ISSN-2249-6335

ANTIMICROBIAL PROPERTIES AND PHYTOCHEMICAL PROFILING OF ESSENTIAL OILS EXTRACTED FROM TRADITIONALLY USED MEDICINAL PLANTS IN BENIN

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Abstract

Essential oils of different medicinal plants including *Clausena anisata*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Eucalyptus camaldulensis*, *Eucalyptus citriodora*, *Lantana camara*, *Lippia multiflora*, *Melaleuca quinquenervia*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, *Citrus aurantium* and *Xylopia aethiopica* were extracted and tested in this work for their antimicrobial properties against various human infectious pathogenic agents. The essential oils were tested against 5 pathogenic strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans)* using the agar diffusion and microdilution methods. *Ocimum gratissimum* essential oil was found to have the strongest antimicrobial effect with the inhibition diameter of 31±2 mm on *Staphylococcus aureus*, while *Lippia multiflora* essential oil was found to have the least antimicrobial effect (inhibition diameter of 10 ± 2 mm) on *Enterococcus faecalis*. The Minimal Inhibitory Concentration (MIC) varies from 0.156 mg/mL to 10 mg/mL depending on the essential oil. Our results showed that *Ocimum gratissimum* have the lowest MIC (0.156 to 2.5 mg/mL) as expected. The chromatographic derived phytochemical profiling analysis showed thymol, 1,8-cineole, sabinene, citral, limonene and estragole to be the major components of the essential oils.

Key words: Antimicrobial activity, Benin, essential oils, medicinal plants.

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Introduction

According to recent survey of the World Health Organization (WHO), more than 80% of the rural and low income world's population uses natural medicinal plants to treat a wide variety of their ailments due to a combination of both high cost, availability and various side effects associated to synthetic drugs. Recently, the growing demand for medicinal plants to treat human diseases has worldwide increased considerably [1]. Furthermore, medicinal plants have been the primary sources of pharmaceutical/drug development, and nowadays, more than 75% of the African population has turned to medicinal plants to address their medical issues [2].

Since 1977, a genuine and health initiative effort has been launched by WHO to promote the development and improvement of traditional medicine [3]. Since then, several of these plants have been studied by individual or concerted groups of scientists around the world to elucidate their bioactive phytochemicals -using a wide range of analytical-based research approaches. However, due to the overwhelming number of natural medicinal plant species available at different habitats around the world, the beneficial values of these important plants are believed to be underexploited. If efficiently tapped, these resources have the potential to adequately treat a wide range of human diseases at relatively low cost and with relatively minimal side effects.

Although several effectively active antibiotics and antifungal drugs have been developed to control different human microbial pathogens, these drugs are often too expensive and beyond the affordable means of ordinary rural people. It is therefore important to explore and characterize the medicinal properties of these valuable plants to promote human health in a more affordable way in the rural regions of the world.

In Benin, one of the smallest developing countries of West Africa, several plants have been known historically for their strong antimicrobial properties and their use is well known and widespread among rural population in the Country. Generally, these plants are often administered orally to patients in the form of decoction, maceration, infusion, or tea [4].

In this study, we investigate the antibacterial activities of a wide range of well known and widely used medicinal plants to treat human infectious disease in Benin and characterize the phytochemical profiling of the plant essential oils.

Material and methods

Plant sampling

The essential oils have been extracted from the different parts of the plants including the leaves, the fruits or the pericarps of fruits. In this study, the following medicinal plants were considered: *Clausena anisata* Willd, *Cymbopogon citratus* (DC) Stapf , *Cymbopogon nardus* L. Rendle, *Eucalyptus camaldulensis* Dehn, *Eucalyptus citriodora* Hook, *Lippia multiflora* Moldenke, *Lantana camara* L, *Melaleuca quinquenervia* S. T. Blak (Cav), *Mentha piperita* L., *Ocimum basilicum* L., *Ocimum gratissimum* L., *Citrus aurantium* L. (pericarps of fruits), *Xylopia aethiopica* A. Rich (fruits). The identification of the plants has been performed at the National herbarium of the University of Abomey-Calavi. The harvest of the plants has been done in geographical regions of Benin including Porto Novo, Sèmè, Adjarra (Ouémé department), Kétou (Plateau department), Save (Zou department) and Kandi (Alibori department).

