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Lethal Action of Essential Oils from *Pentadiplandra brazzeana* Baill., *Allium sativum* L. and *Echinops giganteus* A. Rich. on Cell Membrane Integrity of *Haemophilus influenzae*

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A B S T R A C T

The global rise of antibiotic resistance against bacteria recent years increased the interest of research in alternative antimicrobial agents particularly those of the respiratory tract infection. Among these alternatives, Essential oils (EOs) are these mixtures of volatile compounds from plants more documented for their multiple therapeutic properties against airborne bacteria with multiple irreversible effects in-cell targets of bacteria. This study investigates the effect of EOs from *Pentadiplandra brazzeana* Baill. fresh roots, *Allium sativum* L. bulbs, *Echinops giganteus* A. Rich. dry roots on cell membrane integrity of *Haemophilus influenzae* by correlating their bactericidal action to cell release intracellular biomolecules. The essentials oils were extracted by hydrodistillation using a Clevenger apparatus. Then the inhibition parameters Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) of EOs and Chloramphenicol were investigated using a 96 wells microdilution assay against respiratory tract Gram- bacteria strains of *Haemophilus influenza* ATCC 49247, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 17102. The dynamic action of each EOs on cell membrane integrity was demonstrated by showing their lethal effect on *Haemophilus influenzae* exposed at (MBC) of EOs, by quantifying the DNA release using the Nanodrop 1000 spectrophotometer and number of living cells at different times during 24 h at 37 °C. A correlation coefficient R^2 involved between the DNA released and the number of living cells was established by plotting the curve concentration of DNA release versus the number of living cells counted in a solid medium. The results showed that, compared to Chloramphenicol, *Pentadiplandra brazzeana* and *Allium sativum* EOs exhibited strong activities with MICs of 9.76 µg/mL, and 39.06 µg/mL respectively, while *E. giganteus* EO showed moderate activity with MIC of 156.25 ng/µL. The lethal effect of *Pentadiplandra brazzeana* EO's against *H. influenza* showed the decrease of living cells from 4.7 Log to 2 Log at the same time an increase of DNA concentration from 0 to 290 ng/µL with a symmetric axis of action at half time (280 min). Meanwhile, *Allium sativum* and *Echinops giganteus* EOs exhibited against *H. influenza,* a decrease of living cells from 4.7 Log to 2 Log and from 4.7 to 2.5 Log respectively and an increase of DNA concentration from 0 to 300 ng/µL after 420 min of exposition. In contrast, Chloramphenicol showed only lethal action against *Haemophilus influenza,* with a decrease of living cells from 4.7 Log to 0.2 Log and an increase of DNA concentration from 0 to 40 ng/µL at 500 min. *Allium sativum* and *Echinops giganteus* EOs correlated lethal and cell lysis effects respectively with R^2 = 0.81 and R^2 = 0.89 meanwhile *Pentadiplandra brazzeana* and Chloramphenicol correlated at 0.94 and 0.66 respectively. The action of EOs on *Haemophilus influenzae* cell membrane's reveals a lethal effect directly correlated with cell lysis for *Pentadiplandra brazzeana* EO indirectly correlated with cell lysis for *Allium sativum* and *Echinops giganteus* EOs, depending on their antibacterial activity.

Keywords

Antibacterial activity, Cell membrane, DNA release, Essential oils, Lethal effect.

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Introduction

Essential oils (EOs) are mixtures of volatile bioactive compounds isolated from plants by hydrodistillation and used in aromatherapy as antimicrobials drugs (Dumlupinar *et al.,* 2020; Cavalheiro *et al.,* 2023). The potential for application of EOs in large scale has been increasing due to the higher degree of knowledge and technological advancement and also because of their used by part of population as alternatives to industrial, synthetized antibiotics and food additives (Calo *et al.,* 2015). Their main advantages lie in the fact that they bioactive compounds are natural product with a very broad spectrum of action related to their complex chemical composition and less harmfully (Carson *et al.,* 2002; Silveira *et al.,* 2022). Among the biological activities of EOs, antiviral, anti-inflammatory, antitumoral, immunomodulatory, antioxidant, and

antibacterial properties which facilitate their use as alternatives in the treatment of respiratory affections like asthma, pneumonia, bronchitis and tuberculosis (Horváth *et al.,* 2015; Bedi *et al.,* 2016).

