International Journal of Phytomedicine 9 (2017) 443-450
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Assessment of the antibacterial activity of four essential oils and the biobactericide Neco.

Jean-Fabrice Yala[1*], Rolande Mabika Mabika[1], Brahima Camara[2], Seydou Tuo[2], Alain Souza[1], Alexis Nicaise Lepengue[3], Daouda Koné[2], Bertrand M'batchi[3].

***Corresponding author:**

Jean-Fabrice Yala

[1]Laboratory of Molecular and Cellular Biology, Microbiology-Immunology team; University of Sciences and Techniques of Masuku (USTM), BP 067 Franceville, Gabon

[2]Laboratory of Plant Physiology, Faculty of Biosciences, University Félix HOUPHOUET-BOIGNY Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire

[3]Laboratory of Plant Physiology, Plant Pathology and Plant Breeding, Agrobiology Research Unit, University of Sciences and Techniques of Masuku (USTM), BP 067 Franceville, Gabon

Received: 03 May 2017
Accepted: 11 July 2017
Published: 02 September 2017

Abstract

The purpose of this study was to assess *in vitro* the activity of four essential oils (*Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora, Melaleuca quinquenervia*) and the biobactericide Neco® on Gram-positive bacteria.

The aromatogram and antibiogram were assessed by the agar well diffusion method and the Muller Hinton disk-agar diffusion method, respectively. Also, the minimum inhibitory concentration and the minimum bactericidal concentration were determined by the microdilution method in liquid medium.

The aromatogram showed that the biobactericide Neco® induced the largest inhibition diameters (34.53 ± 11.82 - 43.92 ± 5.38 mm) of all strains combined, followed by the essential oils of *Eucalyptus Citriodora* (30.01 ± 3.02 - 41.89 ± 1.77 mm) and *Lippia multiflora* (20.72 ± 4.72 - 37.61 ± 2.80 mm). However, the essential oils of *Melaleuca quinquenervia* (19.99 ± 3.93 - 26.20 ± 13.27 mm) and *Cymbopogon citratus* (13.52 ± 3.59 - 29.08 ± 2.35 mm) had the smallest inhibition diameters. Moreover, the comparison of the activities of the aromatogram and antibiogram revealed generally that activities were higherwith essential oils than with antibiotics.

At the end of this study, the essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora, Lippia multiflora, Melaleuca quinquenervia* and the biobactericide Neco® had an antibacterial activity on Gram+ bacteria.

Keywords: Essential oils, *S. aureus*, multi-resistance, biobactericide.

Introduction

In recent years, the emergence and spread of antibacterial resistance mechanisms, the growing importance of care-associated infections, combined with the virtual absence of new antibiotics, have generated a real health problem beyond individual health [1, 2]. We also witness the unprecedented emergence of the multi-resistance of Gram-positive bacteria (Gram+) in both hospital and community environments [2-5], placing them in the forefront of bacteria resistant to antibacterials [3, 5]. Indeed, phenotypic and genotypic isolation and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) and other antibiotics have been reported in the literature [6-8]. Similarly, the European Organization for Research and Treatment of Cancer Trials has also highlighted changes within isolated Gram positive microorganisms. Indeed, the percentage of isolated streptococci increased, in particular β-haemolytic *streptococci* and *Streptococcus viridans* [9, 10]. These bacteria are responsible for numerous nosocomial and/or community infections.

Faced with this worrying situation, it seems urgent to ward off and propose new therapeutic approaches in order to efficiently cope with the various bacterial ailments.

So far, several approaches have been proposed, but the one concerning the antimicrobial properties of essential oils from aromatic and medicinal plants seems to be the most envisaged and of major interest. Indeed, numerous works have demonstrated antimicrobial and antifungal activity of multiple species [11-15]. Moreover, these essential oils, concentrated odorous substances, have found applications in the field of food-processing, chemical, pharmaceutical and cosmetic industries [16, 17].

Recent studies, the antibacterial activity of *Eucalyptus citriodora*, *Cymbopgon citrarus*, *Lippia multiflora*, *Melaleuca quinquinervia* L. species, and the biobactericide Neco® has been demonstrated on

DOI:10.5138/09750185.2106

Gram negative (Gram-) bacteria producing or not extended-spectrum β-lactamases [18]. However, it seemed important in this study to assess the antibacterial activity of these plant species on Gram + bacteria.

