Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(5): 271-276
Received: 08-07-2016
Accepted: 09-08-2016

**J Bonou**
(a) Laboratoire National de Contrôle de Qualité des Médicaments et Consommables Médicaux (LNCQ) 06 BP139 Cotonou Benin
(b) Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**F Baba-Moussa**
(a) Laboratoire National de Contrôle de Qualité des Médicaments et Consommables Médicaux (LNCQ) 06 BP139 Cotonou Benin
(b) Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**Z Adéoti**
Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**H Ahouandjinou**
(a) Laboratoire National de Contrôle de Qualité des Médicaments et Consommables Médicaux (LNCQ) 06 BP139 Cotonou Benin
(b) Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**V Dougnon**
Laboratoire de Recherche en Biologie Appliquée, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, 01 BP 2009 Cotonou, Benin

**D Dossa**
(a) Laboratoire National de Contrôle de Qualité des Médicaments et Consommables Médicaux (LNCQ) 06 BP139 Cotonou Benin
(b) Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**JD Gbenou**
Laboratoire de Pharmacognosie et des Huiles Essentielles, FSS-Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire Cotonou, Benin

**F Toukourou**
Laboratoire de Microbiologie et de Technologie Alimentaire, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, ISBA-Champ de foire, 01 BP 526 Cotonou, Benin

**L Baba-Moussa**
Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques/Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Benin

**Correspondence**
**L Baba-Moussa**
Laboratoire de Biologie et de Typage Moléculaire en Microbiologie, Faculté des Sciences et Techniques/ Université d' Abomey- Calavi, 05 BP 1604 Cotonou, Benin

# Antimicrobial activity of essential oils of *Lippia multiflora, Eugenia caryophyllata, Mentha piperita* and *Zingiber officinale* on five oral-dental microorganisms

**J Bonou, F Baba-Moussa, Z Adéoti, H Ahouandjinou, V Dougnon, D Dossa, JD Gbenou, F Toukourou and L Baba-Moussa**

**Abstract**
The present study aimed to determine the chemical composition, the Minimal Inhibitory Concentrations (MIC) and the Minimal Bactericidal Concentrations (MBC) of the essential oils extracted from *Lippia multiflora, Eugenia caryophyllata, Mentha piperita* and *Zingiber officinale* harvested in Benin on five oral-dental germs. It also assessed the influence of different emulsifiers (tweens) on the antimicrobial potential of the four oils on the same microorganisms. Using a Clevenger type machine, the essential oils were extracted by hydrodistillation and their chemical composition was determined by chromatography. The antimicrobial activity of the oils was evaluated by the microdilution method in ELISA microplates. At the end of the study, tween 60 was revealed to be better than the others (20, 40 and 80), because it allows a better diffusion of the oils in both liquid and solid media. The MIC of the essential oils ranged from 0.078 to 5 mg/ml, while their MBC varied from 0.078 to 10 mg/ml. The lowest MIC and MBC were recorded with *Micrococcus luteus* and the highest with *Pseudomonas aeruginosa*. The chemical screening of these extracts revealed the presence of many compounds including linalol, 1,8-cineole and myrtenol in *Lippia multiflora*; eugenol and eugenyle acetate in *Eugenia caryophyllata*; menthone and menthol in *Mentha piperita*; and camphene, beta phellandrene, ar-curcumene and alpha zingiberene in *Zingiber officinale*.

**Keywords:** Essential oil, medicinal plants, antimicrobial activity, mouthwash, tween

## 1. Introduction

Since the ancient times, humans used to appreciate the soothing and analgesic virtues of plants. Throughout centuries, human traditions have found ways to develop their use. Up to date, two third of the pharmacopeia still rely on their curative properties [1]. Traditional medicine occupies an important place despite the progress of modern medicine. The world trade of medicinal plants is currently estimated at more than US dollar 60 billion per year [2].

Furthermore, since 1977, the World Health Organization (WHO) launched an active program for the promotion and development of traditional medicine based on the use of medicinal plants [3]. Therefore, the use of a multitude of plants of various properties is being promoted. A number of such plants were subjected to thorough scientific research and their therapeutic properties were proven. However, very little is known about some of these plants, while others still remain completely unknown.

