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In vitro antibacterial activity of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* essential oils and Neco® on extended-spectrum ß-lactamases producing or non-producing bacterial strains

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This study aims at assessing the *in vitro* activity of *Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora, Melaleuca quinquenervia* essential oils and the biobactericide Neco® on extended-spectrum β-lactamases producing or non-producing bacterial strains (ESBL) isolated at the Armed forces hospital of Libreville. The aromatogram and antibiogram were respectively assessed by the agar well diffusion method and agar disc method. Thus, the minimum inhibitory concentration and minimum bactericidal concentration were determined by the microdilution method in liquid medium.

The aromatogram revealed that the biobactericide Neco® induced the largest inhibition diameters (28.42 - 43.27 mm) in all strains, followed by *E. citriodora* (26.96 - 36.12 mm) and *L. multiflora* (15.32 - 41.42 mm) essential oils. In contrast, *M. quinquenervia* (9.83 - 26.64 mm) and *C. citratus* (6.82 - 14.97 mm) essential oils had the smallest inhibition diameters. Furthermore, the comparison of aromatogram and antibiogram activities generally revealed that activities are better with essential oils than with antibiotics. In addition, no significant differences were observed between ESBL producing or non-producing strains. At the end this study, the antibacterial activity of *C. citratus*, *E. citriodora*, *L. multiflora*, *M. quinquenervia* essential oils and the biobactericide Neco® were highlighted. However, the efficiency of these activities is dependent on the intrinsic composition of the plant.

Key words: Essential oils, extended spectrum β-lactamase (ESBL), multi-resistance, biobactericide, antibacterial activity.

INTRODUCTION

The third millennium is marked by the emergence of cases of bacterial resistance to antibiotics. Indeed, many studies have showed the presence of resistant bacteria (RB), highly resistant bacteria (HRB) and multi-

bacteria (RB), highly resistant bacteria (HRB) and multi-resistant bacteria (MRB) (Fournier et al., 2012; Jing-yi Zhao et al., 2014; Li et al., 2015) to antibiotics, in hospitals as well as in communities. These resistances have a direct impact on the effectiveness of the antibiotherapy by making it difficult. Thus, β-lactamase producing bacteria, by their multi-resistance to antibiotics pose a serious public health problem.

Building on this alarming fact, it seems wise to focus research on the discovery of new molecules. These should be different from conventional antibiotics and have different mechanisms of action (Falconner et al., 2009). Clearly, in recent years, natural resources and more particularly aromatic plants have been the subject of renewed interest in the search for antimicrobial molecules. The choice was directed to essential oils, natural complex mixtures of volatile secondary metabolites, extracted from aromatic plants (Kalemba and Kunicka, 2003) which present numerous bioactive properties. Indeed, essential oils of *Chrysocoma ciliata* L. (Afolayan and Ashafa, 2009), *Cymbopogon proximus* and *Ocimum canum* (Bassolet et al., 2001), of *Prangos asperula* Boissier (Hilan et al., 2009) *Inula viscosa*, *Salvia officinalis* and *Laurus nobilis* (Kheyar et al., 2014) and *Lavandula officinalis* showed antimicrobial activities. Some showed antifungal activity (Kalemba and Kunicka, 2003; Koné et al., 2010; Doumbouya et al., 2012; Kassi et al., 2014; Kouamé et al., 2015). Thus, they are used in food preservation (Mahanta et al., 2007; Kouamé et al., 2015).

Essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Lippia multiflora*, *Melaleuca quinquenervia* and the biobactericide Neco® showed antifungal activity on truck farm mushrooms and banana (*Pythium* sp. and *Fusarium oxysporum*, *Mycosphaerella fijiensis*, *Deightoniella torulosa* etc) in Côte d'Ivoire (Camara et al., 2010; Koné et al., 2010; Doumbouya et al., 2012).

In this study, we propose to assess *in vitro* the antibacterial activity of *C. citratus*, *E. citriodora, Lippia multiflora, Melaleuca quinquenervia* essential oils and the biobactericide Neco® on beta-lactamase producing or non-producing Gram- bacteria. This work falls within the scope of promotion of aromatic molecules for the purpose of using them in medicine.

