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Research Article

Synergistic Effects of Essential Oils and Antibiotics Against Some Bacterial Strains

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Abstract

The emergence of antibiotics resistances become an alarming situation due to the resurgence of cases. Scientific community explore therapeutics alternatives, including essentials oils and their combination with antibiotics. The designed study aimed to evaluate the combined effects of essentials oils from *Drypetes Gosswelleri, Echinops giganteus*, *Melaleuca leucadendron* essentials oils and antibiotics against strains implicated in infectious deseases. The antibacterial effect of essentials oils and antibiotics (Ciprofloxacine and Ceftriaxone) was carried out against *Pseudomonas aeruginosa, Klebsiella pnemoneae, Salmonella enteritidis, Staphylococcus aureus* and *Bacillus cereus* strains using the microdilution method. The synergistic effects were studied by the isobologram method to conclude on the interaction type. The results revealed that the essential oils were active against all the tested bacteria. *Drypetes Gosswelleri* EO showed the strongest activity with MICs ranging from 1.46 µg/mL to 11.71 µg/mL, followed by *Echinops giganteus* essential oil with MICs ranging from 2.92 µg/mL to 23.43 µg/mL and *Melaleuca leucadendron* had the lowest activity with MICs ranging from 5.88µg/mL to 750µg/mL. The combinations realized between *Drypetes Gosswelleri* and *Echinops giganteus* EOs and with two antibiotics use to evaluate the interaction type agains *Salmonella enteritidis* and *Staphylococcus aureus.* MICs obtained with the combinations are lower than those obtained with the EOs and antibiotics tested individually. The isobolograms plotted inform about a synergistic effects of combinations made with *Drypetes Gosswelleri, Echinops giganteus* EOs, Ciprofloxacin and Ceftriaxone against *S. enteritidis* and *S. aureus* strains. Based on the results obtained, the combinations between EOs and the antibiotocs, represent an interesting therapeutic alternative.

Keywords: antibacterial, essential oil, combination, isobolograms, synergistics, Minimale Inhibitory Concentration, Minimale Bactericidal Concentration.

1. INTRODUCTION

Since 1980s, the number of antibiotics resistant strains has increased exponentially, making difficult it in the elimination of pathogens[1]. One alternative explored by the scientific community to overcome multi-drug resistant is antimicrobials combination[2]. This combination approach based on the concept that the more targets a drug have on a microorganism the more difficult it will be for the cell to develop resistance strategies[3,4,5,6]. For this reason, research of medicinal and aromatic plants containing active compounds is increasing[7,8].

Since antiquity, medicinal plants have been used in phytotherapy against several diseases, given their richness in hundreds components with therapeutic virtues[9], but their use was based on traditional practices and applications without scientific bases[10]. Aromatic plants have antimicrobials properties known and used for a long time[11,12], they, are able to synthetize aromatics compounds called essential oils (EOs)[13]. These EOs have a very broad inhibition spectrum including Gram-positive and Gram-negative bacteria, fungi, as well as viruses[1]. Currently, they are used in the fields of food industry, cosmetology and hygiene products sector[15]. In pharmaceutical industry, bacteriostatic, bactericidal, vermicidal, fungicidal, antiseptic, insecticidal, antiviral, anti-inflammatory, antioxidant and many more EOs properties are used[15,16]. antimicrobial power of EOs are due to the diversity of active compounds with antimicrobial properties makes resistances to these EOs difficult or impossible by micoorganisms, because they cannot undergo resistances mutations to all active molecules simultaneously present in EOs[17]. Based on this observation, the combinations of several EOs with each other or with antibiotics would therefore be an effective way to reduce the resistance risk and also increase antimicrobial activity effectiveness [18].

Drypetes Gosswelleri, Echinops giganteus and *Melaleuca leucadendron* are Cameroonian's pharmacopoeia plants. In Cameroon and Congo the powdered stem bark of *D. gosswelleri* is eaten to treat sexual weakness and venereal diseases[19]. The roots of *E. giganteus* are widely used in the traditional medicine of Cameroon and Nigeria for the treatment of various diseases such as heart disease, gastric disorders, stomach pain, and asthma attacks[20]. *M.*

leucadendron is used for antiseptic, antipasmodics, antineuralgics and antirheumatics properties[21]. It would be interesting to assess if these three EOs can potentiate the activity of some antibiotics in the combination approach. Thus, The designed study aimed to evaluate the combined effects of essentials oils from *Drypetes Gosswelleri, Echinops giganteus*, *Melaleuca leucadendron* and antibiotics against strains implicated in infectious deseases.

2. MATERIALS AND METHODS

2.1. Preparation 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). The leaves of *Melaleuca leucadendron* (*M. leucadendron)* were harvested in Yaoundé. EOs were obtained by hydrodistyllation. The plant part used was crushed and immersed in water, then subject to hydrodistillation using Clevenger-type apparatus during 6 to 8 hours. EO was collected, and dried with anhydrous sodium sulfate[18]. 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.

2.2. Antimicrobials agents

For this study, two antimicrobials were used : injectable Ciproflaxacin (CIP) N°RA/DRUGS/RAJ/1594 (BONCIPRO(R)) and injectable Ceftriaxone (CEF) N°GUJ/DRUGS/G/198 (XONE). Antibiotics tested solutions were prepared at 100 µg/mL.

