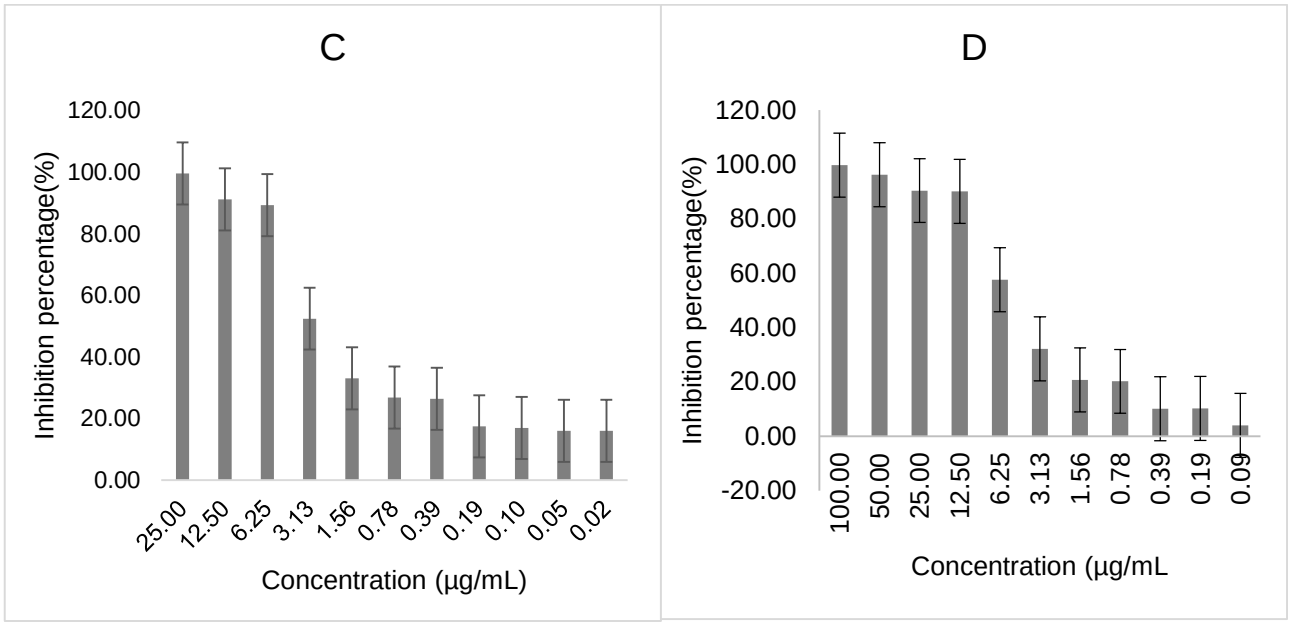
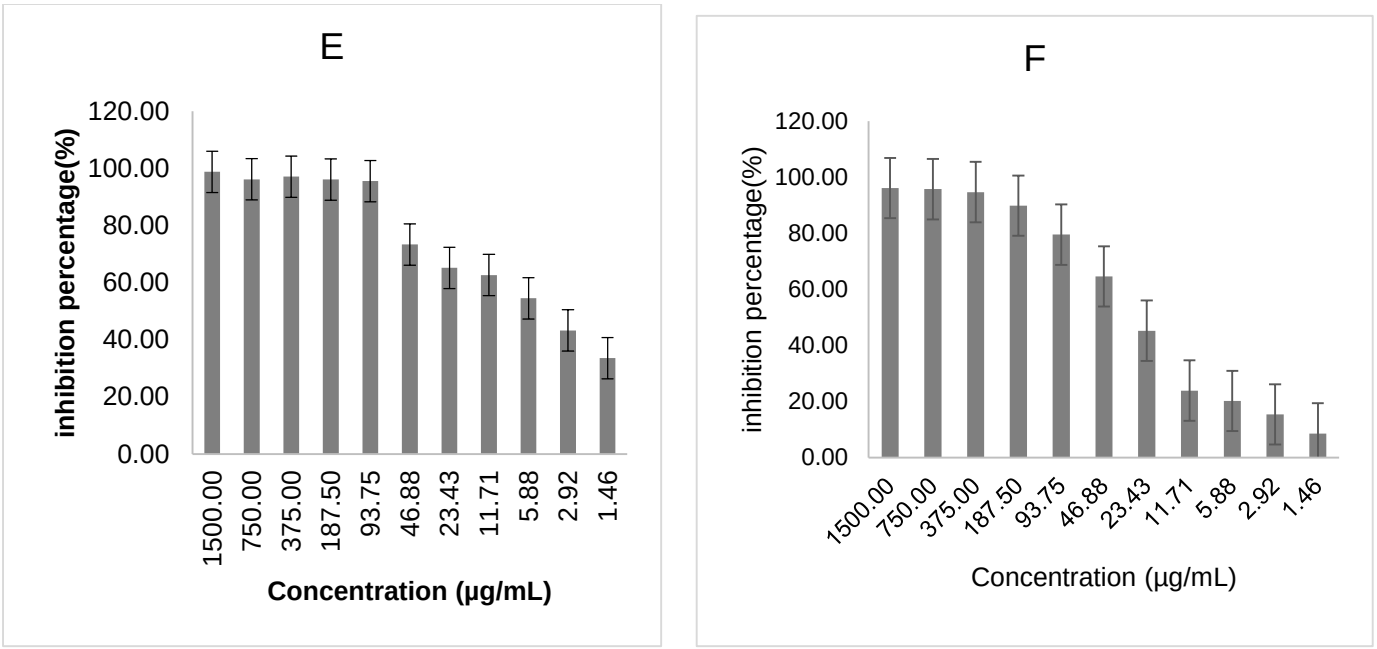