MATERIALS AND METHODS

Essential oils (EH)

In this work, we were provided with five (5) essential oils (EH) by the Laboratory of Plant Physiology, Faculty of Biosciences, University Félix HOUPHOUET-BOIGNY of Cocody-Abidjan (Côte d'Ivoire). The extraction of these species was performed according to the method described by several authors in previous studies (Camara et al., 2010; Koné et al., 2010; Doumbouya et al., 2012). Their different characteristics are noted in Table 1. Among these oils, the biobactericide Neco®, is a product used as a trademark and sold by the University Félix HOUPHOUËT-BOIGNY (Kassi et al., 2014).

Bacterial strains

For this study, eight bacterial strains cryopreserved in the laboratory of Molecular and Cellular Biology (LABMC) were tested (Table 2). Their selection criteria were based on the fact that these bacteria are frequently isolated in hospital environment, and are responsible for various pathologies. Thus, these strains are carrier or non-carrier of antibiotic resistance genes (ATB).

Bacterial cultures or overnight cultures

From cryopreserved stocks, 50 µl were taken and transferred into tubes containing brain-heart infusion broth (BHI) (Bioméreux, France). Then, the tubes were incubated at 37°C for 18 to 24 h.

Assessment of the antibacterial activity of essential oils by the diffusion method

The aromatogram method was used to determine the inhibitory activity of EH. From overnight cultures, successive decimal dilutions were performed at (10^{-1} to 10^{-3}), in order to obtain standardized inocula (approximately 10^5 to 10^6 cells/ml). These were seeded by flooding on Mueller-Hinton agar (MH). Then the plates were dried in the laminar flow hood. Afterwards, wells were made in the agar using Pasteur pipettes and 50 µl of the different pure extracts were deposited. Finally, the dishes were incubated at 37°C for 18 to 24 h. After incubation, the inhibition diameters were measured.

Assessment of antibiotic sensitivity: Comparative test

The determination of resistance phenotypes based on the sensitive, intermediate and resistant trilogy of bacterial strains was made by the diffusion method on MH agar medium. For this purpose, an antibiogram was conducted with antibiotic discs (ATB) belonging to five different families (Table 3).

Determination of the minimum inhibitory concentration (MIC)

The microdilution method in liquid medium in 96-well microplates was used to assess bacteria growth inhibition parameters by essential oils (EH). Indeed, two-fold geometric dilutions were performed (from 1/2 to 1/256). In order to obtain a homogeneous solution, the EHs were diluted in a Tween 20 solution added with the culture medium. The microplate was covered with parafilm and incubated at 37°C for 18 to 24 h. The lowest concentration of essential oils inhibiting any growth visible to the naked eye after the incubation period was the MIC. Thus, three trials were conducted for each of the strains and the MIC value was the average of both tests.

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Table 1. Features of the essential oils studied.

Scientific names	Families	Abbreviation
Cymbopogon citratus	Poaceae	Cymbo
Eucalyptus citriodora	Myrtaceae	Euca
Lippia multiflora	Verbenaceae	Lippia
Melaleuca quinquenervia L.	Myrtaceae	Melaq
Neco®	/	Neco®

Table 2. Bacterial strains tested.

Family	ESBL-producing	ESBL-non-producing
Enterobacteriaceae	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter aerogenes*	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter cloacae*
Acinectobactericeae /Pseudomonaceae	*Acinectobacter baumanii*	*Pseudomonas aeruginosa*

Table 3. Names of the different antibiotic discs.

Antibiotic families	Names of molecules used	Disc initials
Beta-lactamins: 3rd generation cephalosporin	Cefotaxime	CTX
Bêta-lactamines: 4th generation cephalosporin	Cefepime	FEP
Phenicols	Chloramphenicol	C
Tetracyclines	Doxycycline	DO
Aminosides	Gentamicin	GMI
Quinolones	Ofloxacin	OFX

Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration or MBC was determined by seeding on Lauria-Bertani (LB) medium by spreading 100 µl samples of wells not showing growth in the microplate. The lowest essential oil concentration having decimated 99.99% of the starting population, after 24 h of incubation at 37°C corresponded to the MBC. The intrinsic activity of the different EHs was determined based on the ratio MBC/MIC named α (alpha). In fact, if $1 \leq \alpha \leq 2$, the effect is bactericidal and if $4 \leq \alpha \leq 16$, the effect is bacteriostatic (Kpodekon et al., 2013; El amri et al., 2014). Moreover, for any value of α superior to 16, the activity is said to be tolerant.