Thus, this study proposes to assess the antibacterial activity of four essential oils (*Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora, Melaleuca quinquenervia*) and the biobactericide Neco® on *Enterococcus faecalis, Staphylococcus aureus1 and 2, Micrococcus spp*. This work is in line with the prospect of valorization of aromatic molecules in the medical field.

Material and methods

Essential oils (EH)

Essential oils of leaf samples from four plant species (*Eucalyptus citriodora, Cymbopgon citrarus, Lippia multiflora* and *Melaleuca quinquinervia L.*) were obtained by distillation. These extractions were carried out in the Laboratory of Plant Physiology, UFR (Faculty of) Biosciences, University Félix HOUPHOUËT-BOIGNY, using steam distillation as previously described by numerous authors. The biobactericide Neco®, is a product used as a trademark and sold by the University Félix HOUPHOUËT-BOIGNY [14, 19, 20].

Bacterial strains

The microbiological tests were performed in the Laboratory of Molecular and Cellular Biology (LABMC) of the Biology department of the University of Sciences and techniques of MASUKU (Franceville, Gabon). The four (4) bacterial strains teste table-1 were cryopreserved and selected according to several criteria as described by Fontanay, *et al.*, [1]. The latter are not only frequently isolated in hospital environment, but also, responsible for various pathologies.

Table 1. Bacterial strains tested.

	Binomial nomenclature	Abreviation
Enterococcaceae	*Enterococcusfaecalis*	*Ent.faecalis*
Staphylococcaceae	*Staphylococcus aureus1*	*S.aureus 1*
	Staphylococcus aureus 2	*S.aureus 2*
Micrococcaceae	*Micrococcusspp*	*Micro.spp*

Assessment of the antibacterial activities of essential oils by the diffusion method.

In order to determine the activity of these essential oils, over-night cultures were prepared from 50 µl of the cryopreserved stocks and transferred into tubes containing Brain Heart Infusion (BHI) Broth (Biomérieux, France). The different tubes were incubated at 37°C for 18-24 hours. From these preparations, an aromatogram was produced according to the same principle as that of the agar medium diffusion method of a classical antibiogram. The different essential oils were tested without any prior dilution.

Assessment of antibiotic sensitivity: comparative test

The sensitivity of the bacterial strains was assessed by the Mueller Hinton (MH) agar medium diffusion method. For this purpose, an antibiogram was made with discs of five different families of antibiotics (ATB) table-2.

Table 2. Names of the different antibiotic disks.

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

Minimum Inhibitory Concentration (MIC) value determination assay

The MIC was determined by the liquid microdilution method as previously described by several authors [17, 21]. Briefly, two-fold geometric dilutions were carried out (from 1/2 to 1/256) and stock solutions of EHs were made in a preparation of Tween-20 supplemented with the culture medium. The microplate was covered with parafilm and incubated at 37°C for 18-24 hours. On reading, any absence of growth after 18 to 24 hours of incubation at 37°C in the well at the lowest concentration of EH was the MIC. For a better assessment thereof, three tests were carried out for each germ. The value of the MIC was therefore the average of the three tests.

Minimum Bactericidal Concentration (MBC) value determination assay

The minimum bactericidal concentration (MBC) was determined by culturing on the Lauria-Bertani (LB) agar medium through plating 100 µl of samples of the wells that did not grow in the microplate. The lowest essential oil concentration having killed 99.99% of the original population after 24 hours of incubation at 37°C corresponded to the MBC. The intrinsic activity of the different EHs was determined according to the MBC/MIC ratio named . Indeed, if 1 2, the effect is bactericidal and if 4≤ α 16, the effect is bacteriostatic [22]. Moreover, for any value of greater than 16, the activity is said to be tolerant.

Statistical analysis

Single-factor analysis of variance was used to compare the inhibitory activity of essential oils. The Duncan test helped achieve the pair wise comparison of the different essential oils. All this, was made using the software XLSTAT 2014 and Excel 2013.

Results

Assessment of EH activities

The inhibitory effects of the four (4) essential oils (EHs) and of the biobactericide on the growth of four bacterial strains are shown in table-3. The measurements of diameters recorded were done in triplicate..

In this work, a comparison of the antibacterial activity of the volatile extracts of *Eucalptus citriodora, Cymbopogon citratus, Lippia multiflora* and *Melaleuca quinquinervia* L. and the biobactericide Neco® was carried out at the same (pure) concentration. Indeed, a variation in the inhibitory effect was shown depending on the type of germ and the oil present.

Table 3. Antibacterial activity of different essential oil against bacterial strains.