The declaration of Abuja (Nigeria) in April 2001 made traditional medicine research a priority for Africa. This resulted into a high number of studies on medicinal plants across the continent. About 75% of the African population and more than 80% of Beninese use medicinal plants in the treatments of their ailments [4, 5]. With the current techniques of identification and characterization of organic molecules, over 25 to 50% of medicines prescribed nowadays contain bioactive molecules of medicinal plants as active principles [6, 7]. In Africa in general and in Benin particularly, the assessment of active principles of therapeutic plants has increased these last years. The valorisation of natural plant resources has become a preoccupation of paramount importance for the research of new medicinal molecules [8]. These plants are used as decoctions, some are macerated or infused but they can also be used in the form of essential oils. These essential oils are easy to use and mostly very effective [9].

Currently, for economic reasons or because of the scarcity of natural resources the medical industry synthesizes an important amount of essential oils. A number of substances are thus extracted by hydrodistillation and solvent based extractions [10]. Several methods are employed to assess their antimicrobial properties in Benin, however none of them was conducted on the effectiveness or the influence of the emulsifiers used in the detection of the antimicrobial activity of these extracts.

The present study intended to analyse the influence of the emulsifiers used in the assessment of the antimicrobial properties of selected medicinal plants. Therefore, the essential oils of these plants were extracted followed by an analysis of their chemical composition. Thereafter, the *in vitro* bacteriostatic and bactericidal activities of the plants were evaluated on five germs of clinical importance.

## 2. Material and Methods
### 2.1 Material
#### 2.1.1 Plant material
The plant material is constituted of essential oils of the following medicinal plants: *Lippia multiflora* Moldenke (Verbenaceae), *Eugenia caryophyllata* (Myrtaceae), *Mentha piperita* L (Lamiaceae) and *Zingiber officinale* Rosc (Zingiberaceae).

The leaves of *Lippia multiflora* and floral buds of *Eugenia caryophyllata* were harvested from Sèto-Gbowèlè in the Department of Atlantic. The leaves of *Mentha piperita* were bought in the market garden of Cadjehoun in the Department of Littoral, whereas the rhizomes of *Zingiber officinale* were bought in the market of Adjarra in the Department of Ouémé. This collection took place in January and February 2016.

#### 2.1.2 Microorganisms
Five ATCC (American Type Culture Collection) and IP (Institute Pasteur of Strasbourg) reference strains were used in this study (*Micrococcus luteus*; *Staphylococcus aureus* ATCC 29213; *Proteus mirabilis* ATCC 24974; *Pseudomonas aeruginosa* ATCC 27853; *Candida albicans* IP 4872). They were obtained from the Laboratory of Biology and Molecular Typing in Microbiology of the Faculty of Sciences and Techniques of the University of Abomey-Calavi (Benin) and from the National Laboratory of Quality Control of the Ministry of Health in Benin. They were then kept in Muller Hinton broth with glycerol (10%) at -20 °C.

### 2.2 Methods
#### 2.2.1 Extraction of essential oils
Essential oils were extracted by hydrodistillation in a Clevenger type machine [11] as described by Verma *et al*. (2010) [12]. The obtained essential oils were weighed and the yield of each extraction was calculated according to the formula described by Wan *et al*. (1998) and Mazari *et al*. (2010) [13, 14].

#### 2.2.2 *In vitro* assessment of the antibacterial and antifungal activities of the essential oils
The technique of microdilution using 96 wells microplates was employed to analyse the susceptibly of *Micrococcus luteus*; *Staphylococcus aureus* ATCC 29213; *Proteus mirabilis* ATCC 24974; *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* IP 4872 to the different essential oils.

#### 2.2.3 Minimal Inhibitory and bactericidal concentrations of the oils
The Minimal Inhibitory Concentration (MIC) is the smallest concentration for which there is no visible growth for naked eyes, whereas Minimal Bactericidal Concentration (MBC) is the smallest concentration for which there is a maximum of 0.01% of germs. They were determined by the microdilution method described by Ayadi *et al*. (2003) [15].

#### 2.2.4 Chromatographic analysis of essential oils
The chromatographic analyses of the volatile extracts were performed in an electronic gas chromatograph of pressure coupled with a mass spectrophotometer in accordance with the methods described by Bencheqroun *et al*. (2012) [16].

## 3. Results
### 3.1 Data treatment
A datasheet was conceived in Epi-Data version 3.1 software and included the different microorganisms, the different types of tweens, all the essential oils and the different concentrations recorded at each test. All the collected data were then entered in the form and and the final analysis was carried out in EPI-INFO (version 7.0) and IBM SPSS (version 20) softwares. Tables and figures were made in Microsoft Excel (version 2007). A quantitative analysis method was used to compare the different recorded concentrations and presented as tendency curves.