Statistical analysis

The single-factor analysis of variance was used for comparing the inhibitory capacity of essential oils. Duncan's multiple range test was used to compare in pairs the different essential oils. All these were done with the XLSTAT 2014 software and Excel 2013.

RESULTS

Assessment of essential oil (EH) activities

In this study, the antimicrobial activity was assessed by observing the inhibiting capacity of the five (5) essential oils at the same concentration on the bacteria. The results obtained are shown in Table 4. The values recorded are the averages of the three tests.

Indeed, all the essential oils showed a significant inhibitory effect against the studied microorganisms. However, a variation in efficiency in terms of inhibition zones depending on the type of germ and oil concerned was observed.

Activities of oils on *E. coli* strains

The results obtained in this study showed that the 5 tested oils had an inhibitory activity on the ESBL producing or non-producing *E. coli* strains. Indeed, inhibition diameters ranging from 6.96 ± 1.67 to 41.42 ± 8.12 mm and 6.82 ± 1.42 to 28.42 ± 7.08 mm respectively were observed for *E. coli* ESBL**+** and *E. coli* BLSE-. Also, the single-factor analysis of variance showed that there was a significant difference ($p < 0.0001$ and $p = 0.0002$) between the different oils used against *E. coli* ESBL**+** and BLSE- bacteria. As for Duncan's test, it showed that there was no significant difference between the biobactericide

Neco® and *E. citriodora* and *L. multiflora* oils. However, a difference was found between *C. citrates* and *M. quinquenervia* essential oils and the other above-mentioned oils. Similarly, the parity check showed no significant difference between the biobactericide Neco® and *E. citriodora* oil, between *L. multiflora and M. quinquenervia* oils and between those of *M. quinquenervia* and *C. citrates* for *E. coli* BLSE-. Overall, no significant difference (p = 0.33> 0.05) existed between the behavior of ESBL-producing and non-producing strains.

Activities of oils on *Klebsiella*

Considering the results, an inhibitory effect was recorded for all oils regarding the two tested *Klebsiella*. Obviously, inhibition zones ranging between 7.54 ± 1.87 and 29.34 ± 5.22 mm were observed for *K. pneumoniae* carrying resistance genes with diameters ranging from 8.96 ± 2.69 to 31.40 ± 5.61 mm for the other *Klebsiella*. Moreover, for the 5 oils, significant differences (p≥0,002) were obtained for both strains. Thus, Duncan's test showed a significant difference against *K. pneumonia* ESBL**+** respectively between the biobactericide Neco® and *E. citriodora* oil, *C. citratus*, *M. quinquenervia* and *L. multiflora* oils. Moreover, the same result was obtained between species *L. multiflora* and *C. citratus*. Moreover, for *K. pneumoniae* BLSE-, the paired comparison between oils, showed a significant difference between the biobactericide Neco® and *C. citratus*, *M. quinquenervia* and *L. multiflora* oil. Also, the same observation was made between *E. citriodora*, *L. multiflora* and *M. quinquenervia* species. No significant differences (p> 0.05) existed between the behavior of ESBL**+** strains and the other BLSE-.

Activity of oils on bacteria of the genus *Enterobacter*

The average inhibition diameters of species obtained regarding bacteria of the genus Enterobacter ranged from 7.86 ± 1.86 to 43.27 ± 9.32 mm.

Concerning the species *Ent. cloacae* BLSE-, the value of diameters ranged between 7.86 ± 1.86 - 31.00 ± 12.94 mm. From a statistical point of view, there was a significant difference (p = 0.011 <0.05) between the 5 species against this bacterium. The Duncan's test conducted showed that there was a significant difference for *Ent. cloacae* between Neco® volatile and *E. citriodora,* and *C. citratus* and *M. quiquenervia* oils respectively on the one hand. On the other hand, between *L. multiflora* and *C. citratus*.