Figure 3 : percentage of inhibition of biofilms according to the variation of the concentrations of antibacterials against *S. aureus.*

A : Biofilms inhibition by *D. Gossweileri ; B :* Biofilms inhibition by *E. giganteus*, C : Biofilms inhibition by Ciprofloxacin, D : Biofilms inhibition by Ceftriaxone.

The percentages of inhibition of the biofilm formation of *S. enteritidis* by the antibacterials are represented by figure 4. On *S. enteritidis* it was between 98.77% and 33.56 % for *D. gossweileri* EO for the concentrations ranging from 1500 µg/mL to 1.46 µg/mL (Figure 4-E). For *E. giganteus* EO, the percentage inhibition was bettween 96.12% and 18.6% for the same concentration range like *D. gossweileri* (Figure 4-F). With a concentration range from 25 µg/mL to 0.024 µg/mL of Ciprofloxacin, 86.06% at 0% of biofilm inhibition was obtained (Figure 4-G). While, with the concentrations ranging from 100 µg/mL to 0.09 µg/mL of Ceftriaxone, we obtained a percentage inhibition ranging from 98.4% to 1.05% (Figure 4-H).



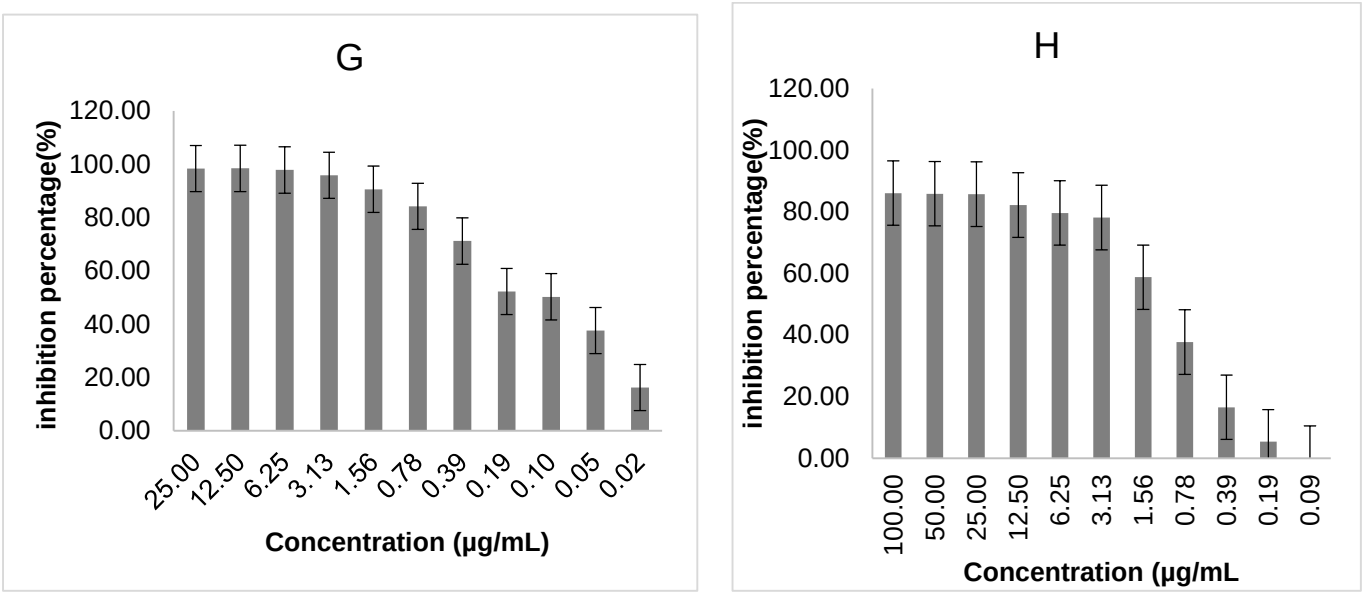


Figure 4 : percentage of inhibition of biofilms according to the variation of the concentrations of antibacterials against *S. enteritidis*.

E : Biofilms inhibition by *D. Gossweileri ;* F *:* Biofilms inhibition by *E. giganteus*, G : Biofilms inhibition by Ciprofloxacin, H : Biofilms inhibition by Ceftriaxone