**Table 1:** Yield of extraction of essential oils

| Plant species | Family | Yield (%) |
|---|---|---|
| *Lippia multiflora* | Verbenaceae | 2.9 |
| *Eugenia caryophyllata* | Myrtaceae | 15.7 |
| *Mentha piperita* | Lamiaceae | 0.6 |
| *Zingiber officinale* | Zingiberaceae | 1.2 |

The highest yield of essential oils was obtained with *Eugenia caryophyllata* (15.7%) and the lowest (1.2%) with *Zingiber officinale*.

**Table 2:** Main chemical compounds of the four analysed essential oils

| Essential oïl | Main compound | Total (%) |
|---|---|---|
| *Lippia multiflora* | Linalol 45.10%, 1,8- cineole 25.40%, Myrtenol 10.40%, α-terpineol 8.20%, α-pinène 5.90% | 95.00 |
| *Eugenia caryophyllata* | Eugenol 85.90 %, Eugenyle acetate 10.20%, beta-caryophyllene 1.98% | 98.08 |
| *Mentha piperita* | Menthone 38.50%, menthol 52.09 %, menthyle acetate 3.21%, 1,8-cineole 2.87% | 96.67 |
| *Zingiber officinale* | Camphene 15.62%, beta phellandrene 8.11%, ar-curcumene 11.85%, alpha zingiberene 30.62%, 1,8-cineole 5.56%, gamma bulgarene 8.26%, beta bisabolene 7.19%, beta sesquiphellandrene 9.02% | 96.23 |

This table shows that most of the major identified constituents are oxygenated compounds.

### 3.2 Influence of different Tweens on the antimicrobial activity of the essential oils

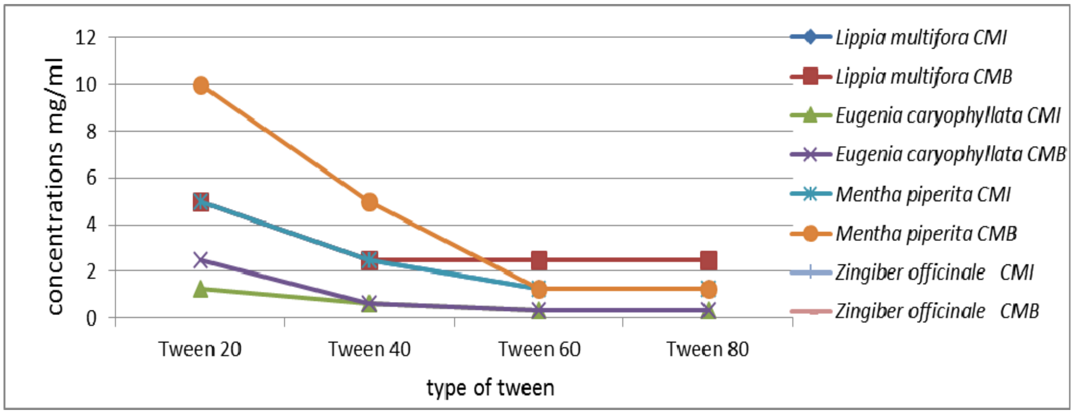


**Fig 1:** Evolution of the MIC and MBC of studied essential oils based on different types of tweens used at 5% on *Pseudomonas aeruginosa*

This figure shows that the curves of MIC and MBC decreased from tween 20 to tween 40 and between tween 40 and tween 60. From tween 60, the curves remained stable up to tween 80. This shows that the MIC and MBC values decreased from tween 20 to tween 60, then become constant from tween 60 to tween 80 on *P. aeruginosa* except for the MBC (2.5 mg/ml) of the essential oil of *Lippia multiflora* obtained with tween 40 that remained constant for tweens 60 and 80. The absence of curves for the MIC and MBC values of the essential oil of *Zingiber officinale* shows its inactivity on *P. aeruginosa* for all different types of tweens used.