For the species *Ent. aerogenes*, which is ESBL+, diameters in the range of 8.12 ± 2.56 to 43.27 ± 9.32 mm were observed. Also, the statistical analysis revealed a significant difference (p <0.0001). Duncan's multiple range test performed, showed no significant difference for species *L. multiflora*, *M. quinquenervia* and *C. citratus*. And also, for *M. quinquenervia* and *C. citrates* oils against *Ent. aerogenes*. In general, no significant difference (p = 0.33) existed between the behavior of ESBL-producing bacteria and the other bacteria.

Activity of oils on *P. aeruginosa* BLSE-

The results revealed that all the species tested showed an activity against *P. aeruginosa* (Table 4). Thus, the diameters observed ranged between 14.97 ±13.29 and 36.12 ± 10.82 mm. Furthermore, the statistical analysis showed that there was no significant difference (p = 0.109) between these oils for this strain.

Activity of oils on *A. baumanii* ESBL+

The five species also showed an effective activity on *A. baumanii*. Diameters varying between 11.50 ± 0.57 and 42.62 ± 11.89 mm were recorded. The best diameter was obtained with the biobactericide Neco® followed by the *E. citriodora, M. quinquenervia, L. multiflora and C. citrates* species which showed a significant difference (p = 0.005). The parity test showed that there was no significant difference between the biobactericide and *E. citriodora*, *M. quinquenervia* and *L. multiflora* oils. The same result was also observed between *M. quinquenervia* and *C. citratus.*

Comparison between aromatogram and antibiogram

The sensitivity of bacteria against six (6) antibiotics (ATB) was assessed by the standard disc diffusion method. The measures of inhibition halos obtained are summarized in Table 5.

Considering the results, it appears that both *E. coli* strains showed a resistance profile to the different antibiotics tested, except for gentamicin for *E. coli* BLSE-. The *Klebsiella* strains, ESBL**+** strains as for it, were resistant to chloramphenicol and cefepime while for BLSE- it was resistant to doxycycline only. In contrast, *Ent. aerogenes* was resistant to cephalosporins, to doxycycline and ofloxacin. Similarly, *Ent. cloacae* was resistant to chloramphenicol only. Concerning the species *A. baumanii*, resistance to two ATB (cefepime and chloramphenicol) was recorded. Furthermore, a resistance phenotype was observed for *P. aeruginosa* against ATBs of the cephalosporin family, doxycycline, chloramphenicol and ofloxacin.

By comparing the activity of essential oils and that of ATBs, the oils showed better inhibition diameters. Indeed, the results recorded revealed that the diameters of antibiogram ranged from 0.00 ± 0.00 and 31.68 ± 0.21 mm (Table 5), while those of aromatogram ranged from 6.82 ± 1.42 to 43.27 ± 9.32 mm for all strains put together (Table 4).

Table 4. Antibacterial activity of different essential oil against bacterial strains.

	Strains	Inhibition diameters of essential oils (mm)				
		Essential oils				
		Neco	Euca	Melaq	Cymbo	Lippia
ESBL+	*E. coli*	38.25± 6.09^{a}	31.92± 0.51^{a}	17.30± 6.71^{b}	6.96± 1.67^{c}	41.42± 8.12^{a}
	K. pneumoniae	29.34± 5.22^{a}	28.89± 1.93^{a,b}	14.75± 5.93^{b}	7.54± 1.87^{c}	15.58± 3.32^{b,c}
	A. baumanii	42.62± 11.89^{a}	32.85± 2.75^{a,b}	26.64± 8.95^{b}	11.50± 0.57^{b}	21.57± 6.67^{b,c}
	Ent. aerogenes	43.27± 9.32^{a}	27.29± 4.07^{b}	10.07± 2.27^{c}	8.12± 2.56^{c}	16.45± 4.68^{c}
ESBL -	*E. coli*	28.42± 7.08^{a}	27.29± 4.13^{a}	9.83± 2.11^{b,c}	6.82± 1.42^{c}	15.32± 4.08^{b}
	K. pneumoniae	34.37± 6.58^{a}	36.12± 10.82^{a}	15.76± 10.12^{a}	14.97± 13.29^{a}	23.43± 12.69^{a}
	Ent. cloacae	31.00± 12.94$_{a}$	28.88± 1.83^{a}	11.05± 2.74^{b,c}	7.86± 1.86^{c}	22.48± 9.76^{a,b}
	P. aeruginosa	31.40± 5.61^{a}	26.96± 0.93^{a,b}	12.83± 2.84^{b,c}	8.96± 2.69^{c}	18.64± 9.61^{a,b,c}