	Inhibition diameters of essential oils (mm)					
	Neco®	Euca	Melaq	Cymbo	Lippia	p value
Ent.faecalis	43.92±5.38	30.79±3.51	19.99±3,93	13.52±3,59	20.72±4.72	5.01E-05
S. aureus 1	40.73±6.07	41.89±1.77	23.23±3,65	29.08±2,35	37.61±2.80	0.00035
S. aureus2	37.89±3.78	33.60±7.37	21.31±4,86	27.57±3,86	26.76±12.35	0.120
Micro. spp	34.53±11.82	30.01±3.02	26.20±13,27	19.08±2,18	26.72±12.80	0.462

Activity of oils on *Enterococcus faecalis*

The analysis of the results shows that *Enterococcus faecalis* strain is sensitive to the four species studied like the biobactericide. Indeed, diameters ranging from 13.52 ± 3.59 to 43.92 ± 5.38 mm were obtained. Moreover, the best activities were observed for the biobactericide Neco® and the species of *Eucalyptus citriodora* with values between 43.92 ± 5.38 mm and 30.79± 3.51 mm respectively. The statistical analysis revealed a significant difference between the different oils (p = <0.000).

Activity of essential oils on Staphylococci

The results obtained in this study indicate an inhibitory effect of the four oils and the biobactericide against the two strains of *Staphylococcus aureus* tested. In fact, diameter values ranging from 23.23 ± 3.65 mm to 41.89 ± 1.77 mm, and from 21.31 ± 4.86 mm to 37.89 ± 3.78 mm were observed for *S. aureus* 1 and *S. aureus* 2, respectively. Furthermore, the greatest activity obtained with respect to these strains was that of *Eucalyptus citriodora* for *S. aureus* 1 with a diameter of 41.89 mm and the biobactericide Neco® for *S. aureus* 2, with a diameter of 37.89 mm. Moreover, the statistical analysis showed that for *S. aureus* 1, a difference amongall the volatile extracts was observed (p = 0.00035). In contrast to *S. aureus* 2 strain, no significant difference was found for *Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora* and *Melaleuca quinquinervia* oils and the biobactericide Neco® (p = 0. 120).

Activity of essential oils on *Micrococcus* spp

All volatile extracts inhibited the growth of the *Micrococcus* spp germ. Measurements of halos of inhibition inferior or equal to 34.53 ± 11.82 mm were recorded; The best activities were obtained with the biobactericide Neco® (34.53 ± 11.82 mm) and *Eucalyptus citriodora* essential oil (30.01 ± 3.02 mm), and the lowest activity with the *Cymbopogon citratus* species (19.08 ± 2.18 mm). The stastical analysis revealed no significant difference between the inhibitory effects of the different essential oils on *Micrococcus* spp (p = 0.462).

Assessment of the sensitivity of the bacterial germs tested

The phenotypic profile of the different bacterial germs studied against antibiotics (ATB) is recorded in table-4.

Table 4. The antibiogram.

	Inhibition diameters (mm)					
	CTX (30 µg)	FEP (30 µg)	CHL ()	DO (30 U.I)	GMI (15 µg)	OFX (5 µg)
Ent.faecalis	12.07±0.71^{R}	18.69±0.97^{I}	11.76±0.84^{R}	24.48±3.92^{S}	19.97±0.07^{S}	13.86±0.71^{R}
S. aureus 1	30.71±1.47^{S}	26.81±2.08^{S}	30.71±0,77^{S}	29.16±2.47^{S}	19.60±2.46^{S}	25.53±0.89^{S}
S. aureus2	15.49±4.87^{I}	19.09±2.34^{I}	9.71±3.42^{R}	28.22±0.47^{S}	17.10±1.86^{S}	0.00±0.00^{R}
Micro. spp	17.43±1.44^{I}	17.61±0.74^{I}	8.83±0.75^{R}	13.68±1.62^{R}	20.71±1.00^{R}	0.00±0.00^{R}

S : sensitive ; **R** : resistant ; **I** : intermediate

The results revealed that all strains do not have the same behavior against the different antibiotics. Indeed, *Ent. faecalis* is resistant to cefotaxime and chloramphenicol, while it is intermediate to cefemipine, and oflaxicin. Likewise, *Micro* Spp shows intermediate resistant phenotypes. Thus, this strain is intermediate to beta-lactams and resistant to other ATB families tested in this study. *S. aureus* 1 is sensitive to all molecules whereas *S. aureus* 2 is only sensitive to doxycycline and gentamicin. On the other hand, this bacterium is resistant to chloramphenicol and oflaxicin and shows an intermediate profile to beta-lactams.