The values of $MBIC_{50}$ are reported in Table 4. *D. gossweileri* EO is the one with the smallest $MBIC_{50}$ against *S. aureus*, this represent the best power of inhibition. To inhibit 50% of the biofilm formation, it takes a *D. gossweileri* concentration lower than 1.46 µg/mL.The two Eos had the best $MBIC_{50}$ against *S. enteritidis* (6.62 µg/mL for *D. gossweileri* EO and 6.73 µg/mL for *E. giganteus* EO). For Ciprofloxacin and Ceftriaxone, the $MBIC_{50}$ are better on the *S. aureus* strain (5.36 µg/mL for Ciprofloxacin and 5.85 µg/mL for Ceftriaxone) than on the *S. enteritidis* strain (8.7 µg/mL for Ciprofloxacin and 9.39 µg/mL for Ceftriaxone). The results obtained showed like preview results that, the antibiofilm effect of antbacterials against *S. aureus* strain was stronger compared to *S. enteritidis.*

Table 4 : $MIBC_{50}$ (µg/mL) Legend : <1.46 : $MBIC_{50}$ are lower than 1.46 µg/mL

	D. gossweileri	*E. giganteus*	Ciprofloxacin	Ceftriaxone
S. aureus	<1.46	8.62	5.36	5.85
S. enteritidis	6.62	6.73	8,7	9.39

Effect of essential oil on biomelecules release.

The OD values of the absorbing material at 260nm (DNA and RNA), measured for the study of the effect of *D. gossweileri* and *E. giganteus* EOs on cell release on *S. aureus* and *S. enteritidis* are presented in figure 5.