*All the values shown are the averages of 3 trials (n=3) avec ± standard deviation. * On the same line the values having the same letter are not significantly different from a<b<c.

Table 5. The antibiogram.

Strains		Inhibition diameters (mm)					
		CTX (30 µg)	FEP (30 µg)	C (30 µg)	DO (30 U.I)	GMI (15 µg)	OFX (5 µg)
ESBL+	*E. coli*	7.79±0.71^{R}	12.39±0.71 ^{R}	14.13±0.08 ^{R}	11.55±2.29^{R}	12.9±5.95^{R}	17.23±1.92^{I}
	K. pneumoniae	31.66±0.71^{S}	9.57±0.62^{R}	21.25±0.77^{I}	0.00±0.00^{R}	18.52±1.99^{S}	29.53±1.44^{S}
	A. baumanii	18.94±0.09^{I}	13.61±0.56^{R}	0.00±0.00 ^{R}	23.54±4.18^{S}	20.68±1.77^{S}	27.97±1.46^{S}
	Ent. aerogenes	9.83±0.25 ^{R}	14.12±0.71 ^{R}	25.79±0.04^{S}	11.57±0.64 ^{R}	16.53±0.46^{S}	0.00±0.00 ^{R}
ESBL -	*E. coli*	12.27±0.86 ^{R}	18.13±1.00^{I}	14.00±0.64 ^{R}	8.80±3.96^{R}	18.63±0.35^{S}	0.00±0.00 ^{R}
	K. pneumoniae	28.30±3.42^{S}	29.45±2.20^{S}	26.93±1.35^{S}	10.03±1.80^{R}	17.70±0.32^{S}	23.79±2.00^{S}
	Ent. cloacae	27.76±0.71^{S}	31.13±0.71^{S}	18.05±0.57 ^{R}	18.04±1.97^{I}	17.32±2.00^{S}	31.68±0.21^{S}
	P. aeruginosa	9.93±0.74 ^{R}	15.05±0.40^{I}	10.40±0.88 ^{R}	12.90±1.24^{R}	21.96±2.33^{S}	10.70±1.84 ^{R}

R, Resistant bacterium; I, intermediate bacterium; S, sensitive bacterium.

Determination of MICs

The different minimum inhibitory concentrations (MICs) obtained for each of the oils tested are shown in Table 6. Each of these oils showed different activities depending on their nature, but also on the bacterial strains tested. Thus, the biobactericide Neco® and the natural species *E. citriodora* and *L. mutiflora* showed strong inhibitory capacities except for *K. pneumoniae* and *P. aeruginosa* respectively for Neco® and *L. mutiflora*. Furthermore, these three (3) oils, gave interesting MICs ranging between 3 ± 1.41 and 96 ± 45.25 µl.ml^{-1}. In contrast, *C. citrates* and M. *quinquenervia* species showed very high MICs or even an absence for some strains (Table 6). And therefore, they had low and very low inhibitory capacities (Table 6).

Determination of MCB and assessment of the MBC/MIC

In general, the biobactericide Neco® and the *E. citriodora* species had a bacteriostatic activity against the strains tested (Table 8). The values of minimum bactericidal concentrations (MBCs) were included in a range of 16 ± 0.00 to 96 ± 45.25 µl/ml for the first oil. Similarly, the MBC values were in the range 12 ± 5.66 to 192 ± 90.51 µl/ml, respectively (Table 7).