Determination of MICs

Table: 5 : values of the minimum inhibitory concentrations of the essential oils tested.

	CMI (µl.ml^{-1})				
	Neco®	Euca	Melaq	Cymbo	Lippia
Ent.faecalis	12±5,66	12±5,66	64±0,00	12±5,66	12±5,66
S. aureus 1	12±5,66	12±5,66	6±2,83	3±1,41	6±2,83
S. aureus2	12±5,66	6±2,83	48±22,63	10±8,49	12±5,66
Micro. spp	4±0,00	10±8,49	24±11,31	96±45,25	96±45,25

∗∗Interpretation of sensitivity tests in aromatogram
CMI < 12 µl.ml-1 : very excellent inhibition power
12 µl.ml-1 < CMI < 48 µl.ml-1 : excellent inhibition power
48 µl.ml-1 < CMI< 96 µl.ml-1 : medium or intermediate inhibition power
CMI > 96 µl.ml-1 : low inhibitory power

The smallest different inhibition spectra obtained for each volatile extract tested are shown in table-5. Each of these oils has variable activities which are closely related to their nature and to the bacterial strain present. The natural species of the biobactericide Neco® and of the *E. citriodora* essential oil showed very excellent inhibitory effects on all strains. The same is true for the oils of *C. citratus* and *L. multiflora*. However, for these two species, low inhibitory effects were recorded for the *Micrococcus* spp germ. Generally, their MIC ranges are better and in the order of 3 ± 1.41 to 12 ± 5.66 µl.ml^{-1}. In contrast, *M. quinquinervia* L. essential oil, although presenting a very strong inhibitory activity against *S. aureus* 1, had MIC values ranging from 24 ± 11.31 to 64±0.00 µl.ml^{-1} table-5. Consequently, its spectrum of activity is considered to be intermediate.

Determination of MBCs

Table 6. Essential oils minimum bactericidal concentrations (MBCs).

	CMB (µl.ml^{-1})				
	Neco®	Euca	Melaq	Cymbo	Lippia
Ent.faecalis	64±0.00	96±45.25	96±45.25	64±0.00	32±0.00
S. aureus 1	96±45.25	80±67.88	96±45.25	24±11.31	64±0.00
S. aureus2	48±22.63	32±0.00	48±22.63	32±0.00	192±90.51
Micro. spp	96±45.25	128±0.00	32±0.00	192±90.51	192±90.51

Table 7. Nature of the intrinsic activities of the different essential oils.

Nature of the effect					
	Essential oils				
	NECO®	**Euca**	**Melaq**	**Cymbo**	**Lippia**
Ent.faecalis	bactériostatic	bactériostatic	bactéricidal	bactériostatic	bactéricidal
S. aureus 1	bactériostatic	bactériostatic	bactériostatic	bactériostatic	bactériostatic
S. aureus2	bactériostatic	bactériostatic	bactéricidal	bactéricidal	bactériostatic
Micro. spp	Tolerant	bactériostatic	bactéricidal	bactéricidal	bactéricidal

After analysis of the results, it appears that the values of the minimum bactericidal concentrations (MBCs) of the four species studied and of the biobactericide vary from 32 ± 0.00 to 192 ± 90.51 μl.ml^{-1} table-6. For the biobactericide Neco® and the essential oil of *E. citriodora* as well as the essential oils of *C. citratus* and *L. multiflora*, the MBC/MIC ratio points out that these natural species are generally bacteriostatic with respect to the bacterial strains tested. However, a bactericidal effect was observed for two of these four species. Obviously, the bactericidal effect was observed for the bacterial strains of *S. aureus* 2, *Micrococcus* spp. and *Ent.faecalis* respectively to essential oils *C. citratus* and *L. multiflora* table-7. Furthermore, the species *M. quinquinervia L*. was the only oil with a proven bactericidal effect against all strains with MBCs ranging from 32 ± 0.00 to 96 ± 45.25 μl.ml^{-1}.

Discussion

The antimicrobial properties of the biobactericide Neco® and the essential oils of *Eucalyptus citriodora (E. citriodora)* and*Lippia multiflora (L. multiflora) Melaleuca quinquinervia (M. quinquinervia)* and *Cympopogon citratus (C. citratus)*, although already known [19, 23-26], have been further corroborated by this study. Indeed, these authors through their works have demonstrated mainly an antifungal activity. In contrast, this study focused on the antibacterial particularities of these species.