2.3. Bacterials strains

Five bacterials strains have been used in this study. Two Gram positive: *Bacillus cereus* ATCC 11966 and *Staphylococcus aureus* ATCC 25923 and three Gram negative: *Salmonella enteritidis* 155A, *Pseudomonas aeruginosa* ATCC 27853 and isolate of *Klebsiella pneumoneae* spp,. The strains were obtained from the Pharmacology and Drugs Discovery Laboratory (at the Institute of Medical Research and Medicinal Plants Studies), except isolate of *Klebsiella pneumoneae* spp obtained from the University Teaching Hospital of Yaounde Cameroon. All was stored at -80°C. Before starting, the strains were sub-cultured on solid medium Mueller Hinton Agar (MHA) followed by Mueller Hinton Broth (MHB).

2.4. Evaluation of Antimicrobial activity

Microdilution method was carried out according to the Microplate Alamar Blue Assay as described previously by Collins and Franzblau and modified by Jimenez-Arellanes *et al.*[22,23]. A stock of EOs solution initialy prepared at 6000 µg/mL was added in the wells containing culture medium MHB to reach final samples concentrations ranging from 3000 µg/mL to 2.92 µg/mL for EOs, from 50 µg/mL to 0.048 µg/mL for CIP and CEF. The wells were inoculated with bacteria inocula (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 CIP and CEF. Each 96 wells microtiter plates were mixed and incubated at 37 °C for 24 hours. After incubation periods, 20µL of 0.02 % resazurin salt solution were added to individual wells and the plates were reincubated for 20 minutes and color changing was checked. The resazurin changing colour from blue to pink indicated reduction of the indicator and thus bacterial growth. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of samples at which the microorganism did not demonstrate growth[24]. The Minimal Bactericidal Concentration (MBC) of promising EOs was assessed by sub-culturing MIC test microtiter plates on MHB medium. The MBC was considered as lowest concentration at which no growth occurred in the medium. The value of the ratio MBC/MIC was used to classify antimicrobial activity : MBC/MIC ≤ 4 for a bactericidal effect, 4<MBC/MIC≤16 for a bacteriostatic effect and MIC/MBC>16 for tolerance effect[25].

2.5. Synergistic effect of combinations

2.5.1 Determination of the minimal inhibitory concentration of combinations

The MICs of combination was determined using isobologram method[18,26,27]. This method is base on the inhibitory concentration determination of two antimicrobials combination on a 96-wells microplate followed by the isobologram construction. The tests was done against the two bacterials strains showing the best sensitivity to EOs one Gram positive and one Gram negative (*Staphylococcus aureus* and *Salmonella enteritidis*). The combinations was realized between *D. gossweilleri* and *E. giganteus* EOs, which showed the best activities against bacteria and with two antibiotics (Ciprofloxacin and Ceftriaxone). For the combined solutions, one antibacterial was at a variable concentrations (MIC/16, MIC/8, MIC/4, MIC/2 and MIC) and another at a fixed concentration (MIC).

Thirty solutions was obtained and tested, of which five solutions of each concentration of EOs and antibiotics combinations below: Dg*Eg (variable concentration of *D. gossweilleri* and a fixed concentration of *E. giganteus*), Eg*Dg (variable concentration *E. giganteus* and a fixed concentration for *D. gossweilleri*), Dg*CIP (variable concentration of *D. gossweilleri* and a fixed concentration of CIP), Eg*CIP (variable concentration of *E. giganteus* and a fixed concentration of CIP), Dg*CEF (variable concentration of *D. gossweilleri* and a fixed concentration of CEF) and finally Eg*CEF (variable concentration of *E. giganteus* and fixe concentration of CEF).

In the width direction of microplate, from wells 1 to 10, after adding 100 µl of culture medium MHB, the solutions initially prepared at different concentrations of each combination were added in duplicate in the following order : MIC/16 *MIC, MIC/8*MIC , MIC/4* MIC, MIC/2* MIC, and MIC*MIC. Successive dilutions following a geometric progression of 1/2 ratio were carried out from wells 1 to 10 to the well G. 100 µL of bacterial inoculum at 6 Log CFU/mL were added. The wells of columns 11 and 12 were used as a negative control for combined solutions which contained MHB culture medium and solutions at decreasing concentrations. The wells of row H were used as a positive control for bacterial growth and contained MHB medium and inoculum (Figure 1). The microplate was closed, covered with film paper and incubated at 37°C for 24 hours. The results were read by turbidity observation of wells: turbidity indicating bacterial growth.