("Atlantic" department).

Microorganisms

In this study, the following microorganisms ATCC (American Type Culture Collection), IP (Institute Pasteur de Strasbourg) reference strains, responsible for common infections, which are not complicated to culture, as well as *Staphylococcus aureus* ATCC 29213, *Enterococcus*

faecalis ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* IP 4872 were used for the microbial tests.

Essential oil extraction

The essential oils were extracted by hydrodistillation with a Clevenger - type apparatus during 180 and 240 min according to the part of the plants. The essential oils were then dried on anhydrous magnesium sulfate and stored at 4° C in brown colored storage bottles.

Essential oil analysis by Gas Chromatography coupled with mass spectrometry

The phytochemical profiling of the essential oils was performed by gas chromatography analysis coupled with mass spectrometry. The gas chromatography (DELSI IGC 121°C) was equipped with the capillary column CP WAX 52 CBS (25 m of length and 0,3 mm of interior diameter) and a flame ionization detector (FID). The vector gas was nitrogen with 1 mL/min debit in the exit of the column, the report of leak was1/60 = 1mL/min and the pressure was 0,8 bar. The combustible gas was hydrogen (debit, 30 mL/min and pressure, 1 bar).The oxidizing gas was compressed air with a debit of 300 mL/min under a pressure of 1 bar. The injection was performed at 240° C and the detector temp was 250° C. The isotherm landing was at 50° C for 5 min with a pressure gradient of 2° C/min for 85 min until a final temperature of 220°C was reached. The analysis was carried out with 0,05 µLS of injected oil.

The chromatography analysis was coupled to a mass spectrometric analysis with the following parameters. The mass detector used was HEWLETT PACKARD (HP) type 5970 with electronic impact (70 eV) ionization, equipped with a non-polar capillary column (DB-1) in melted silica of 25 mm long and 0,23 mm of interior diameter. The vector gas used was helium with a debit of 0,9 mL/min. The isotherm landing was at 1 min in 60° C, and the pressure gradient was 3°C/min until 180° C. The volume of injected oil was 1 µL.

In vitro antibacterial and antifungal activity

The sensitivity of the germs to the different essential oils was tested with the agar diffusion and the microdilution method. The dose-response effect of the extract was studied with *Ocimum gratissimum* and of E*ucalyptus camaldulensis* essential oils. We recorded the inhibition diameter zone at different concentrations of the essential oils. A set of five concentrations obtained by serial dilution were tested (320; 160; 80; 40; 20 mg/mL).

The microbial organisms were plated on agar plates and incubated at 37°C for 24 hours for the bacteria and at 27°C for 48 hours for the yeast. After 24 hours or respectively 48 hours, the microorganisms were streaked onto Mueller Hinton Agar for the bacteria and on Sabouraud agar for *Candida albicans*. Under sterile conditions, an appropriated colony was mixed with 5 ml sterile distilled water. The surface of the Mueller Hinton Agar or Sabouraud was inoculated with 5 ml of suspended strains. The discs were inoculated with 20 µL of essential oils with above indicated concentrations. Three discs were deposited per Petri dish and 3 cm of space was left to separate two disks. Every concentration was tested five times. Plates were incubated at 37°C during 24 hours for the bacteria and at 27°C during 48 hours for *Candida albicans*. The inhibition diameter was recorded after microbial incubation.