An ethnobotanical survey from Cameroon carried out in the locality of Nkam sub-division (Littoral Region of Cameroon) reported the use of *Pentadiplandra brazzeana* fresh roots, *Allium sativum* bulbs and *Echinops giganteus* roots crude extracts for the treatment of respiratory aliments such as cough, asthma, tuberculosis and pneumonia (Moni, 2019). Among the pathogen of respiratory tract affection, *Haemophilus influenzae* (Hi) is an important cause of lower respiratory tract infection including childhood meningitis and bacterial pneumonia in children. This bacterium is one of the most important bacterial colonizers of the nasopharynx and is a common cause of acute otitis media and sinusitis (Hashida *et al.,* 2008; Ortiz-Romero *et al.,* 2017).

Although population-based incidence data are scarced, it is estimated that *H. influenzae* causes at least 3 million cases of severe disease and hundreds of thousands of deaths annually worldwide (WHO, 2023). Antibiotic therapy remains the WHO recommended treatment to cure the disease. However, the widespread use of antibiotics and the positive rate of β-lactamase in *H. influenzae* are increasing the rate of antimicrobial resistance (Wang *et al.,* 2021). In addition to various side effects, antibiotics treatment failures favor the emergence of multi-resistant strains, this current incidence makes these infections being a real public health problem (Goossens *et al.,* 2005).

Hence, the use of natural products from medicinal plants, such as essential oils (EOs) as an alternative treatment is more investigated in aromatherapy as treatments of respiratory tract affections (Gillissen *et al.,* 2013; Horváth *et al.,* 2015).

The lipophilic nature of EOs is important for their antimicrobial properties, as it allows them to pass through the bacterial cell membrane (phospholipid bilayer), leading to increased permeability and loss of intracellular contents, exerting the inhibitory effect on in-cell targets (Lee *et al.,* 2013; Henrik and Jeff, 2017). Although the antibacterial efficacy of essential oils has been well reported, less is known about their mechanism of action on the cell membrane of bacterial which directly correlate to the disruption and release of intracellular material. Nevertheless, potential sites of their antibacterial effects on the cytoplasmic membrane have been defined, i.e. damaging the cell wall, inhibiting the enzyme of the respiratory chain, and decreasing the proton motrice force (Dai *et al.,* 2020). When the bacterial cell membrane is disrupted, internal electrolytes leak into the culture medium, increasing the conductivity of the culture medium (Lambert *et al.,* 2001; Xu *et al.,* 2016). However, these effects of EOs, which have been reported in several studies, cannot explain if the cause the lethal effect on the bacteria is correlated to the disruption and loss of intracellular material. However, few studies have focused on evaluating the loss of membrane integrity or cell death with the release of biomolecules over time to demonstrate cell lysis (Lambert *et al.,* 2001; Xu *et al.,* 2016).

The aim of the present study is to investigate the antibacterial activity of EOs from *P. brazzeana* Baill. fresh roots, *A. sativum* L. bulbs and *E. giganteus* A. Rich. dry roots against *H. influenzae* and their mechanism of dynamic action on cell membrane integrity by correlating their bactericidal activity with cell releasing of DNA.

Material and Methods

Essential oils

P. brazzeana Baill. fresh roots, *A. sativum* L. bulbs and *Echinops giganteus* A. Rich. dry roots were harvested at the Western Region of Cameroon and identified by the Cameroon National Herbarium under the identification numbers 44810/HNC, 23647/SRF/CAM and 42918/HNC, respectively during a previous study carried out by Moni (2019). The essential oils were obtained by hydrodistillation using the Clevenger apparatus (Betote *et al.,* 2020).

Antibacterial Activities

Culture Media, Chemical Reagents and solvents

Dimethylsulfoxide (DMSO) and Tween 80 were purchased from Sigma-Aldrich (France). The Mueller Hinton Broth (Rapid Labs; CM-MHA135) and Mueller Hinton Agar (Rapid Labs; CM-MHB056) media were purchased from Becton Dickinson (USA). The Alamar Blue reagent was purchased from Sigma-Aldrich in France.

Essential oils and Antibacterial drug stock solutions

Essential oils were dissolved into the mixture tween 80 (7 %)-DMSO (7 %) (v:v). The stock solutions of obtained EOs were kept at 20 °C until use. Chloramphénicol® (Chl) at 1.000 µg/mL was used as standard antibacterial drug.