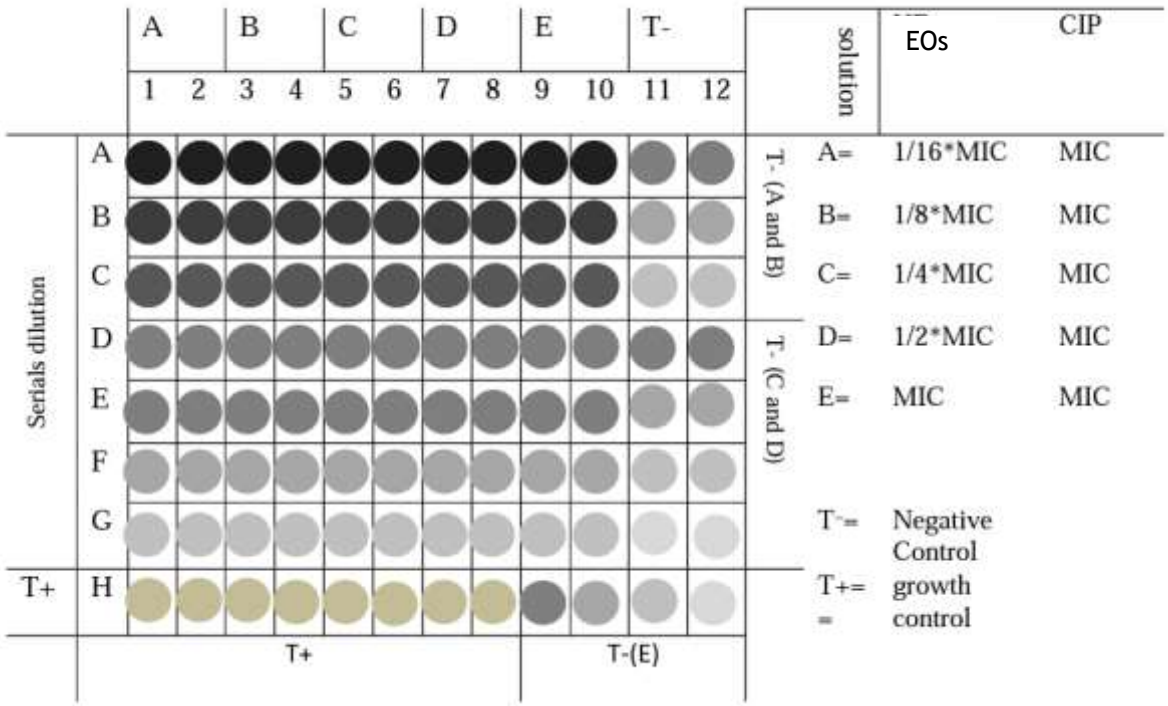

Figure 1: protocol of antimicrobials combination on 96 wells microplate.

The wells of line A are those in which differents solutions of combination (A, B, C, D and E) are deposited, the series of dilutions are carried out in direction A to G (these wells contain bacterial strains, MHB medium and antimicrobial solutions). wells H1 to H8 are used as a growth control (without antimicrobials, just MHB medium and bacterial innoculum), the negative controls of solutions (just antimicrobial and MHB medium) are represented in wells A11 to C11, A12 to C12, D11 to G11, D12 to G12 and H9 to H12 respectively for solutions A,B,C,D and E.

2.5.2 Isobolograms construction

Isobologram is a two-dimensional graph with, on the axis, the concentrations of each compound used in the association are represented[18,28,29]. This method aims to define on a geometric graph the type of interaction between substances. According to this method, the points connecting to form diagonal represent the MIC values of antimicrobials tested individually, one on x-axis and the other on y-axis. This diagonal is the isobole of additivity. The junction points position of MICs of two antimicrobials in combination (at different concentration : five points) allow us to conclude on interaction type. Thus, a point located at the top of the right side of the isobole indicates an antagonism. Those below indicates a synergy and the point near or on isobole indicates additivity[18,26,30].

3. RESULTS

3.1. Antimicrobial activity

Antibacterial parameters (MIC and MBC) of essential oils (EOs) against bacterials cells are reported in Table 1. EOs from *D. gosswelleri* showed the strongest activity against all the bacterials strains with MICs of 1.46 µg/mL against *S. aureus, S. enteritidis* and *B. Cereus,* 5.88 µg/mL against *K. pneumoneae* and 11.71 µg/mL against *P. aeruginosa.* Followed by *E. giganteus* EO with MICs ranging from 2.91 µg/mL to 23.43 µg/mL. *M. leucadendron* had the lowest activity with MICs ranging from 5.88 µg/mL to 750µg/mL.

CEF did not showed any activity against *P. aeruginosa* at the maximal concentrations tested against others strains (1500 µg/mL), against the others strains, the MICs of CEF equal to 0.19 µg/mL. The MICs of CIP ranging from 0.048 µg/mL to 0.19 µg/mL. It should also be notice that *P. aeruginosa* is a strains against which EOs had a less strong activity.

For all antibacterial tested the bactericidal effect was obtained against *P. aeruginosa* , except for CEF. *D. Gosswelleri* EO that had a bactericidal effect against most strains except against *B. Cereus.*

The more sensitive strains at EOs was *S. aureus* strain among the Gram positive bacteria and *S. enteritidis* among the Gram negative bacteria. against these strains, *D. gosswelleri* had MIC equal to 1.46 µg/mL and 2.91 µg/mL and *E. giganteus* EOs, MIC equal to 1.46 µg/mL and 5.88 µg/mL for.

Table 1: inhibition parameters against bacterial strains in µg/mL.