Minimal Inhibitory Concentration (MIC) Determination

Essential oil emulsion preparation

An initial solution of 20 µL/mL (20000 ppm or 20 mg/mL) essential oil was prepared in nourishing broth or Sabouraud. Tween 80 was added at a rate of 5% and homogenized by vortexing the mixture.

Preparation of microbial solution

The microorganisms were plated and incubated for 24 hours for bacteria cultures and 48 hours for *Candida albicans*. One microbial colony was suspended in 9 ml of Mueller Hinton Broth or Sabouraud in a test tube. After incubation at 37 °C and during 24 hours, we obtained a broth with 10^9 germs /mL for the bacteria and 10^7 germs/mL for *Candida albicans* (after incubation at 27°C during 48 hours). The suspended strains were calibrated to 10^6 germs/mL allowing the determination of the antimicrobial activity of essential oil.

MIC determination

For this test, 96 well plates were used. 950 µL of media was put in each well and 50 µL of the initial concentration of essential oil (20 mg/mL) was added in the wells of the first column and 950 µL of media was then added in each well of the third column of the plate. And successive dilutions were made horizontally. Finally, all wells, except those of the first column, were inoculated with 50 µL of microbial inoculums at 10^6 bacteria/mL. The microplates were covered and incubated at 37°C during 24 hours for the bacteria under rocking condition. For *Candida albicans*, the culture was made on Sabouraud broth at27° CS during 48 hours. The comparison between the control and test wells was made. The diameters of inhibition obtained have been compared to those of the antibiotics to which the strains are usually sensitive.

Results

Essential oil extraction

The highest yield (2.86%) was obtained with *Xylopia aethiopica* A. Rich essential oil and the lowest yield (0.26%) was obtained with *Lantana camara* L.

Preliminary study

Our preliminary investigation performed with 40 mg/mL of essential oils revealed no antimicrobial activity with *Clausena anisata, Melaleuca quinquenervia* and of the pericarps of fruits of *Citrus aurantium* essential oils. The averages of the inhibition diameters obtained have been measured. The highest diameter of inhibition (31±2 mm) was detected with *Ocimum gratissimum* essential oil on *Staphylococcus aureus*; while the smallest inhibition zone (10 ±2 mm) was obtained with *Lippia multiflora* essential oil on *Enterococcus faecalis.*

Dose-responsive effects of essential oil assays

Three of the five microorganisms (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*) tested were found to be very sensitive to *Ocimum gratissimum* and *Eucalyptus camaldulensis* essential oils. is *Staphylococcus aureus* was found to be very susceptible to the effect of *Ocimum gratissimum*, while *Escherichia coli* and *Pseudomonas aeruginosa* were found to be resistant to *Eucalyptus camaldulensis*. The antimicrobial effects were found to be dose-responsive dependent. All tested microorganisms were susceptible to the essential oils at higher concentrations as indicated by the diameter of the inhibition zone (Table 1).

Table 1. Dose-responsive effects of essential oil assays

Essential oils		*Ocimum gratissimum*					*Eucalyptus camaldulensis*				
Concentrations (C) in mg /ml		20	40	80	160	320	20	40	80	160	320
Average diameters of inhibition (mm)	S.a	21 ± 2	31 ± 2	37 ± 1	40 ± 1	45 ± 2	17 ± 1	22 ± 2	25 ± 1	31 ± 1	37 ± 2
	E.c	14 ± 1	25 ± 1	31 ± 2	35 ± 2	40 ± 2	15 ± 1	24 ± 2	30 ± 1	34 ± 2	38 ± 1
	C.a	20 ±2	29 ± 2	31 ± 1	35 ± 2	38 ± 2	14 ± 1	20 ± 2	23 ± 2	27 ± 1	32 ± 1
	E.f	13 ± 1	19 ± 1	22 ± 1	27 ± 1	30 ± 1	11 ± 2	15 ± 1	18 ± 1	21 ± 1	26 ± 2
	P.a	09 ± 1	16 ± 2	20 ± 1	22 ± 1	25 ± 2	08 ± 1	11 ± 1	13 ± 2	15 ± 1	17 ± 1

