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Auteur correspondant : tolba.hadjer@hotmail.fr

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Essential oil of Algerian *Eucalyptus citriodora*: Chemical composition, antioxidant and antimicrobial activities

TOLBA Hadjer[a,*], MOGHRANI Houria[a], ABOUN Assia [b], MAACHI Rachida[a]

[a] *Laboratoire de Génie de la Réaction, Faculté de Génie mécanique et Génie des procédés, USTHB, BP 32 El Alia, 16075 Bab Ezzouar, Alger, Algérie.*
[b] *laboratoire de bactériologie et Sérologie Vétérinaire, Institut Pasteur, Kouba, Alger, Algérie*

Abstract

This study was carried out to assess the chemical composition, antioxidant and antimicrobial activities of *Eucalyptus citriodora* essential oil growing in Algeria. The chemical composition of the oil was analyzed by GC and GC/MS and revealed the presence of 22 compounds which accounted for 97.13 % of the oil. The main compounds was Citronellal (69.77 %) followed by Citronellol (10.63 %) and Isopulegol (4.66 %). Antioxidant activity was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The free radical scavenging activity of the oil was found to be inferior (IC_{50} values = 0.896 mg/ml) as compared to butylatedhydreoxytoluene (BHT), (IC_{50} value = 0.041 mg/ml). The antimicrobial activity of *Eucalyptus citriodora* essential oil against twelve bacteria and two yeasts was determined via both the disc diffusion method and the Disc volatilization method. Higher antimicrobial activity was observed in the liquid phase. The minimum inhibitory concentration (MIC) was found to vary from 0.6 to 20 µl/ml for bacteria while for yeasts it was equal to 1.25 µl/ml. The most sensitive strains were the Gram positive bacteria *Staphylococcus epidermidis* and the two yeasts while the most resistant bacterium was *Pseudonomas aeruginosa*. This is the first report on the antioxidant and antimicrobial properties of the essential oil of *Eucalyptus citriodora* growing in Algeria.

Keywords: Antimicrobial activity; Antioxidant activity; Chemical composition; Essential oil; *Eucalyptus citriodora* Hook.

1. Introduction

Aromatic plants, such as those from *Myrtaceae* family are known for their wide spread use both traditionally and commercially to increase the shelf-life and safety of foods. They have demonstrated several therapeutic properties, mainly antioxidant, antimicrobial and anti-inflammatory. This usefulness of plants is due to their complex mixture of compounds including aldehyde, and phenolic compounds [1]. In particular, the antimicrobial properties of plant essential oils and their constituents have been widely demonstrated [2]. *Eucalyptus*, a native from Australia, belongs to *Myrtaceae* family and includes about 800-900 species and subspecies [3, 4]. Today, *Eucalyptus* may be found almost everywhere in the world since it is grown in many countries [5] including tropical, subtropical and even sub-temperature countries [6]. Aromatic plants and their essential oils have been used since antiquity in flavor, fragrance, embalmment, preservation of foods, antimicrobial remedies, medecine, perfumery, and pharmaceutical industries [7, 8]. *Eucalyptus* has been largely studied as a source of essential oil, extracted through steam or hydrodistillation, which has diverse biological activities. Several works have focused on the functional aspects of *Eucalyptus* essential oil, such as antimicrobial, antifungal, insecticidal, and acaricidal activities [7]. The antimicrobial effects against a wide range of microorganisms have been considered in several studies. The most often considered species include *Eucalyptus globulus* [9], *Eucalyptus camaldulensis*, *Eucalyptus terticornis*, and *Eucalyptus citriodora* [3, 10, 11]. In the present work, we focused on *Eucalyptus citriodora*, which is one species of eucalyptus widely used in perfumery, and as an important ingredient of cosmetics, food and pharmaceutics. Its essential oil exhibits various biological properties. These effects are attributed to the monoterpenes, which are the major chemical components of this essential oil. *Eucalyptus citriodora* has been shown to contain about 70 % of monoterpenoid, Citronellal, which is a monoterpene product from the plant secondary metabolism. different studies have demonstrated that *Eucalyptus citriodora* essential oil possesses a wide spectrum of biological activities including antimicrobial

activity against bacteria, fungi, and yeasts [12-17], analgesic and anti inflammatory effects [18, 19], antioxidant activity [6] and insecticide effects [20]. On the other hand, biological and antioxidant properties of its major constituent monoterpene, Citronellal, have been very poorly explored [6, 15, 16]. To the best of our knowledge, no study has been conducted to evaluate the essential oil from Algerian E. citriodora for antioxidant and antimicrobial activities. Moreover, there are not the studies of the antimicrobial activity in both liquid and vapour phases of the *Eucalyptus citriodora* oil. The aim of the present study is to investigate the chemical composition of *Eucalyptus citriodora* essential oil, its antioxidant activity and its effectiveness against Gram negative and positive bacteria and yeasts using antimicrobial tests: qualitative study (disc diffusion method and Disc volatilization method) and quantitative study (minimal inhibitory concentration MIC).