Demonstration of the activity and justification thereof

To this end, all the strains tested showed growth inhibition in the presence of the essential oil from the selected plantspecies, which could be explained by their intrinsic chemical compositions. By analogy with other studies, these oils, like other essential oils, are composed of molecules belonging to the groups of terpenes and oxygenated molecules (aldehydes, phenols, alcohols, ketones) [27-29]. The latter might provide their antibacterial power [30, 25].
The antibacterial activity of the oils might also be correlated, on the one hand, by the proportions and the chemical structure of the aforementioned components. Among them are the so-called majority components and other minority ones [31].Indeed, the works of Zayyad *et al.*, [32], on the EH of thymus showed that the combined action of majority components such as thymol and α-terpinen and minority ones (carvacrol and borneol), had inhibited the growth of Gram positive and negative bacteria (*Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Bacillus subtilis,* and *Erwinia chrysanthemi*). And on the other hand, this activity might also be due to the different chemotypes present in this study. To this end, the scientific literature reports several examples [33-35]. These authors demonstrated in their various works, a causal link between the spectrum of activity and the abundance of specific chemical elements of the species.Also, Djenane*et al.*, in 2011 asserted that EHs containing large proportions of 1.8-cineole are more excellent antibacterial agents than those which do not. In analyzing these results, the strong antibacterial activities of the EHs obtained in this work could be attributed to their chemotypes.

Aromatogram and antimicrobial comparison

In view of the results obtained, the bacterial strains studied appear to be resistant to most of the antibiotics (ATB) tested, with resistance percentage of 58.33%. By comparing the activity spectra of the antibiogram and the aromatogram, the best inhibition diameters are obtained with the essential oils of the selected plant species. This could be explained by the multiple mechanisms and sites of action that interact [36]. They are related to the number of molecules present and the chemical complexity of the volatile extracts.In contrast, antibiotics have a well-defined spectrum of action in the bacterial cell. The latter act either by inhibition of protein synthesis, bacterial wall, nucleic acids or by inhibition of metabolism [37]. On the other hand, although not explicit enough, essential oils have a very wide spectrum of action. Indeed, by their chemical variability and the synergistic effects of the components, several cellular actions are set up within the bacterium [17, 31].

MIC, MBC, ratio

The high activity obtained in this study was confirmed by the microdilution method. The MIC values for the biobactericide Neco® and the essential oils generally range between 3 and 96 μl.ml^{-1}. For the essential oil of *Cymbopogon citratus*, with the smallest MIC (3±1.41 μl.ml^{-1}) obtained for *S. aureus* 1; in this study, is contrary

to that reported by Koba *et al.*, [40]. Indeed, the latter reported a MICs≥ 500µl.ml^{-1}, respectively for bacterial germs *Staphylococcus entermedius and Pseudomonas aeruginosa.* About the biobactericide Neco®, the fungicidal activity is the most mentioned in the literature, according to Doumbouya *et al.,* (2012); Kassi et al., (2014) [19, 39]. In contrast, this study confirmed the antibacterial activity of Neco®. Although having a very excellent inhibitory activity, this Neco species is bacteriostatic against the bacterial strains tested and can be adapted in case of bacterial infection. Similarly, for the oil stemming from *Melaleuca quinquinervia L*., the works of Camara *et al.*, [14] and Doumbouya *et al.*, [19] have demonstrated its fungicidal activity. Thus, the study of Doumbouya et al., assesses the inhibitory concentrations (ICs) IC_{50} and IC_{90} of this species on two fungi *Fusarium oxysporum f.* sp *radicis lycopersici* and *Pythium* sp. Their respective values are 600 and 3700 ppm for IC_{50} and 2700 and 4675 ppm for IC_{90}. Moreover, IC_{50} is also the assessed parameter in the work of Camara *et al.* (2010). As a result, the essential oil of *Meulalauca quinquinervia L*. has an IC_{50} of 3158.900 ppm on the fungus *Mycosphaerella fijiensis*.
However, Yala, *etal.,* [18] demonstrated the antibacterial activity of this species. The MIC values ranged from 96 µl. ml^{-1} to 256 µl.ml^{-1} with the exception of *E.coli ESBL*- (32µl.ml^{-1}). The MIC values reported in their study are very high compared to those obtained in the present study (6- 64 µl.ml^{-1}). The same authors assessed the activity of four (4) other oils with regard to Gram (-) bacteria. These species displayed considerable antibacterial activity. The MIC values ranged from 3 to 96 µl.ml^{-1} for 75% (3/4) of the studied essential oils, biobactericide Neco®, *E. citriodora* and *L. multiflora* as obtained in this work. However, the MICs values obtained with *C. citratus* (128 to 256 µl.ml^{-1}) are higher to those of this study.
By comparing these two studies, the action of these species on Gram (-) appears to be lower than the one on Gram (+). This finding corroborates the arguments of many previous studies [20, 29, 40].
The activity of EHs Neco and *E. citriodora* appears to be bacteriostatic on cocci, while *L. multiflora* and *C. citratus* seem to be both bactericidal and bacteriostatic. And this depends on the strain present. Furthermore, the EH *M. quinquinervia L*. appears to be bactericidal on the latter. Also, this bactericidal activity is more important on the strain *Staphylococcus aureus*, tested whose MBCs are equal to MICs.