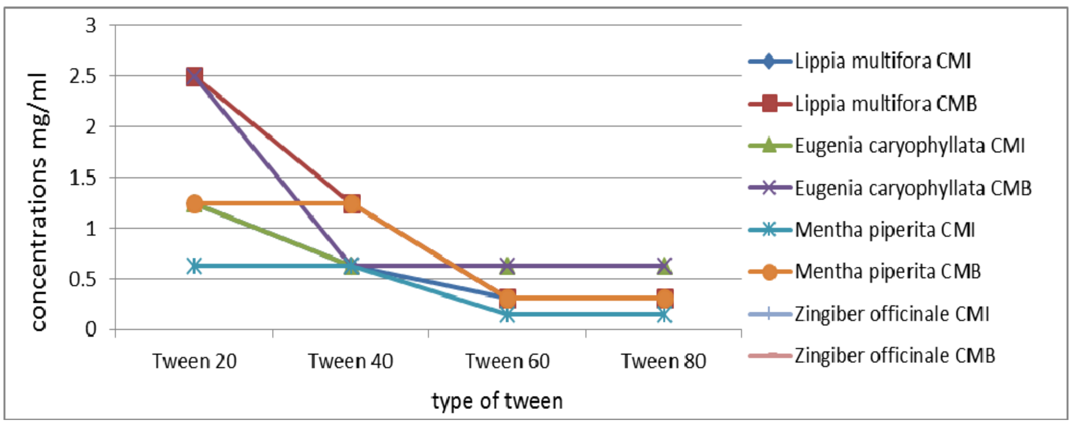


**Fig 2:** Evolution of the MIC and MBC of studied essential oils based on different types of tweens used at 5% on *Proteus mirabilis*

The analysis of this figure shows that the curves of MIC and MBC decreased from tween 20 to tween 40 and tween 40 to tween 60. However, the curves remained constant from tween 60 to tween 80. This means that the values of MIC and MBC decreased from tween 20 to tween 60, and became constant from tween 60 to tween 80 on *P. mirabilis* except for the MIC (0.625 mg/ml) and CMB (0.625 mg/ml) of the oil essential of *Eugenia caryophyllata* recorded with tween 40 that remained steady between tweens 60 and 80. The absence of curves for MIC and MBC values of the essential oil of *Zingiber officinale*, translates its inactivity on *P. mirabilis* regardless of the tweens used.

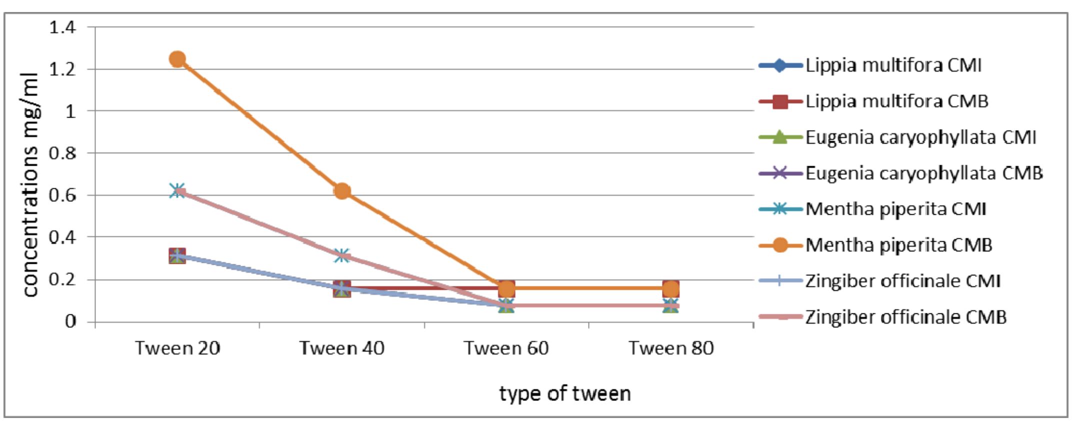


**Fig 3:** Evolution of the MIC and MBC of studied essential oils based on different types of tweens used at 5% on *Micrococcus luteus.*

For *M. luteus*, findings reveal that regardless of the essential oil mixed with the tween, the obtained curves of MIC and MBC decreased from tween 20 to tween 40 and tween 40 to tween 60. However, from tween 60 the curves remained constant up to tween 80. This shows that the values of MIC and MBC decreased from tween 20 to 60 and constant between tween 60 and 80.