2. Material and methods

2.1. Plant material

In September 2014, Fresh leaves of *Eucalyptus citriodora* were collected from the National Institute of Agronomy. The leaves were identified by the herbarium, Department of Botany, by the herbarium, at the National Institute of Agronomy (Algiers, Algeria).

2.2. Essential oil extraction

Samples of 20 g from the species were subjected to steam distillation [21]. Preliminary tests made it possible to fix the duration of extraction to 90min. The extracted essential oil was dried over anhydrous sodium sulphate and, stored at 4 °C until used. The essential oil yield was expressed on the basis of dry vegetal matter.

2.3. Gas chromatographic (GC) and Gas chromatographic/mass spectrometry (GC/MS) analysis

Essential oil was analyzed using an HP (Agilent Technologies) 6800 plus, equipped with a HP-5MS column (length 30m, internal diameter 0.25 mm and film thickness of 0.25 µm), coupled to a quadrupole HP mass spectrometer (Agilent Technologies) MSD5973. During the analysis, the furnace temperature was maintained at 60 °C for 8 min, and then programmed to 250 °C with a heating speed of 2 °C/min; the temperature was maintained at 250 °C for 15 min. The selected injection mode was Split with a Split ratio equal to 1/50 with an injection volume set at 1µl.The N60 purity helium was used as carrier gas with a flow rate of 0.5 ml/min. The mass spectra were recorded at 70 eV. The relative percentages of the components were electronically calculated from GC-FID peak areas. The Components identification was achieved in comparing respective retention indices relative to C_8-C_{24} (n-alkanes); and respective mass spectra to those reported in the literature [22] and in NIST and WILEYS mass spectral libraries.

2.4. Antioxidant activity

2.4.1. Free radical scavenging capacity (RSC)

The antioxidant activity of *Eucalyptus citriodora* oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent [23]. 25 µl of various concentrations of the sample in methanol were added to 975 µl of a 60 µM methanolic solution of DPPH. Absorbance measurements were read at 517 nm, after 30 minutes of incubation time at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. BHT (Butylated hydroxytoluene) was used as positive control. Sample concentration capable of scavenging 50 % of the DPPH radicals (IC50) was obtained by plotting the percentage inhibition as a function of the concentration of EO. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_B - A_A)/ A_B] \times 100$$

where A_B is the absorption of the blank sample (t = 0 min) and A_A is the absorption of the tested oil or substance solution (t = 30 min).

2.5. Microorganisms

In the present study, fourteen microbial strains were used to assess the antimicrobial properties of *Eucalyptus citriodora* essential oil, including twelve bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis*, *Salmonella Typhi Murium, Salmonella Gallinarum Pullorum, Salmonella seftenberg*, *Enterococcus faecalis, Corynebacterium striatum, Bacillus subtilis*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis, Staphylococcus saprophyticcus*), and two yeasts (*Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae).* All strains were

procured from Pasteur Institute, Algiers, Algeria (IPA). The bacterial strains were grown in Mueller-Hinton agar (MHA) and incubated at 37 °C, while the yeasts were grown in Sabouraud Dextrose Agar (SDA) added with chloramphenicol and incubated at 25 °C.

2.6. Antimicrobial tests

2.6.1. Disc diffusion method

The disc diffusion method was employed to determine the qualitative antimicrobial activity of the *Eucalyptus citriodora* essential oil. The microbial suspension (10^8 CFU/ml) was streaked on MHA for bacteria and on SDA for yeasts using a sterile cotton swab. The absorbent disks (diameter 6 mm) were impregnated with 3 different concentrations (10, 20 and 30 µl per disc) of essential oil and placed on the surface of the media. The inhibition diameters were measured after incubation for 24h at 37 °C for bacteria and for 48h at 25 °C for yeasts [10-24]. All tests were performed in triplicate.