Bacterial Strains and Growth Conditions

The bacterial strains used for this study are *Haemophilus influenza* ATCC 49247 and two others Gram negative strains responsible of pneumonia such as *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 17102.

Preparation of the *Haemophilus influenza*e inoculum

A volume of 5 mL suspension was prepared using a fresh culture on agar Mueller Hinton Agar and adjusted to 0.5 McFarland (1.5×10^8 CFU/mL), then, diluted (1:10) before use. The inoculum was then homogenized and maintained on flask at 37 °C until use (CLSI, 2019).

Antibacterial Assessment of Essential oils

Determination of the Minimal Inhibitory Concentration (MIC)

The antibacterial activity of antimicrobial EOs was evaluated by the liquid microdilution method according to the CLSI 2019 guidelines (CLSI, 2019). In 96-well plates, each well received 100 µL of supplemented Mueller Hinton Broth (Rapid Labs; CM-MHB056 UK). Then, 100 µL of each bioactive essential oil was added in every first well. A geometric serial dilution ranging from 50 to 0.044 mg/mL was then carried out and subsequently, 100 µL of inoculum was added to all wells to yield concentration from 25 mg/mL to 0.022 mg/mL. The plates were then incubated at 37 °C for 24 h. 20 µL of Alamar Blue solution (0.1%) was used to reveal the bacterial growth.

All tests were carried out in triplicate and the plates were covered and sealed with parafilm, then, incubated at 37 ° C for 24 h. The MIC is defined as the lowest concentration of EOs, for which there is no bacterial growth visible to the naked eye (CLSI, 2019).

Determination of the Minimal Bactericidal Concentration (MBC)

From each well with EOs ≥ MIC, 50 µL was subcultured in 150 µL of Mueller-Hinton Broth and incubated at 37 °C for 24 h, the bacterial growth was revealed by adding 20 µL of Alamar Blue (0.1%). The MBC was defined as the lowest concentration of antimicrobials at which no visible growth of the bacteria was observed. The antibacterial activity of EOs was classified according to the Aligiannis *et al.,* (2001): that an antimicrobial have a strong activity when MIC is ≤ 100 µg/mL; moderate when 100 µg/mL ≥ MIC ≥ 1000 µg/mL and a weak when MIC ≥ 1000 µg/mL.

Dynamic action of essential oil on *Haemophilus influenzae* membrane integrity

The dynamic action of EO affecting membrane integrity consisted to demonstrate the bacteriolysis effect of EOs correlate to lethal action using the time-kill assay and DNA leakage assay of EO on *Haemophilus influenzae* strain exposure at 1×MBC. Chloramphenicol was used as standard drug.

Exposition phase of *Haemophilus influenzae* to MBC of EOs or Chloramphenicol

Into a tube, containing 1 mL of Mueller-Hinton Broth and EOs or Chloramphenicol at 1×CMB, 1 mL of *Haemophilus influenzae* inoculum (1.5×10^7 UFC/mL) was added to yield a final volume of 2 mL. Afterward, the mixture was cultured during 24 hours at 37 °C and shaked at different interval of times.

The time-kill assay and DNA quantification

Then, at seven-intervals (0 min, 45 min, 90 min, 165 min, 240 min, 360 min and 495 min), a 100 µL aliquot of the EO/Chl and inoculum mixture was 10-fold serially diluted across six tubes numbered from 1 to 6. Over times, 100 µL of the 2 numbered last tubes was seeded in triplicate on Mueller Hinton Agar. The Petri dishes were covered with parafilm paper and incubated at 37 °C for 24 h. An Aliquot (50 µL) of mixture was inoculated into a sterile cryotube then, covered with parafilm paper and stored at 4° C for DNA quantification at 260 nm using NanoDrop 1000 spectrophotometer (Thermo Fischer®) (Moni, 2019). A negative control (Inoculum + Medium) was used to compare the effects obtained by the test and

the blank (containing the culture medium). The reading assays were repeated tree times and the DNA concentration express in ng/µL. The inhibitory or lethal effect of EOs consisted to express the number of living cells in log cell/100µL over time, to get the time-kill curve (Rhayour, 2002; Moni, 2019). The DNA concentration and number of living cells were plotted versus time.