Essential oils or antibiotics	Antibacterial Parameters	*P. aeruginosa*	*S. aureus*	*S. enteritidis*	*K. pneumoneae*	*B. cereus*
D. gosswelleri.	MIC	11.71	1.46	1.46	5.88	1.46
	MBC	11.71	5.88	2.92	23.43	23.43
	effect	bactericidal	bactericidal	Bactericidal	bactericidal	bacteriostatic
E. giganteus	MIC	23.43	2.91	5.88	11.71	2.91
	MBC	46.87	23.43	46.87	46.87	46.87
	Antibacterial effect	bactericidal	bacteriostatic	bacteriostatic	bacteriostatic	bacteriostatic
M. leucadendron	MIC	750	11.71	5.88	23.43	23.43
	MBC	750	93.75	93.75	187.5	375
	Antibacterial Effect	bactericidal	bacteriostatic	bacteriostatic	bacteriostatic	bacteriostatic
Ciprofloxacin	MIC	0.05	0.19	0.19	0.19	0. 1
	MBC	0.19	0.39	0.39	0.39	0.39
	Antibacterial Effect	bactericidal	bactericidal	bactericidal	bactericidal	bactericidal
Ceftriaxone	MIC	>25	0.10	0.10	0.10	0.10
	MBC	ND	1.56	1.56	1.56	1.56
	Antibacterial Effect	ND	bacteriostatic	bacteriostatic	bacteriostatic	bacteriostatic

Legend : MIC : minimal inhibitory concentration, MBC : minimal bactericidal concentration, >1500 : no activity at consentration tested, ND : no determined.

3.2. Study of synergistic activity of combinations

The MIC combinations results were reported in Tables 2. In the combinationsm the MICs of antibacterial combined was reduces compared to MICs obtained when they were tested individually. The best reduction of MICs was obtained by the combination using *D. gosswelleri* EO against the two strains. For the combination of *D. gosswelleri* EO and CEF, the MIC obtained against the strains equal to 0.04 µg/mL for each antibacterial initialy equal to 1.46 µg/mL and 0.09 µg/mL respectively. The combination of *D. gosswelleri* EO with CIP showed MIC equal to 0.19 µg/mL for *D. gosswelleri* EO and 0.02 µg/mL for CIP against the strains, initialy equal to 1.46 µg/mL et 0.01 µg/mL respectively.

Table 2: Minimal inhibitory concentration of the Combination on bacterials strains in µg/mL.

Combination		MIC against *S. aureus*		MIC against *S. enteritidis*	
		Alone	in combination	Alone	in combination
*Dg*Eg*	Dg	1.46	**0.02**	1.46	**0.02**
	Eg	2.92	**0.73**	2.92	**0.73**
*Eg*Dg*	Eg	2.92	**0.02**	2.92	**0.01**
	Dg	1.46	**0.18**	1.46	**0.09**
*Dg*CIP*	Dg	1.46	**0.01**	1.46	**0.01**
	CIP	0.19	**0.02**	0.19	**0.02**
*Dg*CEF*	Dg	1.46	**0.04**	1.46	**0.04**
	CEF	0.09	**0.04**	0.09	**0.04**
*Eg*CIP*	Eg	2.92	**0.01**	2.92	**0.09**
	CIP	0.19	**0.01**	0.19	**0.09**
*Eg*CEF*	Eg	2.92	**0.02**	2.92	**0.02**
	CEF	0.09	**0.01**	0.09	**0.01**

Legend : Dg: *D. gosswelleri*., Eg: *E. giganteus* and Ml: *M. leucadendron*, CIP: Ciprofloxacin, CEF: Ceftriaxone, Dg*Eg: combination of *D. gosswelleri* EO at varying concentrations and *E. giganteus* EO at a fixed concentration equal to the MIC. Eg*Dg: combination of *E. giganteus* EO at varying concentrations and *D. gosswelleri* EO at a fixed concentration equal to the MIC. Dg*CIP: combination of *D. gosswelleri* Eo at varying concentrations and Ciprofloxacin at a fixed concentration equal to the MIC, Dg*CEF: combination of Eo from *D. gosswelleri* at varying concentrations and Ceftriaxone at a fixed concentration equal to the MIC, Eg*CIP: combination of *E. giganteus* EO at varying concentrations and ciprofloxacin at a fixed concentration equal to the MIC, Eg*CEF: combination of *E. giganteus* EO at varying concentrations and Ceftriaxone at a fixed concentration equal to the MIC.

Using these MICs, the type of interaction of different combined concentration was determined using the isobologram construction (figure 2, 3 and 4). The results obtained showed the synergistic action of all contrations of the combinations tested against *S. aureus* and *S. enteritidi* strains.

Figure 2 represent the isobolograms for the combination between the EOs. Synergistic action was obtained for the combined concentrations against *S. aureus* and *S. enteritidis.* For the combinaison Eg*Dg, the MICs obtained with *D. gosswelleri* EO was the same against *S. enteritidi* (0,09 µg/mL) and against *S. aureus* (0.18 µg/mL) for all the solutions. While, the MICs values of *E. giganteus* EO ranging from 0.09 µg/mL at 0.73 µg/mL *S. aureus* and *S. enteritidis* for all the solutions, the interaction type was the same (synergistic) in any case. It for this reason, all point appear in the same posistion on the isobolograms of this combination (figure 2 : C and D).