2.6.2. Disc volatilization method

This method describes the diffusion of the essential oil in vapour phase. Three different volumes (10, 20 and 30 µl per disc) of the essential oil were pipetted on the paper disc (diameter 6 mm) and placed on the inside surface of the upper lid of Petri dishes. The lids were immediately closed with the plates containing the solid media (MHA and SDA for bacteria and yeasts respectively), previously inoculated with microbial suspension (10^8 CFU/ml) and sealed with parafilm to prevent leakage of the EO vapour. Plates were inoculated at 37 °C during 24h for bacteria, and 48h at 25 °C for yeasts and the diameter of the inhibition zone was measured [9]. Tests were carried out in triplicate.

2.6.3. Minimum inhibitory concentration (MIC)

The MIC was determined against the microorganisms which were sensitive to the essential oil and the zone of inhibitory was ≥ 12 mm in the disc diffusion method [25] using the agar dilution method. The tests were performed using MHA for bacteria and SDA for yeasts. Various concentrations of *E. citriodora* essential oil (i.e.: 20 µl/ml to 0.3 µl/ml) were added to the media (MHA/SDA) supplemented with Tween 80 0.5 (*v/v*) to enhance the oil solubility. The resulting MHA and SDA agar solutions were immediately mixed and poured into Petri plates. All the plates were spot inoculated with the microbial suspension 10^8 CFU/ml. Plates with Tween-80 but without essential oil were used as control. After incubation for 24h at 37 °C for bacteria and for 48h at 25 °C for yeasts, the MIC was red as the highest dilution (lowest concentration) of oil where no visible growth of microorganisms was noticeable [26].

2.7. Statistical analysis

All determinations were expressed by mean ± Standard Deviation (± SD). ANOVA was conducted to determine significant differences ($P < 0.05$), and multiple comparison Tukey's post hoctest was applied between the averages. Statistical analysis of the data was performed using software XLStat (XLStat, Paris, France).

3. Results and discussions

3.1. Extraction of *Eucalyptus citriodora* essential oil

The variation of the extraction yield versus extraction time is shown in figure1. Initially, the yield increases rapidly, characterizing the extracted essential oil obtained during the first 30 min. then, the yield stabilizes (Horizontal line), marking the end of the extraction procedure, which is reached after 120 min. The extraction time was chosen to be 90min and corresponded to a time when no more drops of oil were observed in the distillate.

Steam distillation of *Eucalyptus citriodora* leaves yielded a pale-yellow colored oil (yield: 2.26 %, *w/w*, dry weight basis).

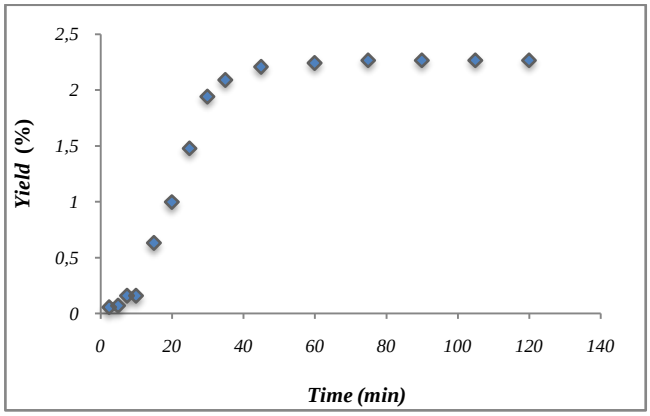

Figure1: Yield profile of essential oil as a function of the extraction time.

3.2. Chemical composition

Table 1 provides both qualitative and quantitative analyses for the *Eucalyptus citriodora* essential oil volatile profiles. A total of 22 compounds which represented 95.27 % of the oil, were identified. The dominant compounds of the essential oil was citronellal (69.77 %) followed by citronellol (10.63 %) and isopulegol (4.66 %). The remaining constituents amount to less than 2 %.

Table 1
Chemical composition of *Eucalyptus citriodora* essential oil.

N°	Compound[a]	RI[b]	Percentage %
	Monoterpene Hydrocarbons		
1	α-pinene	929	0.08
2	β-pinene	973	0.43
3	limonene	1029	0.25
	Oxygenated Monoterpenes		
4	1,8 cineole	1035	0.14
5	*cis*-Rose oxide	1112	0.27
6	*trans*-Rose oxide	1128	0.13
7	Isopulegol	1152	4.66
8	Citronellal	1167	69.77
9	Neo-isoisopulegoI	1176	0.24
10	α -Terpineol	1199	0.33
11	Citronellol	1242	10.63
12	geranial	1262	0.32
13	Isopulegyl acetate	1276	0.16
14	p-Menthane-3,8-diol	1350	2.76
15	Citronellyl acetate	1355	2.16
16	Jasmone <(Z)->	1398	0.23
	Sesquiterpene Hydrocarbons		
17	β-Caryophyllene	1415	1.34
18	α-Humulene	1450	0.10
19	BicycIogermacrene	1493	0.07
	Oxygenated Sesquiterpenes		
20	Spathulenol	1574	0.07
21	Caryophyllene oxide	1583	0.22
	Other Compounds		
22	2,6-Dimethyl-5-heptenal	1055	0.91
	Total identified components		95.27
	Not identified		4.73