For *S. aureu*, strain, the ODs of control ranging from 0.038 to 0.048 over time. For *D. gossweileri* treatment, OD value ranging from 0.07 to 1.57, for *E. giganteus* from 0.0057 to 1.25, for Ceftriaxone from 0.0024 to 0.89 and for Ciprofloxacin from 0.001 to 0.012 (Figure 5-A).

For *S. enteritidis*, the OD value of control ranging from 0.007 to 0.012. For *D. gossweileri* treatment, from 0.04 to 0.98; for *E. giganteus* treatment, from 0.003 to 0.57, for Ceftriaxone treatment, from 0.0017 to 0.81 and for Ciprofloxacin treatment from 0.002 to 0.012 (Figure 5-B).

The OD values measured and the non-evolving trend of the curves obtained with the control and with Ciprofloxacin treatment inform us of an absence of release of the absorbing materials (DNA and RNA) over time ($p>0.005$, materializing a no significant difference of Ciprofloxacin treatment with the control). While, for the treatment with *D. gossweileri*, E. *giganteus* EOs and Ceftriaxone the OD obtained for the two strains of increasing evolution over time and the trend of the curves informed us about the presence of the absorbent material released ($p<0.005$ materializing a significant difference of these treatment with the control).

The greatest release of biomolecules was observed on the *S. aureus* strain for *D. gossweileri* EO, *E. giganteus* EO and Ceftriaxone treatments. *D. gossweileri* was the antimicrobial that led to the highest release of biomolecules on the two strains (OD=

0.98 for *S. enteritidis* and 1.57 for *S. aureus*), followed by Ceftriaxone (OD= 0.71) for *S. enteritidis* and by *E. giganteus* (OD= 1.25) on *S. aureus.*