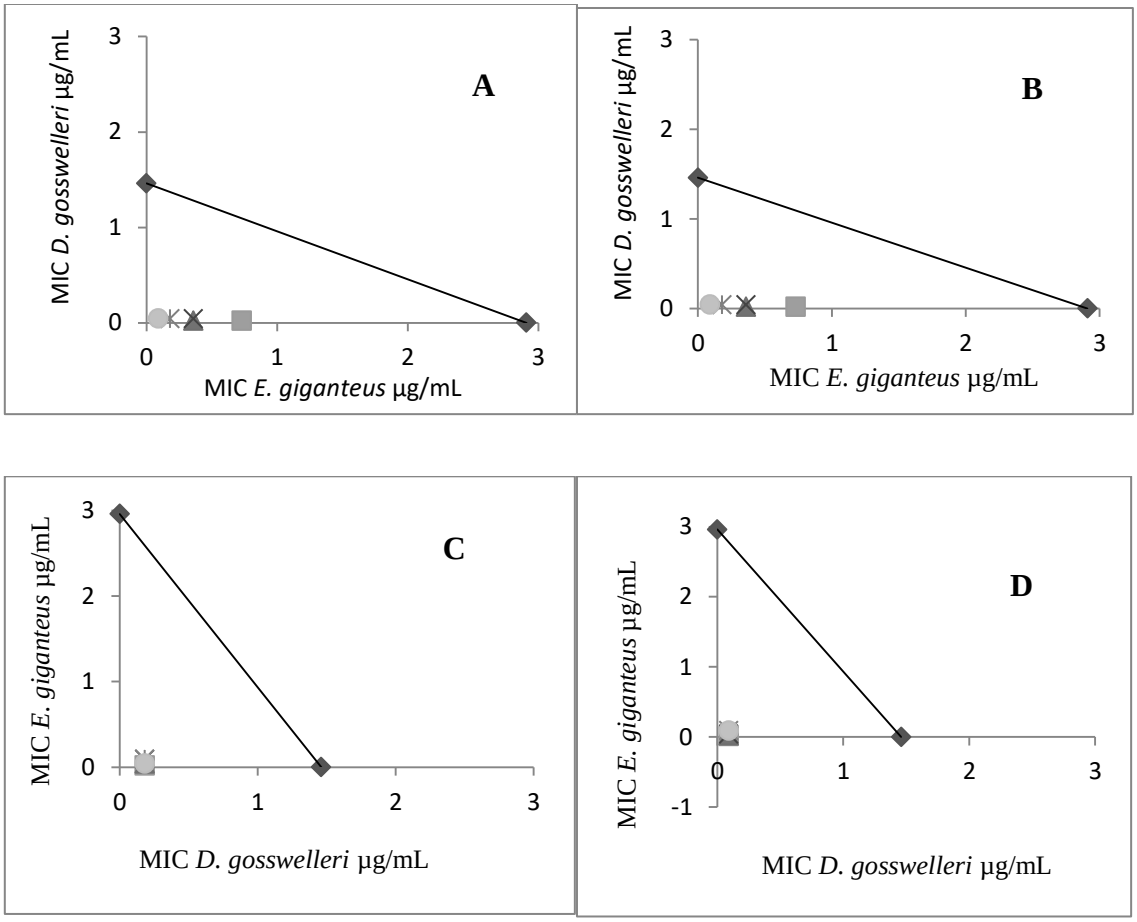

Figure 2 : synergytic effect of combination between D. *gosswelleri* EO and *E. giganteus* EO.

A : Dg*Eg: combination of D. *gosswelleri* EO at varying concentrations with *E. giganteus* EO at a fixe concentration equal to the MIC against *S. aureus*. B: Dg*Eg: combination of *D. gosswelleri* EO at varying concentrations with *E. giganteus* EO at a fixe concentration equal to the MIC against *S. enteritidis*.C: Eg*Dg: combination of *E. giganteus* EO at varying concentrations with *D. gosswelleri* EO at a fixe concentration equal to the MIC against *S. aureus*. D: Eg*Dg: combination of *E. giganteus* EO at varying concentrations with *D. gosswelleri* EO at a fixe concentration equal to the MIC against *S. Enteritidi.*

Figure 3 represent the isobolograms for the combination of EOs with CIP. Synergistic action was obtained for the combined concentrations against *S. aureus* and *S. enteritidi* strains. Against *S. aureus* strain, the range of the MICs values of CIP for the combinaison of Eg*CIP (0.006 µg/mL to 0.01 µg/mL) was lower than MICs obtained by combination of Dg*CIP (0.003 µg/mL to 0.02 µg/mL). While against *S. enteritidi* strains, the range of the MICs values of CIP for the combinaison of Dg*CIP (0.003 µg/mL to 0.023 µg/mL) was lower than MICs obtained by combination of Eg*CIP (0.003 µg/mL to 0.09 µg/mL).