[a] *Essential oil compounds sorted by chemical families and percentages on non-polar HP5MS™ capillary column.*

[b] *Retention indices relative to C8-C24 n-alkanes calculated on non-polar HP5MS™ capillary column.*

The results of our study are similar to those carried out by [14, 20, 27, 28]. The chromatographic analysis of the *Eucalyptus citriodora* essential oil showed that citronellal was the prevailing compound with amounts ranging from 46.2 % to 86 %, followed by citronellol with amounts ranging from 4.5 % to 11.86 %. This result is in contrast with a recent study of Elaissi *et al.* [3] on the *Eucalyptus citriodora* essential oil from Tunisia which showed significant differences in composition, consisting of 1,8 cineole (54 %) and α-pinene (23.7 %) with minor amounts of p-cymene, α-Terpineol (3 %), and limonene, trans-pinocarveol (2.3 %). Recently, Mittal and Ali [29] found α-pinene (38.6 %), β-pinene (25.7 %), sabinene (19.6 %) and α-thujene (11.9 %) as the major compounds in their *Eucalyptus citriodora* essential oil. In our study, these constituents were present at very low levels and accounted for less than 2 % of the oil. Furthermore, trans-pinocarveol was altogether absent. The wide variations in the oil chemical composition could be attributed to environmental and agronomical factors as well as to the extraction procedure [30].

3.3. Antioxidant activity

The oil was screened for its possible antioxidant activity by using in vitro inhibition of DPPH free radical. The DPPH radical is a free radical, which has been widely used as a tool to estimate free radical scavenging activity of antioxidants. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character [31]. The scavenging ability of essential oil and positive control (BHT) are presented in table 2. The oil (0.05-1 mg/ml) scavenged DPPH radicals by (12.1-51.91 %), with IC_{50} = 0.896 mg/ml. RSC activity was less than that of the positive control BHT (IC_{50} = 0.041 mg/ml). further, the oil exhibited moderate antioxidant activity. Similar results were shown by Singh *et al.* [6]. A few studies have been

conducted to investigate the antioxidant potential of essential oil from various *Eucalyptus* species [32-37].

Table 2
Scavenging ability of *Eucalyptus citriodora* essential oil and BHT on DPPH radicals (%).

Sample	Scavenging ability (%, mean ± SD), concentrations (mg ml^{-1})							IC_{50} (mg/ml)
	0.05	0.1	0.2	0.4	0.6	0.8	1.0	
EO	12.1	17.51	25.62	34.08	36.06	48.2	51.91	0.896
BHT	-	43.4 ± 0.7	55 ± 0.7	81.4 ± 2.1	84.8 ± 0.7	86 ± 1.4	90 ± 0.7	0.041

3.4. Antimicrobial activity

The antimicrobial activity of *Eucalyptus citriodora* essential oil was determined by both qualitative (in liquid and vapour phase) and quantitative assays (determination of MIC). The diameter of the inhibition zone (DIZ) and the minimum inhibitory concentration (MIC) are given in table 3.