S.a : *Staphylococcus aureus* ; E.c : *Escherichia coli* ; C.a : *Candida albicans* ;
E.f : *Enterococcus faecalis* ; P.a : *Pseudomonas aeruginosa*

Minimal Inhibitory Concentration research

The minimal inhibitory concentration (MIC) assays showed research revealed that the oil of *Ocimum gratissimum* essential oil to have the strongest antimicrobial effect with the smallest MIC value (0,156 to 2.5 mg/mL). It is followed by *Eucalyptus camaldulensis*, *Lantana camara* and *Lippia multiflora* essential oils.

The most sensitive microorganisms were *Escherichia coli* (MIC 5 mg/mL), *Candida albicans* (MIC 10 mg/mL) and *Staphylococcus aureus* (MIC 10 mg/mL), while the most resistant strain was *Pseudomonas aeruginosa* (Table 2).

Table 2. Results of the determination of MIC of essential oils

	MIC (mg / ml)				
	Staphylococcus aureus	*Escherichia coli*	*Candida albicans*	*Enteroucoccus faecalis*	*Pseudomonas aeruginosa*
Ocimum gratissimum	0 .156	1.25	0.312	0.625	2.5
Eucalyptus camaldulensis	2.5	1.25	2.5	5	5
Lantana camara	2.5	1.25	2.5	10	ND
Lippia multiflora	10	5	5	10	ND
Xylopia aethiopica	2.5	5	ND	ND	ND
Cymbopogon citratus	5	ND	ND	5	ND
Mentha piperita	ND	5	10	ND	ND
Cymbopogon nardus	ND	2.5	ND	ND	ND
Ocimum basilicum	>1O	>10	2.5	>10	>10
Eucalyptus citriodora	ND	ND	5	ND	ND
Melaleuca quinquenervia	ND	ND	ND	ND	ND
Péricarpe d'orange	ND	ND	DN	ND	ND
Clausena anisata	ND	ND	ND	ND	ND

The essential oil analysis

The chromatographic analysis of *Ocimum gratissimum*, *Eucalyptus camaldulensis*, *Lantana camara* and *Lippia multiflora* essential oils are summarized in Tables 3 - 6.

Essential oil of *Eucalyptus camaldulensis*

More than 98.28% of the phytochemical components of this extract were identified, of which the major compound (71.41%) was found to be 1,8-cineole (Table 3).

Table 3. Chemical composition of essential of *Eucalyptus camaldulensis*

N°	Retention time (min)	Kovats Indice	Identified components
8,66	935	a- pinene	3,12
9,97	978	E3 -pineme	1,94
10, 33	992	a- pellandrene	0,09
11,51	1033	P- cymene	0,34
11,60	**1033**	**Limonene**	**10,57**
12,06	**1050**	**1,8-cineole**	**71,41**
13,57	1091	y - terpinene	0,80
14,77	1122	fenehol	0,23
16,63	1166	Pinocampophe	0,39
16,91	1188	Terpinen - 4 - ol	0,94
17,41	1212	a-terpineol	1,88
18,68	1250	transpinocarveol	0,21
27,97	1440	aromadendrene	0,19
28,72	1575	elemol	0,16
29,50	1603	spathulenol	1,12
29,71	**1625**	**globulol**	**4,23**
30,02	1625	vicidillorol	0,37
30,53	1625	δ-endesmol	0,15
30,96	1675	E3-endesmol	0,14

Identified components: 98.28%, Non-Identified components: 1.72%

Essential oil of *Lippia multiflora*

For this extract we identified about 96, 30% of the chemical components. Linalol (51.1%) was the major component of Lippia multiflora (Table 4).