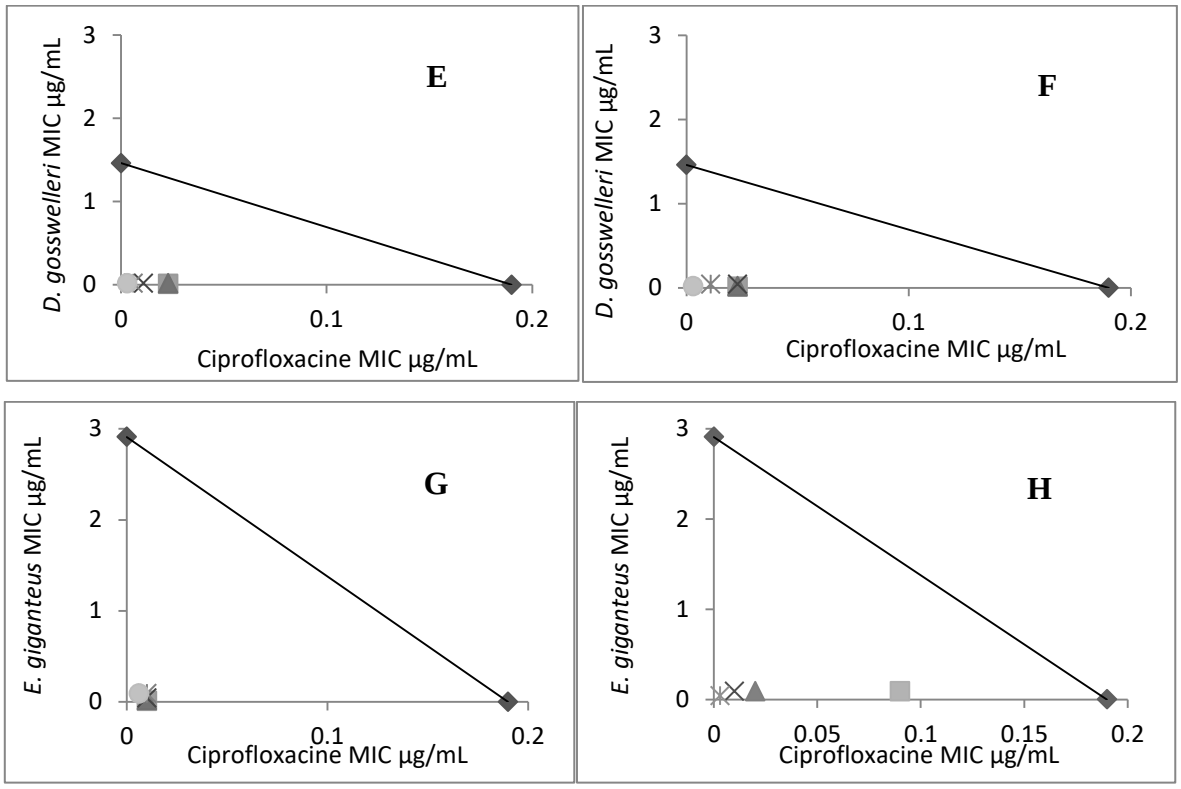

Figure 3 : synergytic effect of the combination between Eos and CIP.

E: Dg*CIP: combination of *D. gosswelleri* EO at varying concentrations with Ciprofloxacin at a fixe concentration equal to the MIC against *S. aureus*, F: Dg*CIP: combination of *D. gosswelleri* EO at varying concentrations with Ciprofloxacin at a fixe concentration equal to the MIC against *S. enteritidis*, G : Eg*CIP: combination of *E. giganteus* EO at varying concentrations with Ciprofloxacin at a fixe concentration equal to the MIC against *S. aureus*, H: Eg*CIP: combination of *E. giganteus* EO at varying concentrations with Ciprofloxacin at a fixe concentration equal to the MIC against *S. enteritidis.*

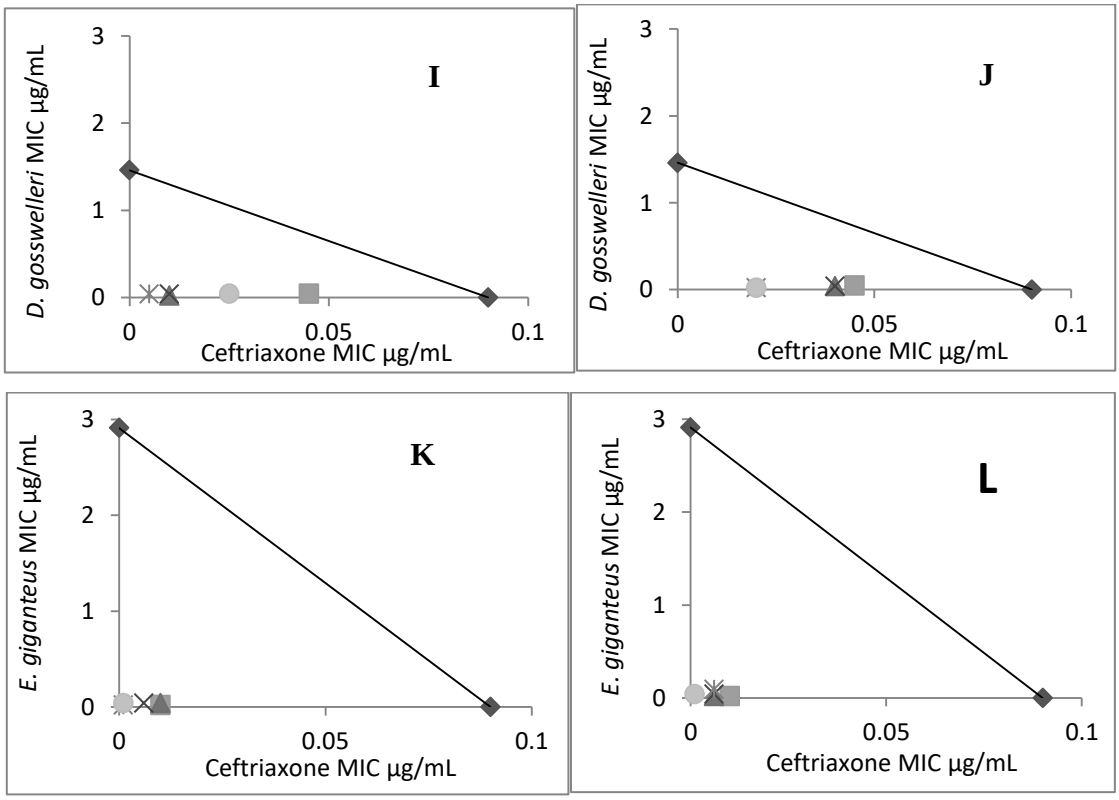

Figure 4 : synergytic effect of combination between EOs and CEF.