Conclusion

The analysis of the antimicrobial activity of the essential oils of *Cymbopogon citratus, Eucalyptus citriodora, Lippia multiflora* and *Melaleuca quiquinervia L*. and the biobactericide Neco® revealed a proven effect on Gram positive cocci showing resistance to Some antibiotics. This activity is generally bacteriostatic in all species, although bactericidal activity has been recorded on the strains of *Enterococcus faecalis, Micrococcus* spp. and *Staphylococcus aureus* 2 for *C. citratus, L. multiflora* and *M. quinquinervia L*.
The relatively average MICs show a certain therapeutic interest in spite of the fact that the cytotoxic studies have not been carried out. Also, based on these results *in vivo* studies are being conducted to explore the full pharmacological potential of these essential oils.

Acknowledments

Brahima Camara, one of the co-authors, was a recipient of the HAAGRIM project grant for his stay at the University of Sciences and Technique of Masuku (Gabon). The grant was awarded in the framework of the Intra-African Academic Mobility Scheme funded by the European Union.
The authors would like to thank the Coordinating team of the HAAGRIM project, particularly Dr Saïdou Nourou Sallfrom Gaston Berger University, for the efforts made towards the implementation of researchers mobility. The authors also thank the EU for the fundings which allowed us to reinforce the networking of research teams between Felix Houpouet Boigny university (Ivory Coast) and the University of Sciences and Techniques of Masuku (Gabon).

References

[1]. Fontanay S, Mougenot ME, Duval R. Évaluation des activités antibactériennes des huiles essentielles et/ou de leurs composants majoritaires.Hegel. 2015; 5(2):109-118.
[2]. Berger-Bachi B. Resistance mecanisms of Gram-positive bacteria. International Journal of Medical Microbiology. 2002; 292:27-35
[3]. Quincampoix J. Mécanisme de résistance des cocci Gram positifs. Réanimation. 2001; 10:267-275.
[4]. Loeffler A, Pfeiffer D, Lloyd D, Smith H, Soares-Magalhaes R, Lindsay J, Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups. The Journal of Hospital infection. 2010; 74(13):282-288.
[5]. Fernandez-Gerlinger M, Mainardi J. Nouvelles associations dans le traitement des infections graves à cocci Gram positifs. Journal des Anti-infectieux. 2014; 16(11):18-23.
[6]. Montesinos L, Delgado T, Riverol D, Salido E, Miguel M, Jimenez A, Sierra A, Changes in the epidemiology of meticillin-resistant Staphylococcus aureus associated with the emergence of EMRSA-16 at University hospital.

Journal of Hospital infection. 2006; 64(13):257-263.

[7]. Nimmo G, Fong J, Paterson D, McLaws M. Changing epidemiology of meticillin-resistant S. aureus in Queensland, Australia, 2000-2006: use of passive surveillance of susceptibility phenotypes. Journal of Hospital Infection. 2008; 70(14):305-313.

[8]. O'Hanlon S, Enright M. A novel bactericidal fabric coating with potent in vitro activity against meticillin-resistant *Staphylococcus aureus* (MRSA). International Journal of Antimicrobial Agents. 2009; 33(15):427-431.

[9]. CormicanMet Jones R. Emerging resistance to antimicrobial agents in Gram-positive bacteria. Enterococci, Staphylococci and Nonpneumococcal streptococci. Drugs, Supplement 1. 1996; 51:6-12.

[10]. Klastersky J. Science and pragmatism in the treatment and prevention of neutropenic infection. Journal of antimicrobial chemotherapy, Suppl D. 1998; 41:13-24.

[11]. Nakamura TU-N CV, Bando E, NegrãoMelo AF, Cortez DAG, Dias Filho BP. Antibacterial Activity of Ocimumgratissimum L. Essential Oil. Memorias do Instituto Oswaldo Cruz, Rio de Janeiro.1999; 94: 675-678.