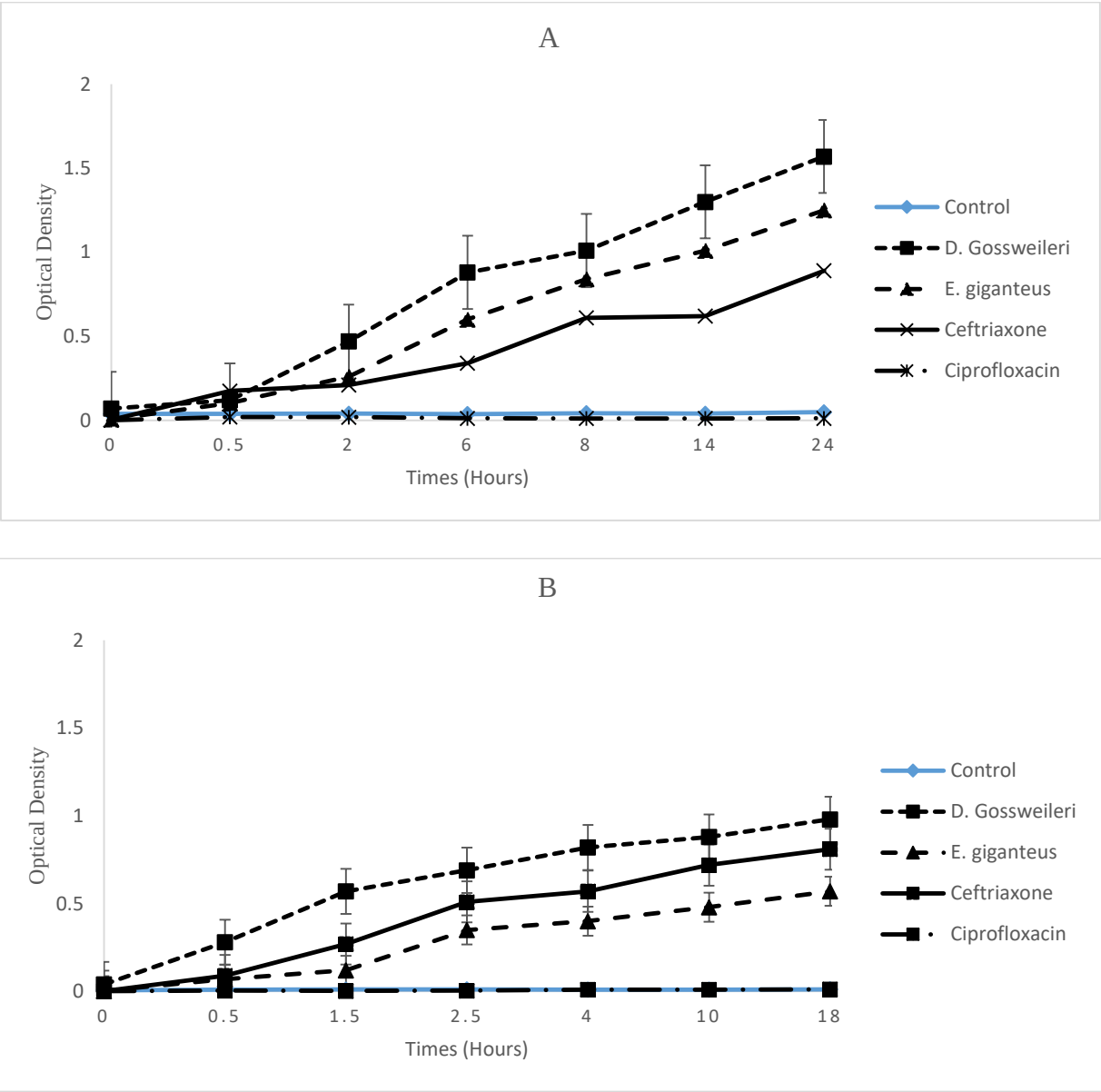


Figure 5 : Optical Density of absorbent biomolecules at 260nm for *S. aureus* (Figure 5-A) and *S. enteritidis* (Figure 5-B).

DISCUSSION

In this study, the time kill kinetics of *D. gosswelleri* and *E. giganteus* EOs and of two antibiotics (Ciprofloxacin and Ceftriaxone) against the bacterials strains of *S. aureus* and *S. enteritidis* over time during 24 hours was studied. We obtained a progressive desactivation of the cells by the four antibacterials. It is important to note that, this bactericidal effect has a permanent effect, as even after the neutralization of the agent, the microbial cells are not capable of growth and reproduction [26].

The ability of plants to inhibit microbial growth or kill the cells would be influenced by chemical composition of each EO [27]. Synergy action between these different majority and minority compound would therefore be responsible of this bactericidal activity [22].

D. gosswelleri EO is mainly composed of benzyl isothiocyanate noted in previous works at 91.28% [28] and also by the others studies (86.7% and 63.19%) [30, 31]. Sesquiterpenes are the compounds mainly present in *E. giganteus.* The presence of these compounds at 94.3% was revealed by Menut *et al.*, 1997 [31] and at 93% by Pavela *et al*., 2016 [32]. These chemical compounds, have long been known to have biological activities including various pharmaceutical benefits among which the bactericidal effects [31, 33].

The antibiofilm activity of the two EOs and two antobiotics was study against *S. aureus* and *S. enteritidis* strains. We was

observed the inhibition of biofilms formation by *D. gosswelleri* and *E. giganteus* Eos and the two antibiotics.

The antibiofilm activity of Ciprofloxacine and Ceftriaxone are study by several studis which had revealed the good potential of inhibition of biofilm formation by these antibiotics [34, 35].