Considering the results, the *M. quinquenervia* species therefore had a bactericidal intrinsic activity overall. However, the values of its concentrations were very high and were between 64 ± 0.00 and ≥ 256 ± 0.00 µl/ml. However, the EH of *L. multiflora* also had a bactericidal activity against most strains studied. With the difference that the assessment of MBCs showed values ranging from 32 ± 0.00 to 192 ± 90.51 µl/ml, which are close to the MIC values obtained for this essential oil (Table 6). Furthermore, the only oil of which no effect was observed during this study was *C. citratus*. Thus, no MBC was recorded for all tested bacteria.

DISCUSSION

Through this study, the antibacterial activities of five (5) essential oils (HE) were assessed. In the light of the

Table 6. Different values of essential oils minimum inhibitory concentration (MICs).

	Strains	MIC (µl.ml^{-1})				
		Essential oils				
		Neco	Euca	Melaq	Cymbo	Lippia
ESBL+	*E. coli*	6 ± 2.83	4±0.00	96±45.25	>256	12±5.66
	K. pneumoniae	64±0.00	6±2.83	160±135.76	>256	6±2.83
	A. baumanii	4±0.00	6±2.83	128±0.00	256±1. 41	12± 22.63
	Ent. aerogenes	3±1.41	6±2.83	96±45.25	>256	48± 8.49
ESBL -	*E. coli*	6±2.83	6±2.83	32±11.31	>256	4±0.00
	K. pneumoniae	4±0.00	16±0.00	192±90.51	>256	12±5.66
	Ent. cloacae	10±8.49	10±8.49	256±0.00	128±0.00	8±0.00
	P. aeruginosa	16±0.00	24±11.31	96±45.25	256±0.00	96±45.25

Interpretation of MICs: MIC < 48 µl.ml^{-1}: Strong inhibitory capacity; 48 µl.ml^{-1} < CMI < 96 µl.ml^{-1}: Average inhibitory capacity; 96 µl.ml^{-1} < CMI < 256 µl.ml^{-1}: Low inhibitory capacity; CMI ≥ 256 µl.ml^{-1}: Very low or nil inhibitory capacity.

Table 7. Essential oils minimum bactericidal concentrations.

Strains		Concentration (µl.ml^{-1})				
		Essential oils				
		Neco	Euca	Melaq	Cymbo	Lippia
ESBL+	*E. coli*	32±0.00	12±5.66	192±90.51	NT	32±0.00
	K. pneumoniae	64±0.00	48±22.63	/	NT	24±11.31
	A. baumanii	32±0.00	16±0.00	256±0.00	/	64±0.00
	Ent. aerogenes	16±0.00	64±0.00	192±90.51	NT	32±0.00
ESBL -	*E. coli*	96±45.25	64±0.00	64±0.00	NT	96±45.25
	K. pneumoniae	16±0.00	64±0.00	256±0.00	NT	48±22.63
	Ent. cloacae	64±0.00	192±90.51	/	/	192±90.51
	P. aeruginosa	24±11.31	64±0.00	128±0.00	/	128±0.00

NT: None tested; /: no MBC.

Table 8. Nature of the intrinsic activity of the essential oils studied.

Strains		Nature of the effect				
		Essential oils				
		Neco	Euca	Melaq	Cymbo	Lippia
ESBL+	*E. coli*	Bacteriostatic	Bactericidal	Bactericidal	ND	Bactericidal
	K. pneumoniae	Bactericidal	Bacteriostatic	Nd	ND	Bactericidal
	A. baumanii	Bacteriostatic	Bacteriostatic	Tolerant	ND	Bactericidal
	Ent. aerogenes	Bacteriostatic	Bactericidal	Bactericidal	ND	Bactericidal
ESBL -	*E. coli*	Bacteriostatic	Bacteriostatic	Bactericidal	ND	Tolerant
	K. pneumoniae	Bacteriostatic	Bacteriostatic	Bacteriostatic	ND	Bacteriostatic
	Ent. cloacae	Bacteriostatic	Tolerant	ND	ND	Tolerant
	P. aeruginosa	Bactericidal	Bactericidal	Bactericidal	ND	Bactericidal

results, it appears that the inhibitory effect of these species, against the studied bacteria, is heterogeneous. Thus, the biobactericide Neco® and the *Eucalyptus citriodora* (*E. citriodora*) and *Lippia multiflora* (*L. multiflora*) oils present the highest efficiencies while *Melaleuca quinquinervia* (*M. quinquinervia*) and *Cympopogon citratus* (*C. citratus*) oils have lower efficiencies as compared to the first three ones.