Table 4. Chemical composition of the essential oil of *Lippia multiflora*

N°	Retention time (min)	Kovats Indice	Identified components
8,91	933	a-thujene	1,1
9,12	937	a-pinene	3,9
9,63	951	camphene	0,5
10,63	980	E3-pinene	1,2
11,24	1001	Myrcene	0,2
11,63	1012	a-pellandrene	1,7
12,06	**1050**	**1,8- cineole**	**17,4**
13,57	**1091**	**Linalool**	**51,1**
14,77	1122	a-terpineol	5,2
16,63	**1166**	**Myrtenol**	**5,4**
16,91	1188	Thymol	0,8
17,41	1212	Carvacrol	0,1

18,68	1250	thymyle acetate	0,2
24,97	1440	carvacryl acetate	0,2
28,72	1575	β-caryophyllene	2,8
29,50	1603	Cis- β- farnesene	0,8
29,71	1625	Germacrene D	2,9
30,02	1625	δ-cadinene	0,3
30,02	1625	caryophyllene oxide	0,5

Identified components:96,3 0%, Non Identified components: 3,7%

Essential oil of *Lantana camara*:

With *Lantana camara* essential oil, we identified more than 98,89% of the chemical components of the plant and sabinene was detected as the major component (21,47%) (Table 5).

Table 5. Chemical composition of the essential oil of *Lantana camara*

N°	**Retention time (min)**	**Kovats Indice**	**Identified components**
3,16	930,1	α –Thujene	0,45
3,24	937,6	α -pinene	3,56
3,43	953,5	Campene	1,47
3,75	**978,3**	**Sabinene**	**21,47**
3,79	981,1	β-pineme	1,36
3,94	992,0	Myrcene	1,65
4,21	1012,7	α-pellandrene	1,93
4,42	1029,0	α-terpinene	1,05
4,47	1032,8	p- cymene	2,48
4,7	**1019,1**	**1,8-cineole**	**16,88**
5,238	1085,5	γ-terpinene	1,35
5,735	1120,0	linalol	0,52
6,405	1166,7	camphre	1,64
6,657	1183,0	bomeol	0,92
6,793	1191,6	terpinen – 4 - ol	1,05
7,015	1206,5	α-terpinol	1,96
10,125	**1428,9**	**β-caryophyllene**	**14,34**
10,578	1463,4	α-humulene	6,73
11,137	1504,8	viridiflorene	1,72
11,995	1573,7	Trans nerolidol	3,58
12,068	1579,3	spathulenol	0,45
12,255	1593,6	Caryophyllene oxide	2,04
12,293	1596,5	globulol	1,64
12,407	1605,9	Guaiol	3,20
12,47	1611,5	Humulene epoxde II	0,74
12,723	1633,5	γ-eudesmol	1,07
12,94	1652,0	α-eudesmol	2,79
13,03	1658,7	α-cadinol	0,85

Identified components:98,89 %, Non-Identified components: 1,11%

Essential oil of *Ocimum gratissimum*

As shown in Table 6, 95,68% of the chemical components of this essential oil were identified and thymol (40,45%) was detected to be the major component of the extract.

Table 6. Chemical composition of the essential oil of *Ocimum basiicum*

N°	**Retention time (min)**	**Kovats Indice**	**Identified components**
8,91	933	a-thujene	0,17
9,12	937	a-pinene	0,59
9,63	951	camphene	0,09
10,63	980	3-pinene	0,16
11,24	1001	myrcene	3,18
11,63	1012	a-pellandrene	0,24
11,82	1016	~-3-carene	0,16
12,08	1029	a-terpinene	3,12
12,43	**1039**	**Para cymene**	**14,94**
12,52	1047	Limonene	1,34
12,83	1050	1,8- cineole	0,24
13,17	1058	(Z)-b-ocimene	0,15
13,59	**1071**	**γ-terpinene**	**15,52**
13,95	1096	terpinolene	0,28
14,52	1105	Para cymenene	2,02
14,95	1128	linalol	0,37
15,35	1135	trans-p-2,8-menthadien-1-ol	0,26
17,31	1205	Terpinen-4-ol	3,7
17,76	1212	a-terpineol	0,4
17,87	1221	Estragol	0,28
18,88	1253	Thymolmethylether	0,46
21,06	**1358**	**thymol**	**40,45**
21,23	1364	carvacrol	1,18
23,93	1435	3-caryophyllene	1,97
24,25	1453	Trans a-bergamotene	0,06
24,76	1471	a-humulene	0,36
25,47	1503	Germacrene D	0,17
25,6	1506	3-selinene	2,32
25,78	1515	a-selinene	1,02
26,31	1528	y-cadinene	0,21
26,4	1534	~-cadinene	0,27