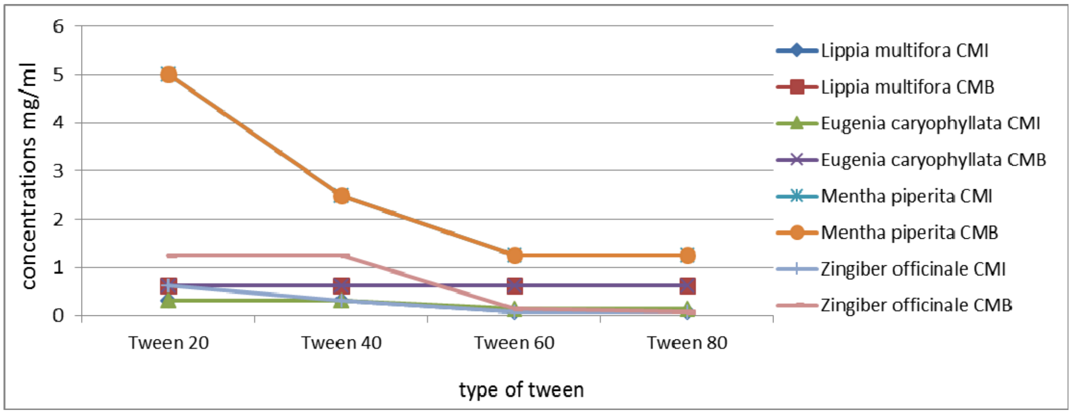


**Fig 4:** Evolution of the MIC and MBC of studied essential oils based on different types of tweens used at 5% on *Staphylococcus aureus*

When used on *S. aureus*, the essential oils mixed with the different tweens generated MIC and MBC curves that decreased from tween 20 to tween 40 and from tween 40 to tween 60. From tween 60 to tween 80, the curves remained constant. This shows that the values of MIC and MBC decreased from tween 20 to tween 60, but stayed constant between tween 60 and 80 on *S. aureus*. Figure 4 also reveals that the MBC value (0.625 mg/ml) of the essential oil of *Lippia multiflora* remained invariant for the different types of tweens.

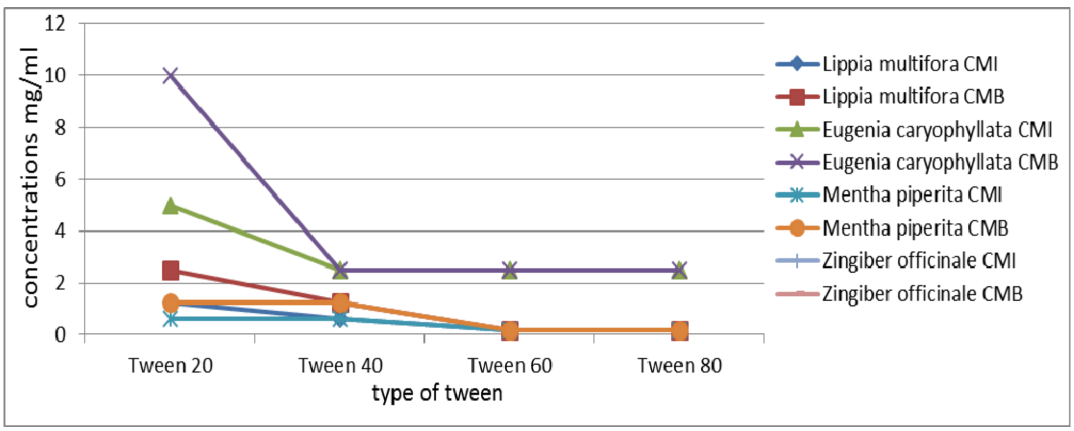


**Fig 5:** Evolution of the MIC and MBC of studied essential oils based on different types of tweens used at 5% on *Candida albicans*

The analysis of figure 5 shows that the curves of MIC and MBC values decreased from tween 20 to tween 40 and from tween 40 to tween 60, but remained constant between tween 60 and tween 80. This means that the MIC and MBC values decreased from tween 20 to tween 60, but stayed steady between tween 60 and tween 80 on *C. albicans*. The absence of MIC and MBC curves for *Zingiber officinale* shows that this extract does not have any effect on the studied yeast.

**Table 3:** Effect of the different tweens at 5% on the germs

| | GERM | | | | |
|---|---|---|---|---|---|
| **Type of tween** | ***Pseudomonas aeruginosa*** | ***Proteus mirabilis*** | ***Micrococcus luteus*** | ***Staphylococcus aureus*** | ***Candida albicans*** |
| Tween20 | ND | ND | ND | ND | ND |
| Tween40 | ND | ND | ND | ND | ND |
| Tween60 | ND | ND | ND | ND | ND |
| Tween80 | ND | ND | 1/2 | ND | ND |

ND : Not Determined

The half value (1/2) corresponding to a concentration of 10 mg/ml recorded with tween80 on *M. luteus*, shows its influence on the antimicrobial activity of this plant.