Correlation between lethality and bacteriolysis (DNA leakage)

The correlation between lethality and cell lysis was established by plotting and analyzing the number of living cells (Log UFC/mL), and the concentration of DNA (ng/µL) versus time, using GraphPad Prism 8 software; followed by the determination of the correlation coefficient R^2 using Microsoft Excel software. The closer the values of R^2 are equal to 0, the weaker the correlation between cell lysis and inhibition (lethal) is, and the closer these values are equal to 1, the stronger the correlation is.

Results and Discussion

Inhibition parameters (MIC and MBC) of EOs and Chloramphenicol

The inhibition parameters of EOs and Chloramphenicol against *H. influenzae* are reported in Table 1. The results allow us to quantify and classify the antibacterial activities according to Aligiannis *et al.,* (2001). From this table, it appears that against *H. influenzae*, *P. brazzeana* and *A. sativum* EOs exhibited strong activities with MIC ranging from 9.76 µg/mL to 39.06 µg/mL respectively, meanwhile *E. giganteus* exhibited moderate activity with MIC of 156.25 µg/mL. Compared to the two other reference strains, *P. brazzeana* showed strong activity against *P. aeruginosa* and *K. pneumoniae* with MIC at 4.88 µg/mL.

A. sativum exhibited strong activity with MIC ranging from 39.06 µg/mL to 78.12 µg/mL while *E. giganteus* EO exhibited moderate activity with MIC ranging from 156.25 µg/mL to 312.5 µg/mL, respectively against *P. aeruginosa and K. pneumoniae*. We found that *H. influenzae* is less sensitive to Chloramphenicol with a MIC of 31.25 µg/mL, compared to *K. pneumoniae* and *P. aeruginosa* (7.81 µg/mL).

Dynamisms of antimicrobials on *Haemophilus influenzae* membrane integrity

After been exposed for 560 min to antimicrobials, we noticed that the number of viable cells of *H. influenzae* decreases while the quantity of extracellular DNA increases for all EOs except Chloramphenicol (Figure 1). The dynamic actions of these two effects on bacteria were spotted versus time to yield the curves presented by Figure 1.

Each EO and Chloramphenicol exhibited three types of curves appearances describing their dynamism action on bacteria causing cell lethality and DNA leakage. First, a synchronic curve represented by a symmetric appearance between lethality and DNA leakage over time (Fig. 1a), reflecting a synchronic action of EO between lethality and cell lysis. Then, an asynchronic curve represented by the asymmetric appearance between lethality and DNA leakage (Fig. 1b and Fig. 1c), which shows that cell lysis effect occurs after the lethal action of EO. Lastly, a non-synchronic curve represented by the single appearance of lethality with absence of DNA release (Fig. 1d), reflecting EO lethal action without cell lysis. Based on all mentioned above, *Pentadiplandra brazzeana* EO exhibited simultaneous lethal and lysis actions against *H. influenzae* (Fig. 1a), with a symmetric point of action at half time (280 min). Meanwhile, *Allium sativum* and *Echinops giganteus* EOs exhibited lethal action, then cell lysis over time with joint point of action at 420 min (Fig. 1b and Fig. 1c). In contrast, Chloramphenicol exhibited only lethal action against *H. influenza* (Fig. 1d) with a joint point between living cells and DNA released when there are no living cells.

Correlation between lethality and DNA leakage

After plotting the number of living cells versus DNA concentration over the time, the correlation coefficients ($R^{2)}$ involved enable us to put in evidence a strong, moderate and weak correlation as show in Table 2. From this table, it appears that a strong correlation between lysis and inhibition was observed with the EO of *P. brazzeana* with R^2 of 0.94, showing a symmetric evolution between living cells and DNA released. *A. sativum* and *E. giganteus* EOs exhibited moderate correlation between lysis and inhibition with R^2 at 0.81 and 0.89 respectively. A weak correlation was observed with the reference antibiotic, Chloramphenicol, with R^2 at 0.66.