Identified components: 95, 68%, Non-Identified components: 4, 32%

Discussion

The highest yield (2.86%) was obtained from *Xylopia aethiopica*, while the lowest yield (0.26%) was obtained from *Lantana camara*. The preliminary study of the antimicrobial activity using the agar diffusion method performed on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida albicans* was very promising. It is evident from our findings that *Ocimum gratissimum* and *eucalyptus camaldulensis* essential oils efficiently inhibit the growth of all microorganisms tested in this study. At a concentration of 40 mg/mL, the diameters of inhibition zone ranged from11 to 24

mm and 16 to 31 mm for eucalyptus *camaldulensis* and *Ocimum gratissimum* essential oils respectively. These two oils showed an inhibition zone of about 20 mm for *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* respectively. Our results differed from those of Nakamura *et al*. [5] who, in 1999, had shown that *Staphylococcus aureus* and *Escherichia coli* were more sensitive to the essential oil of *Ocimum gratissimum* at a concentration of 48 mg/mL with corresponding inhibition diameters of 21 mm and 17 mm respectively. This difference could be explained by the chemical composition of the different essential oils of *Ocimum gratissimum*. Apart from the essential oils of *Clausena anisata*, *Melaleuca quinquenervia* and of pericarps of *Citrus aurantium* which revealed no antimicrobial effect, all the other extracts tested in this study showed an inhibition zone of about 20 mm on the microorganisms.

The measurement of the dose-responsive effect for the two essential oils (*Ocimum and Eucalyptus*) showed a dose dependent effect of the extracts on the microorganism growth. Among the microorganisms, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were the most susceptible to the essential oils followed by *Enterococcus faecalis*. *Pseudomonas aeruginosa* was found to be the most resistant microorganism. The MIC assays showed that the active essential oils were efficient from varying concentrations between 0.156 and 10 mg/mL. *Ocimum basilicum* essential oil was active at concentration starting from 10 mg/mL on the bacteria. However in agar plate test, the bacterial growth inhibition started to be obvious at 40 mg/mL of the essential oil. The MIC detected in this study was identical to those of Quenum and Baloïtchas (0.126 mg/mL for *Ocimum gratissimum*) [6]; and they differ from those reported by Gbenou J. D. [7] for *Eucalyptus camaldulensis* (2.5 mg/mL against 5 mg/mL); for *Eucalyptus citriodora* (5 mg/mL against 1.25 mg/mL) and for *Melaleuca quinquenervia* (reported to have no inhibition effect against our report with 2.5 mg/mL MIC). These differences could be explained by the fact that the tested essential oils could have had different chemical compositions due to the different analytical procedures used to extract and identified the compounds in these extracts by respective studies.

The chromatographic analysis of active essential oils has shown to contain the following major compounds: thymol (43.45%), gamma -terpinene (12.11%) and p-cymene (12.26%) for *Ocimum gratissimum*; limonene (10.57%) and the 1,8-cineole (71.41%) for *Eucalyptus camaldulensis*. These main components have been observed in *Ocimum gratissimum* essential oil from Cameroon at a rate of 46.2% for thymol and 20% for terpinene [8]. On the other hand *Ocimum gratissimum* essential oil from Rwanda was reported to contain a large amount of eugenol [9]. However, in most cases, thymol was the major component of this extract [10, 11].