### 3.3 Antimicrobial power of the essential oils

Table IV shows the MBC/MIC ratio of each essential oil on the studied germs. This ratio is used to determine the bactericidal or bacteriostatic activity of the tested oils. When the ratio is lower than or equal to 4, the oil is considered bactericidal but when it is higher than 4, the oil is reported bacteriostatic.

**Table IV:** MBC/MIC ratios obtained with tween 60

| Plant species | MBC/MIC | | | | |
|---|---|---|---|---|---|
| | ***P. aeruginosa*** | ***P. mirabilis*** | ***M. luteus*** | ***S. aureus*** | ***C. albicans*** |
| *Lippia multiflora* | 2 | 1 | 2 | 8 | 1 |
| *Eugenia caryophyllata* | 1 | 1 | 1 | 4 | 1 |
| *Mentha piperita* | 1 | 2 | 2 | 1 | 1 |
| *Zingiber officinale* | ND | ND | 1 | 2 | ND |

The analysis of this table reveals that with tween 60, all essential oils have a bactericidal power on the germs except for *Lippia multiflora* that had a bacteriostatic activity on *S. aureus*. *Zingiber officinale* was inactive on *P. aeruginosa, P. mirabilis* and *C. albicans.*

## 4. Discussion

The present study revealed that the highest yield of essential oils extraction was obtained with *Eugenia caryophyllata* (15.7%) and the lowest (1.2%) with *Zingiber officinale*.

The antimicrobial activity of the essential oils of *Lippia multiflora*, *Eugenia caryophyllata, Mentha piperita* and *Zingiber officinale* was evaluated *in vitro* on two Gram+ bacteria (*M. luteus, S. aureus*), two Gram- bacteria (*P. aeruginosa, P. mirabilis*) and one yeast (*C. albicans*). It came out that the Gram- bacteria were more resistant to the tested oils than the Gram+ bacteria. The architectural structure of the cellular membrane of Gram+ bacteria is less complex than the one of Gram- bacteria. This structural difference makes them more sensitive to the essential oils and other antimicrobial substances [17]. *M. luteus* was the most susceptible microorganism to the tested oils. The high sensitivity of Gram+bacteria to the compounds of essential oils was previously reported by other authors [17-19]. *P. aeruginosa* was the most resistant germ of the study. This resistance of *P. aeruginosa* was also reported in many other studies [20-22]. The resistance of Gram- bacteria can be attributed to their hydrophilic external membrane that can block the penetration of hydrophobic compounds in the targeted membrane [23]. Moreover, the study revealed for all the essential oils mixed with tweens that the curve of MIC values decreased from tween 20 to tween 40 and from tween 40 to tween 60. However, between tween 60 and tween 80 the curves remained constant. This explains that MIC values decreased from tween 20 to tween 60 but stayed constant between tween 60 and tween 80 on all the five tested microbes. Similar observations were recorded for the MBC values.

The MIC values obtained with tweens 20 and 40 at 5% varied from 0.156 to 5 mg/ml, whereas the use of tween 60 in the same conditions induced MIC values that ranged from 0.078 to 1.25 mg/ml on the same microorganisms. It can therefore be concluded that the performances of the oils vary according to the type of tween used. Previous studies demonstrated that the nature and the concentration of an emulsifier can influence the antimicrobial activity of an essential oil [16]. The results of tweens 20 and 40 at 5% produced very high MIC and MBC values. This could be due to their amphiphilic structure that prevents them from diffusing at the interface of the hydrophilic and hydrophobic phases of the essential oils. Besides, the amphiphilic structure of tweens 60 and 80 allows their adequate dispersion at the interface of the hydrophilic and hydrophobic phases of the essential oils leading to the formation of molecular films [24]. This results into a good diffusion of the oils in the liquid and solid media [25-27].