This study investigated first of all the bactericidal activity of EOs from *Pentadiplandra brazzeana* fresh roots, *Allium sativum* bulbs and *Echinops giganteus* dry roots then, their dynamic effects on the cytoplasmic membrane integrity of *Haemophilus influenzae*. Out of the essential oils tested against *H. influenzae*, *P. brazzeana* and *A. sativum* exhibited strong activities with MICs at 9.76 µg/mL and 39.6 µg/µL, meanwhile *E. giganteus* showed moderate activity at 156.25 µg/mL. The same EO's activities profiles were obtained against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* but with slight difference in MIC. The difference between EOs activities is directly related with the chemical composition of each essential oil (nature of their main compounds), the virulence factors like biofilm formation ability of some strains to exhibit a higher resistance to EOs compare to planktonic cells (Bazargani *et al.,* 2016).

The highest EO activity of *P. brazzeana* is probably due to the action of isothiocyanate compounds (ITC at about 100 % of the EO) with benzyl-isothiocyanate at 91.3 %. Voundi *et al.,* in 2015, reported that *D. gossweileri* EO which contains 94.5 % of benzyl isothiocyanate exhibited strong antibacterial activity against four *Bacillus spp* (MIC ranging from 0.44 to 9.7 µg/mL) and anti-germinating activity against their spores at 2 µg/mL (Voundi *et al.,* 2015). In fact, it has been suggested that ITC functional group (R–N=C=S) can covalently bind to the cellular targets due to the high electrophilic effect of their central carbon with the nucleophilic centers of nucleic acids or proteins (Tajima *et al.,* 1998; Wilson, 2011, Zhang *et al.,* 2006). Concerning *A. sativum* EO, its strong antibacterial activity comes from their high amount of allicin derivatives like allyl sulfide, allyl disulfide or trisulfide in contrast to their crude extract which highly contained allicin, ajoem as main compounds (Moni, 2019). These organosulfur compounds can pass through the cell wall to induce DNA replication inhibition, loss of energy substrate (glucose, ATP) and electron flow resulting to coagulation of the cell content and bacterial death (Tang *et al.,* 2021; Ahn *et al.,* 2001; Lin, *et al.,* 2000). The moderate activity of *E. giganteus* EO is due to the antibacterial potential of cyclic triterpenes such as silphiperfol-5-ene (27.4%), silphiperfolan-6-α-ol (11.3%), silphiperfolene (7.4%) 7-epi-silphiperfol-5-ene (6.0%) and caryophyllene (7.0%) (Moni, 2019). This moderate activity of these sesquiterpenes against *Mycobacterium tuberculosis* $H_{37}R_v$ has however been mentioned by Pinto *et al.,* (2009). Most of those cyclic triterpenes are heavy and lipophilic compounds which usually inhibit the synthesis of peptidoglycan and increase the membrane permeability according to (Cowan, 1999).

The action mechanism of essential oils related with the disruption of membrane integrity is complex and often not fully explained. In fact, most of classic methods revealed the action mechanism of EOs on cytoplasmic membrane and cell wall by investigating their biomolecules (DNA, RNA and proteins) leakage effect at the end of incubation. However, in this way we cannot confirm that direct action of EO bioactive compounds leads to bactericidal effect in tandem with cell release of biomolecules, whereas indirect action leads first to bactericidal effect and then to release of biomolecules. In this study, the dynamic action of EOs on membrane integrity was put in evidence by the correlation between decreasing viable bacterial cell counts and increasing extracellular bacterial DNA. Depending to the appearance curves and the correlation coefficient (R^2), *P. brazzeana* EO exhibited bactericidal and lysis action at the same time against *H. influenzae*. Many studies reported these both properties of ITC, especially relating to leakage of neither ATP molecules nor damage resulting to the cell alteration of the cell membrane permeability, but in addition to that, ITC inhibits thioredoxin synthesis, a molecule that plays an important role in DNA synthesis (Lin *et al.,* 2000; Ahn *et al.,* 2001). The authors hypothesized that Benzyl ITC, having both lipophilic and electrophilic properties, might penetrate through the outer bacterial membrane and hamper the ability of the bacterium to maintain its membrane potential, similar to the effect observed with cationic peptides (Sofrata *et al.,* 2011). These data indicated that ITC has most similar to polymyxin B with respect to its antibacterial effect on cell membranes and on leakage of cellular metabolites.

The bactericidal action of *A. sativum* and *E. giganteus* EOs on *H. influenzae* occurs after the linkage of DNA thus, the disruption of membrane integrity. In general, the antibacterial activity of diallyl polysulfides from garlic is due to inhibition of proteolysis or protease activity (Bakri and Douglas, 2005). Due to their proteolysis action, we can suggest that these compounds interacted with enzymes involved in cell wall and membrane synthesis, expressed through further bacteriolysis effect. This hypothesis explains the asymmetry between lethality and DNA leakage.