I: Dg*CEF: combination of *D. gosswelleri* EO at varying concentrations with Ceftriaxone at a fixe concentration equal to the MIC against *S. aureus*, J: Dg*CEF: combination of *D. gosswelleri* EO at varying concentrations with Ceftriaxone at a fixe concentration equal to the MIC against *S. enteritidis*, K Eg*CEF: combination of *E. giganteus* EO at varying concentrations with Ceftriaxone at a fixe concentration equal to MIC against *S. aureus*, L Eg*CEF: combination of of *E. giganteus* EO at varying concentrations with Ceftriaxone at a fixe concentration equal to the MIC against *S. enteritidis.*

Figure 4 represent the isobolograms for the combination of EOs with CEF. Synergistic action was obtained for the combined concentrations against *S. aureus* and *S. enteritidis*. For the combination Eg*CEF, the range of the MICs obtained with *E. giganteus* EO against *S. enteritidi* and *S. aureus* strains (ranging from 0,001 µg/mL to 0,01 µg/mL) are lower than the MICs obtained with the combination Dg*CEF (raging from 0.005 µg/mL to 0.04 µg/mL against *S. aureus* and from 0.02 µg/mL to 0.045 µg/mL against *S. enteritidis*) for the solutions tested.While, the MICs values of *E. giganteus* EO ranging from 0.09 µg/mL at 0.73 µg/mL *S. aureus* and *S. enteritidis* for all the solutions, the interaction type was the same (synergistic) in any case. It for this reason, all point appear in the same position on the isobolograms of this combination (figure 2 : C and D).

4. DISCUSSION

In this study, the antimicrobial activity of three EOs was determined by microdillution method against five strains (*B. cereus, S. aureus, S. enteritidis, P. aeruginosa* and *K. pneumoneae).* The results showed anti-bacterial effects of *D. gossweileri, E. giganteus* and *M. leucadendron* EOs.

The ability of the plants to inhibit microbial growth and the difference in activity that exists between EO of the same plant and plants of the same family or different families would be influenced by chemical composition of each EO also influenced by several harvest factors[31,32]. Synergy action between these different majority and minority compound would therefore be responsible for this microbial inhibition[33].

D. gosswelleri EO showed MICs ranging from 1.46 µg/mL to 11.71 µg/Ml. Moni *et al*[34,35] studied the activity of *D. gosswelleri* EO against *Mycobacterium tuberculosis* strain and isolates. They had obtained MICs ranging from 4.88 µg/mL to 9.86 µg/mL, showing the strong activity of this EO. Similarly, in another study, the activity of this EO against yeasts showed MICs equal to 62.5µg/mL[36]. *D. gosswelleri* EO is mainly composed of benzyl isothiocyanate noted in previous work of our research team[34,35] at 91.28% and, also by the others studies (86.7%[37] and 63.19%[38]) in their studies. Isothiocyanates (ITCs) are compounds with the great antimicrobials prospects. Particular ITCs, have long been known to have biologicals activities including various pharmaceutical benefits to human health (anticarcinogenic, antimicrobial and antioxidant properties) and plants defense (against insects, fungi and microbial infections)[39,40,41]. Kaiser *et al*.[42], studied the activity against *P. aeruginosa* strain of benzyl isothiocyanate present in *D. gosswelleri a*nd obtained the MIC equal to 2.14 µg/mL[42], similar to MICs obtained in this study against S*. aureus, S enteritidis* and *B. cereus* strains (1.46 µg/mL), this represents the best inhibition among all isothiocyanates inhibitions tested in their study.

Regarding the *E. giganteus* EO, the MICs value obtained ranging from 2.91 µg/mL to 23.43 µg/mL. Tekwu *et al*[43]studied the extracts activites of *E. giganteus* plant against *Mycobacterium tuberculosis* and obtained MICs between 16µg/mL to 32µg/mL, inhibition by *E. giganteus* extract was the best observed among the plants extracts tested. Sesquiterpenes are the compounds mainly present in *E. giganteus*. The presence of these compounds at 94.3% was revealed by Menut *et al.*[20] and at 93% by Pavela *et al*.,[44].

The activity obtaines with *M. leucadendron* EO ranging from 5.88µg/mL to 750µg/mL respectively against *S. enteritidis* and *P. aeruginosa*. The antibacterial and anti-inflammatory properties of *M. leucadendron* have been demonstrated against various pathogens with MICs between 4µg/mL and 8µg/mL by Monzote *et al*.[45] (2020). This EO are mainly composed to Sesquiterpenes like *E. giganteus* EO. Rajendra *et al* was revealed the presence of thesecoumpounds in this EO at percentage between 81.23% and 93.50% from different locations and harvest seasons[46]. These compounds could be of great pharmacological utility[20]. Their antimicrobials activities have been the subject of the preview studies, while, one showed the antimicrobial activity of sesquiterpenes on fungi with MICs between 128µg/mL and 256µg/mL[47].