Furthermore, the study revealed that tween 80 inhibited the growth of *M. luteus* at half dilution (1/2). The use of this tween could have a synergic action with some essential oils and thus bias the actual antimicrobial activity of the essential oils. The study also demonstrated that tween 60 presents the best characteristics. These differences could be due to the influence of the type of tween used. They can also be explained by the fact that the tested essential oils do not have the same chemical composition. The chromatographic analysis of the essential oils showed the diversity and the complexity of their chemical composition. Some are rich in linalol, 1,8-cineole and myrtenol (*Lippia multiflora*); eugenol, eugenyle acetate (*Eugenia caryophyllata*); and others in menthone, menthol (*Mentha piperita*); camphene, beta phellandrene, ar-curcumene and alpha zingiberene (*Zingiber officinale*).

In this study, the essential oils of *Eugenia caryophyllata* rich in eugenol, *Lippia multiflora* rich in linalol, 1,8-cinéole and *Mentha piperita* rich in menthone and 1,8-cineole were the most active oils. The antimicrobial activity of these compounds was reported by several other authors [28-31]. Similarly, Knobloch *et al*. (1989) and Deena *et al* (2000) demonstrated that 1,8-cineole is very active on *S. aureus* [32, 33]. The essential oil of *Eugenia caryophyllata,* very effective on the tested germs, is at 85.90% constituted of eugenol. This result confirms those of Oussalah *et al*. (2007) that proved that essential oils can count more than sixty different compounds with over 85% of the main compound [34]. Although the essential oil of *Zingiber officinale* contains many major compounds, it had no antimicrobial activity on *P. aeruginosa, P. mirabilis* and *C. albicans*. Nevertheless, it was very active on *S. aureus* and *Mr. luteus*. These observed differences show that the antimicrobial activity of a plant depends on the nature of its components and the microorganisms on which it is used.

## 5. Conclusion

The results generated by the present study revealed that the antimicrobial activity of essential oils can be influenced by the type of emulsifier used. Out of the four tested emulsifiers, tween 60 showed the best characteristics and tween 80 although presenting good results seems to have a synergic action with the essential oils. With tween 60, all microorganisms were susceptible to the four oils. The essential oils of *Eugenia caryophyllata*, *Lippia multiflora* and *Mentha piperita* were the most effective oils.

## 6. References

1. Encyclopedia of Medicinal Plants; version 2$^{end}$ Edition. 2001, 10.
2. Bourkhiss M, Hnach M, Bourkhiss B, Ouhssine M, Chaouch A. composition chimique et propriétés antimicrobiennes de l'huile essentielle extraite des feuilles de *Tetraclinis articula* (vahl) au Maroc, Afrique Science. 2007 ; 03(2) :232-242.
3. Organisation Mondiale de la Santé. Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005. Genève. 2002.