The antimicrobial activity observed in this study could be due to the active effect of one or a combination of these compounds. *Eucalyptus camaldulensis*, which is rich in 1,8-cineole (71.41%), was found to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* with respective MIC's of 2.5 and 1.25 mg/mL. Also, LENS LISBOA *et al* [12] have shown that 1,8-cineole and p-cymene inhibited *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* with MIC > 1 mg/mL. At the same time, they also showed that thymol had a MIC of 0.10; 0.10 and 0.20 mg/ml on *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis,* respectively [12]. In this study, the essential oil of *Ocimum gratissimum* essential oil, which is rich in thymol (43.45%) was found to have a similar MIC on the same microorganisms (0.156; 1/25 and 0.624 mg/mL, respectively) tested by LENS LISBOA *et al* [12]. The work of SIMEON de BOUCHBERG [13] confirmed our findings with thymol MIC about 0.13 mg/mL and 1 mg/mL, and p-cymene MIC of about 2 mg/mL. The inhibitory activity of these two essential oils on *Candida albicans* was very interesting (MIC≤ 2.5 mg/ml). The different major components of the two oils were suggested to have potential antifungal properties. Inouye S. *et al.* [14] have shown in 2001, that thymol and 1,8-

cineole were active against *Candida albicans*. In the same way, KNOBLOCH K. *et al*.[14] demonstrated in 1989, that 1,8-cineole is active on *Candida albicans*.

The essential oil of *Lantana camara* was found to have a MIC from 1.25 to 10 mg/mL in this study. However, Quenum and Baloïtchas [4] and Deena M. J. *et al*. [16] found this plant to have a MIC from 0.115 to 22.546 mg/mL. On the other hand, Bassole H. N. *et al.*[17] have found inhibition diameters ranging from 6 to 11mm for the essential oil of *Lippia multiflora*, which is more or less in agreement with our inhibition diameters ranging from 10 to 12 mm for the same plant extract.

The essential oils from *Clausena anisata* and *Melaleuca quinquenervia* orange fruit pericarps showed no inhibition effect. Chemical analysis of these oils showed the following major components: estragole (83.19%) for *Clausena anisata*; 1,8-cineole (30.01%), viridiflorol (11.89%), and limonene (11.29%) for *Melaleuca quinquenervia* and limonene (92.00%) for the orange pericarps. Our results differ from those of Gbenou J. D. [8] who showed that *Melaleuca quinquenervia* inhibited the growth of some bacteria and *Candida albicans*. Dongmo P. M. J. *et al.* [18], in 2001, found that the most active essential oils extracted from Citrus were rich in neral and geranial. The difference between these findings could be due to the chemical composition of these oils. Comparing the diameters of inhibition zones with positive controls [19], we found that our diameters of inhibition zones were comparable to those of ampicillin, co-trimoxazole, norfloxacin and oxacillin on plate grown microorganisms. We hypothesized that the purified antimicrobial compounds from our essential oils would be better that the antibiotics used here as positive controls. A combination of various compounds in essential oils especially antagonistic compounds could lead to significant reduction of the active compounds. The present study showed that no correlation exist between the yield and the activity.

Conclusion

In this study we evaluate the antimicrobial activity of essential oils from thirteen plants against five microorganisms causing a wide variety of human infectious diseases, by the agar diffusion and microdilution methods. The chemical analysis showed several major components of which thymol;1,8-cinéole; geranial; limonene and estragole were the common compounds found in the tested oils. Some plants found in the flora of Benin possessing antimicrobial properties are here revealed. The tested plant species were selected due to their widespread use in traditional medicine. The fact that we observed weak or no antimicrobial activity with some oils can be attributed to their composition.

Conflict of interest

The authors declare no conflict of interest

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