Fig.1 Effects of *P. brazzeana* (a), *E. giganteus* (b) and *A. sativum* (c) EOs, and Chloramphenicol (d) on *H. influenzae* cell membrane during exposition at MBC.

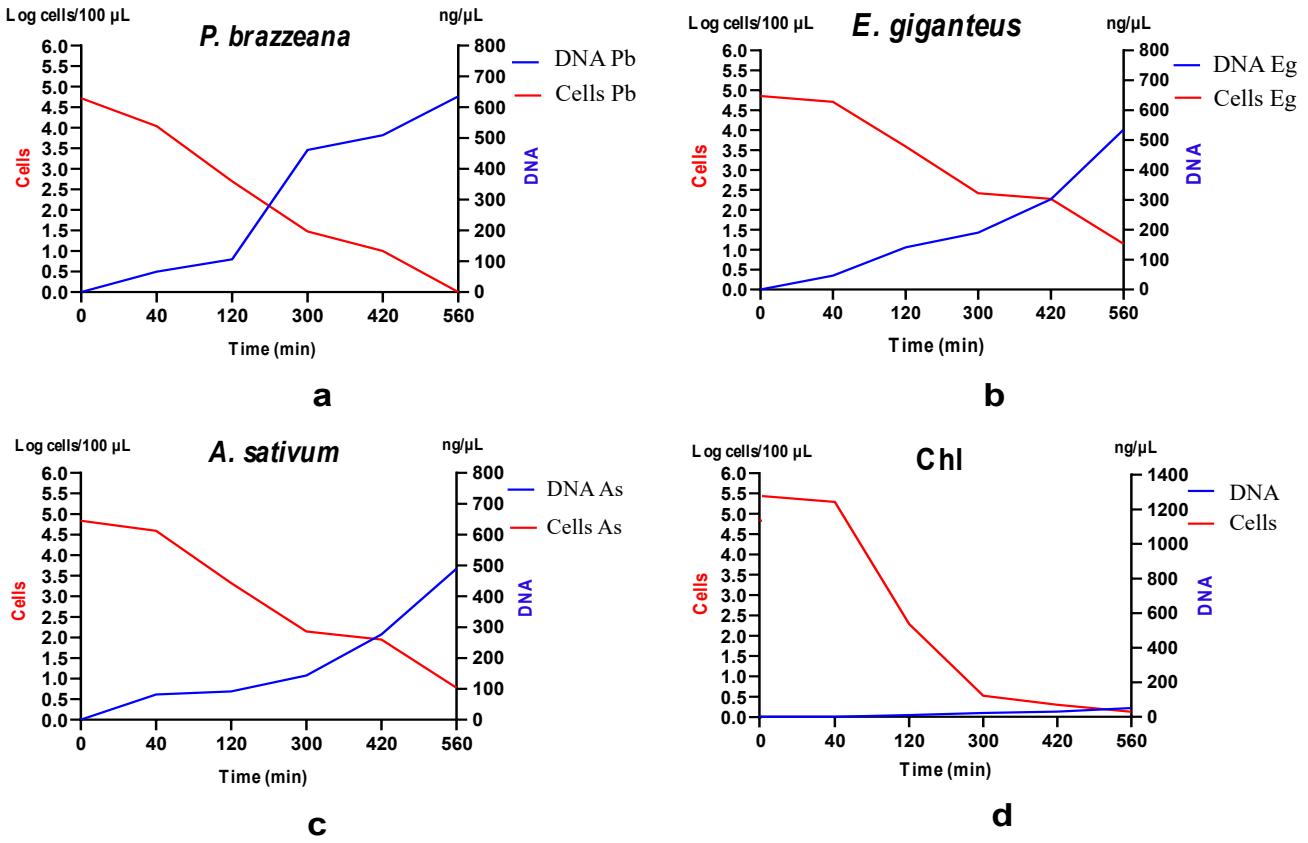

Table.1 Inhibitory parameters of EOs and reference drug against *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*