The MICs obtained with ciprofloxacin (CIP) against the five strains in this study (0,05 µg/mL to 0.19 µg/mL) allow us to conclude on the sensitivity of these strains to this antibiotic. One strain is declared sensible at CIP when the MIC of this is <1mg/l[48].

Ceftriaxone (CEF) have an activity against all strains except *P. aeruginosa.* The sensitivity of strains to CEF are declare when MIC ranging from 0.125 µg/mL to 8 µg/mL[49] this is the case in this study with MICs equal to 0.10 µg/mL. Against *P. aeruginosa,* CEF was not active, that explains the lack of inhibition obtained in this study. This resistance seems to be due to the natural presence of cephalosporinase, efflux mechanisms and the impermeabily developed which protects these bacteria against action of the antibiotics belonging to the family of cephalosporine like CEF[50,51]. This mecanism made also the action of the EOs difficult. *P. aeruginosa* are generally ranked among the least sensitive bacteria to EOs compared to other Gram-negative bacteria[51,52] which explain the results of this study with EOs from *D. Gosswelleri, E. Giganteus*, and *M. leucadendron* (MIC equal to 5.88 µg/mL, 23.43 µg/mL and 750µg/mL respectively). CIP are the fluoroquinolone the most active against *P. aeruginosa* [53], this is justifies by MIC obtained (0.048 µg/mL) in this study with CIP against this strains, represente the best inhibition of this antibiotic.

The activities of EOs against Gram-negative bacteria *(P. aeruginosa, S. enteritidis* and *K. pneumoneae)* and against Gram-positive bacteria (*B. cereus* and *S. aureus*) are not difference in this study. Preview study showed that, the antibacterial activity of EOs does not depend on the Gram type bacteria[54].

In this study, Synergistic effect of D*. gosswelleri, E. giganteus* and antibiotics are study using isobologram method. The combinations realized between the two EOs and the EOs with antibiotics showed a synergistic action for different concentration combined. In their comparative study of methods for evaluating the interaction type of antimicrobial combinations, Kemegne *et al* concluded on a best reliability of isobologram method which was used in this study[18]. The reduction of MICs of antibacterials combinations compared when they are tested alone was obtained with synergistic interaction.

Indeed, several research have been the subject of the combinations between EOs and with antibiotics against several pathogens with the aim to potentiate their respective actions[55,56,57]. Malik *et al*, studied the synergistic action between *P. graveolens* EO and CIP against bacteria responsible for urinary tract infections: *Klebsiella pneumoniae, Proteus mirabilis* and *S. aureus* all initially resistant to CIP, they obtained synergy between two antimicrobials[53]. another study evaluated the antibacterial activity of 11 EOs against *S. aureus, E. coli, K. pneumoniae* and *P. aeruginosa* as well as 50 clinical strains isolated from different infections, alone and in combination with standard antibiotics. The results found were significant because most EO/antibiotic combinations had a synergistic effect[58]. The development of the resistances against CIP increase over time, this is due to its frequently use for her strong antimicribial power with broad spectrum. To potentilised the action of this antibiotic, the combinations between plants products are much studied in various works and a synergistic activity is frequently obtained.[53].

The mechanisms by which EOs inhibit bacterial growth involve several modes of action. Their hydrophobic nature and their richness in several phenolic compounds allows them to divide the lipid bilayer of bacterial wall making it more permeable. This permeability facilitate antibiotics penetration and improves their action, hence the synergy obtained[59]. The combinations between EOs and with the antibiotics allows to obtain the set of components, affect multiple biochemical processes in the bacteria, producing a plethora of interactive antibacterial effects[9,18]. Moreover, somes EOs act by inhibiting the efflux pumps bacterial, which cause the bacterial resistances to antibiotics if they are used alone. This inhibition increases antibiotic action on bacteria with a reduced effective dose.

Based on the results obtained in vitro, the antibacterial activity of D. *gosswelleri, E. giganteus* and *M. leucadendron* EOs could serve as a source of new antibacterials agents. The reduction of effective dose of antibiotics obtained in this sudy, allows us to reduce undesirable effects attributed to it, this also makes it possible to overcome bacterial multi-resistance problem.

5. CONCLUSION

The results of this study indicated that, aromatherapy as an interesting alternative to antibiotics. That is due to the antimicrobial activities demonstrated against five microbial strains responsible for common infections. The synergistic action of EOs between them and with antibiotics makes more effective for reisistance treatment. The importance of this synergistic action between essential oils and with antibiotics is contributing to the broadening of microbiocidal spectrum and bactericidal activities against several microbial strains and simultaneously, increasing the effectiveness of antimicrobial activity. Therefore, it would be an effective means of reducing the risk of resistance emergence.

Competing interests

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

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Ethic approval and consent to participate

Not applicable in this section.

Availability of data and materials

The datasets supportings the conclusions of this article are presented in this main paper. Plant material used in this study have been identifieds 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 formating of the final version. KGA designed and supervised laboratory works and revised the manuscript. TFC participated in the behavioud experiment. MNE guided to choices the plants and design study. TDA guided to laboratory work and revised manuscript. MGS participated at laboratory works. 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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