[12]. Bassolet I, Ouattara AS, Nebie R, Ouattara C, Kabore Z, Traore S. Composition chimique et activités antibactériennes des huiles essentielles des feuilles et des fleurs de Cympogonproximus (Staps) et d'Ocimumcanum. Pharmacopée et Médecine Traditionnelle Africaine. 2001;11:37-51.

[13]. Camara BD, Kanko C, Anno A, Ake S. Activité antifongique des huiles essentielles de Ociumgratissimum L., de Monodoramyristica (Gaaertn) Dunal et deux produits de synthèses (Impulse et Folicur), sur la croissance mycelienne et la production de spore *in vitro* de *Deightoniellatorulosa*. Revue Ivoirienne des Sciences et Technologie. 2007;09:187-201.

[14]. Camara B, Dick E, Sako A, Kone D, Boye MAD, Aké S, Ano A. Lutte biologique contre Deightoniellatorulosa (Syd.) Ellis, par application des huiles essentielles *d'Eucalyptus platyphylla* F.Muell et de *Melaleucaquinquenervia* L.Phytothérapie et écologie. 2010;8:240-244.

[15]. Kempf M, Kowalczyk F, Rossines E, Panhelleux G, Joly-Guillou M. Etude de la sensibilité de 224 bactéries isolées d'infections hospitalières vis-à-vis des composés JCA250 et JCA251 à base d'huiles essentielles issus de la recherche Aroma Technologies. Pathologie Biologie. 2011; (59):34-43.

[16]. Caillet S, Lacroix M. Les huiles essentielles: leurs propriétés antimicrobiennes et leurs applications potentielles en alimentaire. INRS-Institut Armand-Frappier, RESALA Université de Laval, Québec, Canada. 2007; 1-8.

[17]. El amri J, Elbadaoui K, Zai T, Hayate B, Saïd C, Taj Imolk A. Etude l'activité antibactérienne des huiles essentielles de *Teucriumcapitatium* L et de l'extrait de Silène vulgaris sur les différentes souches testées. Journal of Applied Biosciences. 2014;82:7481-7492.

[18]. Yala JF, Mabika MR, Camara B, Souza A, Lepengue AN, Seydou T, Koné D, M'batchi B. 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. Journal of Medicinal Plants Research. 2016; 10(143):796-804.

[19]. Doumbouya M, Abo K, Lepengue A, Camara B, Kanko K, Aidara D, Kone D. Activité comparées in vitro de deux fongicides de synthèse et de deux huiles essentielles, champignons telluriques des cultures maraîchères en Côte d'ivoire. Journal of Applied Biosciences. 2012; 50:3520-3532.

[20]. Bencheqround HK, Ghanmi M, Satrani B, Abderrahman A, Chaouch A. Activité antimicrobienne des huiles essentielles d'*Artemisiamesatlantica*, plante endémique du Maroc. Bulletin de la Société Royale des Sciences de Liège. 2012; 81:4 - 21.

[21]. Tibyangye J, Okech MA, Nyabayo JM, Nakavuma JL. In vitro Antibacterial Activity of *Ocimum suave* Essential Oils against uropathogens isolated from Patients in Selected Hospitals in Bushenyi District, Uganda. Author manuscript. 2015;8(13):489–498.

[22]. Kpodekon MT, Boko KC, Mainil JG, Farougou S, Sessou P, Yehouenou B, Gbenou J, Duprez JN, et Bardiau M. Composition chimique et test d'efficacité *in vitro* des huiles essentielles extraites de feuilles fraîches du basilic commun (*Ocimum basilicum*) et du basilic tropical (*Ocimumgratissimum*) sur *Salmonella enterica*sérotypes Oakland et Legon. Journal de la Société Ouest-Africaine de Chimie. 2013;41 - 48.

[23]. Koba K, Sanda K, Guyon C, Raynaud C, Chaumont J.-Pet Nicod L. In vitro cytotoxicactivity of *Cymbopogon citratus* L. and *Cymbopogonnardus* L. essential oilsfrom Togo. Bangladesh Journal of Pharmacology. 2009; 4:29-34.

[24]. Kone D, Camara B, BadouOdjoutchoni J, Doumbouya M, Soro S, N'GuessanAya C, Bomisso EL. Fungicides and biological products activities towards fungi causing diseases in Banana and Vegetable in Côte d'Ivoire. Fungicides, O. Carisse, Éd. In Tech. 2010;39-68.