EOs	Inhibitory parameters (µg/mL)								
	Haemophilus influenzae			*Pseudomonas aeruginosa*			*Klebsiella pneumoniae*		
	MIC	MBC	Activity	MIC	MBC	Activity	MIC	MBC	Activity
Pb	9.76	625	Strong	4.88	9.76	Strong	4.88	78,12	Strong
As	39.06	625	Strong	78.12	156.25	Strong	39.6	78.12	Strong
Eg	156.25	1250	Moderate	156.25	312.5	Moderate	312.5	1250	Moderate
Chl	31.25	65.50	Strong	7.81	125	Strong	7.81	7.81	Strong

Legend: MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; Pb: *Pentadiplandra brazzeana*; As: *Allium sativum*; Eg: *Echinops giganteus*; Chl: Chloramphenicol.

Table.2 Correlation coefficient between lethality and DNA leakage

Correlation coefficient (R^2)			
Pb	**As**	**Eg**	**Chl**
0.94	0.81	0.89	0.66

Legend: Pb: *Pentadiplandra brazzeana*; As: *Allium sativum*; Eg: *Echinops giganteus*; Chl: Chloramphenicol.

It can also justify the fact that cell death induced by both essential oils does not seem to be caused by a lytic effect. A recent study established that the antibacterial activity of diallyl polysulfides is due to their ability to react with sulfhydryl groups of various enzymes of *B. subtilis* such as bacillithiol and CoA, and with amino acid cysteine (Kyo *et al.,* 2001).

Concerning the lethal effect of *E. giganteus* EO against *H. influenzae*, a few studies reporting the effect of these cyclic sesquiterpenes on cell membrane integrity. However, according to Sulsen *et al.,* in 2009, the lethal effect of *E. giganteus* EO against the parasite *Trypanosoma cruzi* enhances the apoptosis mechanism leading to the disruption of membrane integrity, loss of mitochondrial membrane potential and DNA fragmentation. Although demonstrated on eukaryotic cells, the results of Sulsen *et al.,* are in agreement with those of our study; it was noted that the extracellular release of DNA occurs after the lethal effect of EO, justifying the asymmetric appearance of the curve obtained. In contrast, Chloramphenicol showed only a lethal effect without affecting the membrane integrity of *H. influenzae*, justifying the inhibition of protein synthesis in the ribosome without affecting the membrane integrity.

In conclusion, the fight against Pneumonia as well as other respiratory tract diseases, involves the use of reference antibiotics whose inhibitory actions face resistance and multi-resistance phenomena. This raises the need to permanently seek therapeutic alternatives, such as EOs. The identification of the therapeutic targets and the understanding of the mechanism of action of these EOs are an asset for the justification of the effectiveness of the drug which they constitute.

During this work we evaluated the antibacterial activities of EOs of three medicinal plants and illustrated one of the mechanisms which justify this activity through not only the evaluation of the kinetics of lethality and of release DNA, but also through the evaluation of the correlation between these two phenomena. It appears that: all the EOs used in this study presented antibacterial properties but the most active is that of *P. brazzeana*. The lytic activity of the EOs allows the release of the DNA revealed by quantification with Nandrop 1000. The positive correlation between cell lethality and DNA release confirms the bactericidal activity of these EOs, which starts with the bioactive molecules of the EOs binding to bacterial membranes, disrupting their structure and permeability, and ultimately causing progressive DNA loss through significant bacterial cell lysis.

Abbreviations

DMSO: Dimethylsulfoxide; Chl: Chloramphénicol®; EOs: Essential Oils; MIC: Minimal Inhibitory Concentration; MBC: Minimal Bactericidal Concentration; CFU: Colony Forming Unit; DNA: Deoxyribonucleic Acid; Pb: *Pentadiplandra brazzeana*; As: *Allium sativum*; Eg: *Echinops giganteus*.

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Author Contributions

G. Amongue II, M-A.N. Diengue, H.SO. Mbarga, G.H.D. Bea and E.D.F.N. Moni: Initiated the project, participated in laboratory research (antibacterial activity and time killing kinetics) and data analysis, and wrote and revised the manuscript article. S.H.E. Riwom: Initiated the project, guided the research work, and revised the manuscript. G.E. Feudjeu and P.H.D. Betote: Participated in the phytochemical analysis, data analysis, and revision of the manuscript article. M.A. Nyegue: Guided the research work and revised the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All the results presented in this study were carried out by authors, and the data used as references were properly cited.

Declarations

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Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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