Indeed, studies have been carried out on the evaluation of the antibiofilm effects of EOs which represent good pharmaceutical alternatives today, with regard to biofilms [11]. These studies revealed the ability of several EOs to degrade and/or eliminate biofilms, Basem et *al.*, 2022 showed the antibiofilm effect of *Thymus syriacus* EO on isolated strains of *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Streptococcus pneumonia*, they obtained respective percentage of inhibition of 43%, 50% and 60% by the concentration of EO equal to 3.12 µL/mL, 1.56 µL/mL, and 3.12 µL/mL respectively. Bacterial behavior in biofilms is regulated by the quorum sensing (QS) system [36]. It is a key regulator of their development, of their resistance to antibiotics [37] and of the expression of their virulence factors [7, 38, 39]. Quorum sensing (QS) is a term first used to describe an environmental sensing system that allows bacteria to monitor their own population density [38]. The interruption of QS system or bacterial cell-to-cell communication, is one example of an antipathogenic effect [39]. The available data indicate that the antibiofilm potential of plant would be due to the fact that, they are able to produce a range of inhibitory compounds of QS signal molecule [40].

According to Famuyide *et al.*, 2019 plant phytoconstituents are responsible of the biofilm inhibition [41]. The antibiofilm activity of benzyl isothiocyanate (ITCs) present in *D. gosswelleri* was demonstrated in previews studies. Borges *et al*., 2013 showed biofilm formation preventive activity and biofilm mass reducing activity of *E. coli, P. aeruginosa* and *L. monocytogenes* by ITCs, they obtained up to 60% reduction in activity of biofilm on the strains tested by the ITCs and the best inhibition was that of benzyl isothiocyane [42]. This preventive action is apparently due to interference with bacterial viability, motility and surface properties by ITCs demonstrated in the same study. The transition from a planktonic to a surface associated life-style, initiates with the transportation (motility) and attachment (adhesion) of microorganisms to a particular substratum [23]. Bacterial motility has also influence on adhesion and biofilm formation processes. For this reason motility inhibition can be correlated with a decreased ability of bacteria to form biofilms.

Sesquiterpenes compounds present in *E. giganteus* hold a good antibiofilm activities also demonstrated in preview study. Four types of sesquiterpenes showed antibiofilm activities against *S. aureus* strain at low concentration in the study conducted by Elmasri *et al.*, 2014 [43]. Similarly, Amaya *et al.*, 2012 showed significant inhibition of biofilm formation of *Pseudomonas aeruginosa* strain by sesquiterpenes at concentrations equal to 0.5 µg/ml and 1.32 µg/ml at a percentage between 42 and 50% [39]. This antibiofilm activity is due to the inhibition of production of N-acyl-homoserinelactones (AHLs: gram negative bacteria QS signal molecule) by sesquiterpens, which explain the antibiofilm activity of *E. giganteus* showed in the same study.

According to the results of this study, *D. gosswelleri*, *E. giganteus* EOs and Ceftriaxone cause leakage of 260nm absorbing materials. This effect is an indicative of irreversible damage to the membrane permeability and cell wall [44]. Ciprofloxacin had no activity on the membrane, due to the absence of biomolecules in the extracellular medium.

Tchinang *et al.*, 2023 showed in their study that *D. gossweileri* EO act by altering the structure of the cell wall and cell membrane of yeast [45]. This action has also been demonstrated by Moni *et al.*, 2019 on the membrane and the wall of *Mycobacterium tuberculosis* [46]. We did not find a review concerning the *E. giganteus* EO, however, the study on *Echinops ritro* EO showed a disruption of membrane integrity [47]. Hydrophobicity is an important property of EOs that leads to the accumulation of oils inside bacterial cell membranes, resulting in modification of a normal metabolic functioning of the bacterial cell, cellular lysis by disturbance of their structure, increased cellular leakage and the inhibition of bacterial growth and also the death of cell [46, 48].