The identification of the antibacterial activities of these natural substances corroborates the antimicrobial capacity of the essential oils described by many works (Benkherara et al., 2011; Chahboun et al., 2015; Yang et al., 2015). In fact, the recorded biological activities can be explained first by the chemical composition of these oils which is very complex, but also, by the quantitative and qualitative variability of its components (Gabriel et al., 2013; El amri et al., 2014). Indeed, according to the works of El Amri et al. (2014), due to the variability of quantities and profiles of essential oil components, it is likely that their antimicrobial activity is not attributable to a single mechanism, but to several sites of action at the cellular level. For these authors, the mode of action of essential oils depends primarily on the type and characteristics of active components, particularly their hydrophobic property that enables them to penetrate the phospholipid bilayer of the bacterial cell membrane. This would therefore induce a conformational change of the membrane, a chemo-osmotic disturbance and ion (K^+) leakage.

The antibacterial property of *E. citriodora* oil was reported by Traoré et al. (2013). This study showed that this EH has an activity against *E. coli* and *S. aureus* and that this activity might come from its high content of aldehyde (76.33%), called citronellal. Moreover, these same authors suggest that improved activity may be observed by testing the pure oil, as confirmed by the results of this study. Indeed, diameters of 10 mm were obtained with dilute species of *E. citriodora* for *E. coli* by Traoré et al. (2013). In contrast, for pure species, diameters of 15.32 ± 4.08 and 41.42 ± 8.12 mm were recorded for both *E. coli* phenotypes tested in our study.

Moreover, the antimicrobial activity of *L. multiflora* might be due to the presence of molecules of sesquiterpene (β-caryophyllene), geraniol, γ-terpinene, para-cymene, thymol, carvacrol, and 1.8-cineole (Soro et al., 2015).

For the biobactericide Neco®, which active matter was obtained from *O. gratissimum* (*O. gratissimum*) fresh leaves and used as pesticide (Kassi et al., 2014). The results of the works of Nakamura et al. (1999) and Kpodekon et al. (2013) have shown that the antibacterial activity might be correlated to its high concentration in phenolic compounds particularly thymol.

As for the species *C. citratus*, its antibacterial virtue might be linked to the presence of major components such as the neral/geranial frequently called citral (Koba et al., 2004; Mahanta et al., 2007; Koba et al., 2009).

However, the analysis of the results clearly highlights a significant difference in activity of the five oils on the bacterial strains tested. These differences can be explained by the intrinsic properties of their constituent molecules. Therefore, the antimicrobial activity would depend on the lipophilicity of the carbon chains and functional groups concerned (phenol>aldehydes>ketones>alcohols>ethers> hydrocarbons) (Folashade and Omoregie, 2012; Gabriel et al., 2013). The fact that the volatile extracts of *L. multiflora* and Neco® are mainly composed of terpene and phenol, and that the one of *E. citriodora*, of aldehyde molecules, might likely explain the large inhibition diameters observed and therefore the best activities obtained during this study. Also, the fact that all strains are sensitive to it might be explained by the wide spectrum of antibacterial activity of these molecules (Kheyar et al., 2014).

However, for the *C. citratus* oil, despite the major presence of an aldehyde, low activities were obtained. Thus, the pure extract tested showed activities ranging from 6.82 ± 1.42 to 14.97 ± 13.29 mm. Similar results were obtained for dilute species of *C. proximus* and *canum* (Bassole et al., 2001). These results suggest that the intrinsic characteristics of the studied strains (Bourkhiss et al., 2007; El Amri et al., 2014), or the volatile extract and the existence of the synergy/antagonist effect between the different majority and minority components (Yang et al., 2015), might modulate the efficiency of the volatile extract. In addition, the variability factor of the chemical composition that could be related to ecology, to the harvest period in the year and especially to soil structure (Kpodekon et al., 2013), might justify the results observed.