4. Pousset JL. Medicinal Plants of Africa. Edisud, La Calade, 2004, 7-10, 65-66.
5. Sekousounon S. Evaluation de la qualité microbiologique des phytomédicaments améliorés vendus dans les officines de pharmacie de la ville de Cotonou (Bénin) thèse de pharm. Univ. Abomey Calavi. 2012, 46-50.
6. Newman DJ, Cagg GM, Snader KM. The influence of natural products upon drug discovery¨ Natural Product Report, 2000; 17:215-234.
7. Calixto JB. Twenty-five years of research on medicinal plants in Latin America: A personal view journal of Ethnopharmacology. 2005; 100:131-134.
8. Moukimou L, Osseni A, Agbangnani DCP *et al*. Activité antiradicalaires et étude des composés volatils de trois plantes de la médecine traditionnelle du Bénin : *Anchomanes difformis*, *Parkia biglobosa* et *Polyathia longifolia*. 2014, 1610
9. Quenum Rosine OA, Baloîtcha Olurémi DR. Contribution to the study of antimicrobial properties of extracts of *Lantana camara* L. and *Hyptis suaveolensis* Poit *Ocimum gratissimum* L. on five coetaneous germs tropism. An MSc Dissertation, University of Abomey-Calavi. 2003.
10. Hmamouchi H. et col. Etudes chimiques et microbiologiques des huiles essentielles de quelques espèces d'Eucalyptus marocaines, Rev. Med. Pharm. Afr. 1992; 6(N°2):109-117.
11. Clevenger JF, Apparatus for volatile oil determination: description of New Type Clevenger. Am Perf Ess Oil Review. 1928, 467-503
12. Verma SR, Rahman UL, Chanotiya SC *et al*. Essential oil composition of Lavandula angustifolia Mill. Cultivated in the mid hills of Uttarakhand, India, Journal of the serbian chemical society. 2010; 75(3):343-348.
13. Wan J, Wilcock A, Coventry M. the effect of essential oils of basil of the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. J Appl Microbiol. 1998; 84:152-158.
14. Mazari K, Bendinerad N, Benkhechi Ch, et Fernandez X. Chemical composition and antimicrobial activity of essential oil isolated from Algerian *Juniperus phoenicea* L and *Cupressus sempervirens*. Medicinal Plants Research. 2010; 4(10):959-964.
15. Ayadi S, Jerribi C, Abderrabba M; Extraction et étude des huiles essentielles de *Rosmarinus Officinalis* cueillie dans trois regions differentes de la Tunisie. J Soc Alger Chim. 2011; 21(1):25-33.
16. Bencheqroun HK, Ghanmi M, Satrani B *et al*. activité antimicrobienne des huiles essentielles d'*Artemisia mesatlantica,* plante endémique du Maroc. Bulletin de la société Royale des sciences de Liège. 2012; 81:4-21.
17. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr. Med. Chem. 2003; 10:813-829.
18. Remmal A, Tantaoui-Elaraki A, Bouchikhi T *et al*. Improved method for determination of antimicrobial activity of essential oils in agar medium. J Ess Oil Res 1993; 5:179-84.
19. Sivropoulou, Kokkini S, Lanaras T. antimicrobial activity of mint essential oil, journal of agricultural and food chemistry. 1995; 43:2384-88.
20. Guinoiseau E. Molécules antibactériennes issues d'huiles essentielles: séparation, identification et mode d'action. Thèse de doctorat, Université de Corse, 2010, 115.
21. Gachkar L, Yadegari D, Rezaei MB *et al*. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chemistry. 2007; 102(3):898-904.
22. Chouitah O, Meddah B, Aoues A *et al*, chemical composition and Antimicrobial Activities of the Essential Oil from *Glycyrrhiza glabra* Leaves, Jeobp. 2011; 14(3)284-88.
23. Ouattara S, Kra MKA, Kporou EK, Guede-Guina F, Evaluation de l'activité antifongique des extraits de *Terminalia ivorensis* (tekam2) sur la croissance *in vitro* de *Candida albicans.* Rev. Ivoir. Sci. Technol. 2008; 12:205-214.
24. Nogarede A, Amram H. Les tensioactifs. Actual. Pharm., 1983; 204:93-95.
25. Lens - Lisbonne C. Assessment of the bacteriological activity of essential oils and their constituents. PhD Thesis. In Pharmacy: University of Marseille, France 1988.
26. Bouchikhi T. activité antimicrobienne de quelques huiles essentielles. These sciences, univ. Blaise Pascal, Clermont-ferrant. 1994, 60-79.
27. Dongmo PM, Boyom FF, Ducelier D *et al*. Chemical composition and in vitro antifungal activity of essential oils of Citrus on mycelial growth of *Phaeoramularia angolensis*. J Books Services Company EDP. 2002; 1:95-104.
28. Bassolé IHN, Lamien-Meda A, Bayala B *et al.* Composition and Antimicrobial Activities of *Lippia multiflora* Moldenke, *Mentha* x *piperita* L. and *Ocimum basilicum* L. Essential Oils and Their Major Monoterpene Alcohols Alone and in Combination; Molecules. 2010; 15:7825-7839.
29. Oussou KR, Yolou SF, Tue Bi B *et al.* Etude Chimique Bio-Guidée de l'huile Essentielle de *Ocimum gratissimum* (Lamiaceae). Eur J Sci Res. 2010; 40:50-59.
30. Baba-Moussa F, Adjanohoun A, Adéoti K *et al*. Antimicrobial properties and phytochemical profiling of essential oils extracted from traditionally used medicinal plants in Benin. International Journal of Natural Product Science. 2012; 2(4):1-11, 47-49.
31. Inouye S, Uchinda K, Yamaguchi H *et al*. Volatile aroma constituents of three Labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact. J Essent Oil Res. 2001; 13:68-72.
32. Knobloch K, Pauli A, Iberl B *et al*. Antibacterial and antifungal properties of essential oil components. J Essent Oil Res. 1989; (1):119-128.
33. Deena MJ, Thoppil JE. Antimicrobial activity of the essential oil of *Lantana camara*. Fitoterapia. 2000; 71(4):453-455.
34. Oussalah M. Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* 0157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control, 2007; 18:414-420.