According to *Carson et al*., the mode of action of EOs depends primarily on the type and characteristics of the active components contained in this oil [49]. Ping *et al.*, 2021 showed in their study that Benzyl isothiocyanate, a majority compound of *Drypetes gossweileri* EO, acted on *Bacillus cereus, Staphylococcus aureus, Samonella enterica* and *Penincillum citrinum* by affecting the integrity of the cell membrane [50]. Shuangshuang *et al.*, 2022 showed the action on the membrane of benzyl isothiocyanate from *Moringa oleifera* [51]. According to Cowan, 1999**,** sesquiterpenes would disrupt the membrane structure of microorganisms through their lipophilic components which was demonstrated in our study [52]. Sülsen *et al.*, 2016 showed an induction of cell death by apoptosis on *Trypanosoma cruzi* by these compounds [53].

Ceftriaxone is an antibiotic family of 3rd generation Cephalosporins. The antibiotics of these family act on bacterial wall, by selective toxicity on the synthesis of peptidoglycan by inhibiting proteins binding penicillin (PLP). PLPs have transpeptidase, carboxypeptidase and transglycolase activity. Inhibition of PLPs leads to inhibition of the formation of the pentacyclic bridges responsible for the reticular structure of the wall. Odd forms (round or filamentous) are thus obtained which result in bacterial lysis [54], which explain the presence of absorbing material in the extracellular medium showed by this study. Ciprofloxacin belongs to the fluoroquinolone family that act on the bacterial cells by the Selective inhibition of DNA synthesis by acting

on two enzymes involved in this synthesis: DNA gyrase and DNA topoisomerase IV, which explain the absence of biomolecules release because this antibiotic don't act on the cells envelops [54, 55].

S. aureus was the strain the most vulnerable to EOs and antibiotics activity according to this study. This was materialized by the death of all the cells of inoculum treated by the antibacterial in time kill kinetics results, the best inhibition of biofilms and the most leakage of absorbent material by antibacterial after exposition. The action was therefore less against *S. enteritidis*. This difference in sensitivity would be due to the fact that Gram negative (*S. enteritidis*) bacteria have an outer membrane which surrounds the cell wall and which limits the diffusion of hydrophobic compounds by the cover lipopolysaccharides [56]. However, for Gram positive bacteria (*S. aureus),* the absence of this barrier make easy the contact with cells, which causes an increase in ion permeability and the passage of constituents or an alteration of vital intracellular bacterial enzyme systems [57].

CONCLUSION

This study assessed the time kill kinetics of cell death, antibiofilm activity and effect on biomolecules release of essential oils (EOs) from *Drypetes gossweileri* barks and *Echinops giganteus* roots against *Staphylococcus aureus* and *Salmonella enteritidis* strains. The findings showed that, these EOs have an ability to kill bacterial cells over time. *Drypetes gossweileri* EO caused the death of all the cells of the inoculum treated before 24h of exposured like Ciprofloxacin for the *Staphylococcus aureus* strains. Against *Salmonella enteritidis*, *Drypetes gossweileri* and *Echinops giganteus* EOs kill all the cells after 24h of treatment. A good inhibition of biofilm formation was observed by the two EOs against the two strains. One mechanism elucidate in this study, responsible for this cellular death by EOs and Ceftriaxone are release material absorbent at 260nm, (DNA and RNA) this materialized a diminution of membrane permeability and cell wall integrity. The good bactericidal properties and antibiofilm properties of *Drypetes gossweileri* and *Echinops giganteus* EOs present a good alternative to treated infections caused by these bacteria and solve the problems caused by biofilm formation.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Ethic approval

Not applicable in this section.

Availability of data and materials

The datasets supporting the conclusions of this article are presented in this main paper. Plant material used in this study have been identified at Cameroon National Herbarium where voucher specimen are deposited.

Author's contributions

FEG carried out the research work, prepared the first draft of the manuscript and participated in revision and formatting of the final version. TFC guided and designed to laboratory work and revised manuscript. KGA designed and supervised laboratory works and revised the manuscript. MNE guided to choices the plants and design study. MGS participated at laboratory works. NWG and ED : attended laboratory tests. GAA conceived the study and revised the final form manuscript. All authors have read, revised and approved the final version of the manuscript.

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