Finally, the antifungal efficiency of the species of *M. quinquenervia*, was demonstrated by the works of Camara et al. (2010), and Doumbouya et al. (2012). Indeed, these authors showed that the fungicidal effect of this EH could be attributed to terpene molecules which are its most constituents and whose main monoterpenes were 1.8-cineole (46.5%), α-pinene (11.9%) and viridiflorol. However, in this study antibacterial activities were revealed. In analogy to the composition of *L. multiflora* oil, this activity observed could be related to the presence of 1.8-cineole, component common to both species, as well as by the presence of α-pinene. Indeed, Ngom et al. (2014) explained that these hydrocarbons such as α-pinene, sabinene are known for their antimicrobial capacity.

Among the eight bacteria tested, four are extended-spectrum β-lactamase producing (ESBLs). Considering the results of antibiogram, most of ESBL**+** strains are not only resistant to cephalosporins, but also to other families of antibiotics tested. The same observation is made for the other strains of the study. Comparing the results of aromatogram and antibiogram, it is clear that the essential oils tested show the best activities. According to the literature, similar results were obtained by Bassole et al. (2001) for *L. multiflora* by comparing its activity with that of gentamicin and penicillin. These authors explained that at a smaller concentration by 2.5 times, the EH induced better activities. Similarly, Kheyar et al. (2014) have obtained the same results with *Inula viscosa* L., *Salvia officinalis* L. species. Indeed, diameters varying from 15.5 to 31.5 mm and 10.12 to 24 mm respectively

for *Inula viscosa* L., *Salvia officinalis* L., oils have been recorded; they were higher than those of the different antibiotics tested (6 to 31 mm).

However, Tibyangye et al. (2015) have determined the inferior inhibition diameters for the species of *Ocimum suave* compared to reference antibiotics ciprofloxacin and nitrofurantoin, tested on uropathogens. In fact, the average activities of this oil ranged from 16 to 22 mm. In contrast, they ranged from 13 to 29 mm and 11 to 26 mm respectively for ciprofloxacin and nitrofurantoin.

These results can be understood by the mechanism of action of essential oils, as the inhibitory activity of volatile extracts might be correlated with the existence of several modes of action related to their active components described by many authors (Kouamé et al., 2015). Indeed, the antibacterial activity appears to be influenced by combined action at many levels on the bacterial structure (Bouhdid et al., 2012). But also by the synergistic effect between the different components in spite of the presence of the majority molecules (Ngom et al., 2014). That certainly might explain the lack of difference in behavior, at the significance level between ESBL **+** and ESBL-.

In the light of the results, the species of *L. multiflora* and *M. quinquenervia* are bactericidal while Neco® and *E. citriodora* are bacteriostatic. However, only *C. citrarus* has no *in vitro* intrinsic activity. The bactericidal and bacteriostatic properties demonstrated during this study are in accordance with those of (Kpodekon et al., 2013) in which the *O. gratissimum* oil is bactericidal and those of Haddouchi et al*.* (2009) with the *Thymus fontanesii* species. Overall, the effect of essential oils is bacteriostatic. However, most of their chemical elements might have bactericidal properties (Benkherara et al., 2011).

Conclusion

Finally, this study has highlighted the *in vitro* antibacterial activity of essential oils of *C. citratus, E. citriodora, L. multiflora, M. quinquenervia*, and Neco® on medically relevant bacteria. Based on these results, these natural species have antimicrobial activities that depend on the intrinsic qualitative and quantitative composition of each species.

Their antibacterial activities on all strains and especially on those referred to as resistant bacteria suggests bits of solutions to deal with the thorny issue of emergence of resistant and multi-resistant strains. These species seem likely to be potential candidates for anti-infective therapies. However, *in vivo* studies are needed in order to assess all their antibacterial capacities.

Conflicts of Interests

The authors have not declared any conflict of interests.

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