[25]. Folashade KO, Omoregie EH. Essential oil of *Lippia multiflora* Moldenke: A review. Journal of Applied Pharmaceutical Science. 2012; 2(11):15-23.

[26]. Traoré N, Sidibé L, Bouaré S, Harama D, Somboro A, Fofana B, Diallo D, Figueredo G, Chalchat JC. Activités antimicrobiennes des huiles essentielles de *Eucalyptus citriodora*Hook et *Eucalyptus houseana*W.Fitzg. ex Maiden.International Journal of Biological and Chemical Sciences. 2013;7(12):800-804.

[27]. Freeman L, Carel Y. Les huiles essentielles,un pouvoir antimicrobien avéré. Nutra News : Sciences, Nutrition, Prévention et Santé. 2006; 8-16.

[28]. Bassolél. HN, Juliani HR. Essential Oils in combination and their antimicrobial Properties. Molecules. 2012;17:3989-4006.

[29]. Haloui T, Farah A, Balouiri M, Chraibi M, Fadil M, Benbrahim KF, Alaoui AB. Bacteriostatic and Bactericidal Profile of Leaves and Twigs Essential oils of Moroccan Pistacialentiscus L. Journal of Applied Pharmaceutical Science. 2015;5(106):050-053.

[30]. Hassane SOS, Satrani B, Ghanmi M, Mansouri N, Mohamed H, Chaouch A. Activité antimicrobienne et composition chimique de l'huile essentielle de *Plectranthusaromaticus* Roxb. de l'Ile de la Grande Comore. Biotechnologie, Agronomie, Société et Environnement. 2011; 15(12):251-258.

[31]. Bouhdid S, Abrini J, Baudoux D, Manresa A, Zhiri A. Les huiles essentielles de l'origan compact et de la cannelle de Ceylan : pouvoir antibactérien et mécanisme d'action. Journal of Clinical Pharmacology. 2012; 31(13):141-148.

[32]. Zayyad N, Farah A, Bahhou J. Analyse chimique et activité antibactérienne des huiles essentielles des trois espèces de *Thymus*: *Thymus zygis*, *T. Algeriensiset T. bleicherianus*. Bulletin de la Société Royale des Sciences de Liège. 2014; 83(118):132, 2014.

[33]. Brada M, Bezzima M, Marlier M, Carlier A, Lognay G. Variabilité de la composition chimique des huiles essentielles de *Mentharotundifolia* du Nord de l'Algérie. Biotechnologie, Agronomie, Société et Environnement. 2007;11(11):3-7.

[34]. Merghache S, Hamza M, Tabti B. Etude physicochimique de l'huile essentielle de *Rutachalepensis* L. de Tlemcen, Algérie. Afrique Sciences. 2009; 05(11):67- 81.

[35]. Soro LC, Grosmaire L, Ocho-AninAtchibri AL, Munier S, Menut C, Pelissier Y. Variabilité de la composition chimique de l'huile essentielle des feuilles de *Lippiamultiflora* cultivées en Côte d'Ivoire.Journal of Applied Biosciences. 2015; 88:8180– 8193.

[36]. Turek C, Stintzing FC. Stability of Essential Oils: A Review. 2013; 12:40-53.

[37]. Courvalin P. Larésistance des bactéries aux antibiotiques: combinaison de mécanismes biochimiques et génétiques. Bulletin Académique Vétérinaire de France. 2008;161(11):7-12.

[38]. Koba K, Sanda K, Raynaud C, Nenonene YA, Millet J, Chaumont JP. «Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogonsp*. africainsvis-àvis de germes pathogènes d'animaux de compagnie d'animaux. Annales de Médecine Vétérinaire. 2004; 148:202-206.

[39]. Kassi F, Badou OJ, Tonzibo ZF, Salah ZAL, N'ognDadé EGD Kone D. Action du fongicide naturel NECO contre la cercosporiose (*Mycosphaerellafijiensis* Morelet) chez le bananier plantain (AAB) en Côte d'Ivoire. Journal of Applied Biosciences. 2014; 75:6183–6191.

[40]. Djenane D, Lefsih K, Yangüela J, Roncalés P. Composition chimique et activité anti-*Salmonella enteritidis* CECT 4300 des huiles essentielles d'*Eucalyptus globulus*, de *Lavandulaangustifolia* et de *Saturejahortensis*. Tests *in vitro* et efficacité sur les œufs entiers liquides conservés à 7 ± 1 C. Pharmacologie–aromathérapie. 2011; 9:343–353.