Table 3
Antimicrobial activity of *Eucalyptus citriodora* essential oil

Microbial strains	Disc diffusion assay Diameter of inhibition zone(mm)*			Vapour diffusion assay Diameter of inhibition zone(mm)*			MIC (µl/ml)
	10 µL	20 µL	30 µL	10 µL	20 µL	30 µL	
Escherichia.coli 25922	*26 ± 0.0e*	*38 ± 0.0b*	40 ± 0.0^{c}	13.5 ± 0.7^{f}	19 ± 0.0^{d}	31 ± 0.0^{d}	5
Salmonella enteritidis	*9 ± 1.41g*	*11 ± 1.41gf*	14.5 ± 0.7^{f}	R^{g}	R^{g}	R^{g}	>20
Salmonella Typhi Murium	9 ± 1.41^{g}	12 ± 1.41^{f}	13.5 ± 2.12^{f}	R^{g}	R^{g}	R^{g}	>20
Salmonella Gallinarum Pullorum	11.5 ± 2.12^{gf}	14.5 ± 2.12^{f}	15.5 ± 0.7^{f}	R^{g}	R^{g}	R^{g}	>20
Staphylococcus.aureus 25923	20 ± 0.0^{ed}	24 ± 0.0^{e}	26 ± 0.0^{e}	13 ± 4.24^{gf}	17 ± 0.0^{ed}	20 ± 0.0^{e}	1.25
Pseudomonas aeruginosa	R^{g}	R^{g}	R^{g}	R^{g}	R^{g}	R^{g}	-
Corynebacterium striatum	20.5 ± 2.12^{ede}	31.5 ± 2.12^{e}	44 ± 1.41^{c}	14 ± 0.0^{f}	16 ± 1.41^{fe}	18 ± 0.0^{fe}	1.25
Bacillus subtilis	16 ± 1.41^{fe}	30 ± 0.0^{de}	41 ± 1.41^{c}	13 ± 0.0^{gf}	48 ± 0.0^{a}	49 ± 0.0^{b}	0.6
S. seftenberg	10 ± 0.0^{g}	14 ± 0.0^{f}	15 ± 0.0^{f}	R^{g}	R^{g}	R^{g}	>20
Staph epidermidis	50 ± 0.0^{a}	51 ± 0.0^{a}	66 ± 0.0^{a}	27 ± 1.41^{e}	38 ± 0.0^{b}	42 ± 0.0^{c}	0.6
Enterococcus faecalis	R^{g}	R^{g}	R^{g}	R^{g}	R^{g}	R^{g}	-
Staph saprophyticcus	23 ± 0.0^{dc}	39 ± 0.0^{b}	43 ± 0.0^{e}	14 ± 0.0^{f}	26 ± 0.0^{c}	32 ± 0.0^{d}	1.25
Candida albicans	42 ± 0.0^{b}	50.5 ± 2.12^{a}	55 ± 2.82^{b}	35 ± 0.0^{d}	36 ± 0.0^{b}	47 ± 1.41^{b}	1.25
Saccharomyces cerevisiae	24.5 ± 3.53^{de}	25 ± 2.82^{ed}	32 ± 0.0^{d}	12 ± 4.24^{gf}	14 ± 0.0^{f}	17 ± 0.0^{f}	1.25

Means within the same column followed by the same small letter are not significantly different (P<0.05) according to Tukey's test Posthoc multi comparison
** Diameter of inhibition zone (mm) including disc diameter of 6 mm; DIZ values are presented as mean (mm) ± standard deviation of triplicates*
-: not tested; R: resistant

3.4.1. Disc diffusion method

The results from the antimicrobial activity of *E. citriodora* essential oil determined via the disc diffusion method are summarized in table 3. According to the width of the diameters of inhibition zone expressed in millimeter, results were appreciated as follows: not sensitive for a diameter equal to 8 mm or below, sensitive for a diameter between 8 and 14 mm, very sensitive for a diameter between 14 and 20 mm and extremely sensitive for diameter equal or larger than 20 mm [38]. The oil showed a variable degree of antimicrobial potential against the strains tested. The zone of inhibition increased with the increasing concentration (*i.e.* 10, 20 and 30 µl) of *E. citriodora* oil. The Gram negative bacterium, *Escherichia coli*, was extremely sensitive to the oil (DIZ = 26 ± 0.0 mm). In contrast, the oil was inactive against the Gram negative bacteria *P. aeruginosa* and *Enterococcus faecalis*. Whereas, *Salmonella enteritidis*, *Salmonella Typhi Murium, Salmonella Gallinarum Pullorum,* and *Salmonella Seftenberg* were resistant to the oil and presented the smallest inhibition zone diameters values which varied between 10-16 mm.

For the Gram positive bacteria, *Staph epidermidis* was considered extremely sensitive to the oil, producing the largest inhibition zone diameters values of 50 ± 0.0 mm. The yeasts showed a higher sensibility to the oil with a larger inhibition zone (42 ± 0.0 mm and 24.5 ± 3.53 mm) for *Candida albicans* and *S. cerevisiae*, respectively. The *E. citriodora* essential oil showed a higher activity against the Gram positive bacteria and the yeasts than the Gram negative bacteria. The EO showed very promising antibacterial activity. Furthermore, the oil was more selective for the Gram-positive bacteria than for the Gram-negative bacteria, which is in accordance with studies reporting that Gram-positive bacteria are generally more sensitive than Gram-negative bacteria to EO [4-10]. It was observed that the *Eucalyptus citriodora* essential oil possess the greatest antimicrobial activity on *S. aureus* and *E. coli* [39]. It seems that no previous attempt has been made to explore the antibacterial potential of *E. citriodora* essential oil against the Gram positive bacterium *Staphylococcus epidermidis*, which was the most sensitive to the *Eucalyptus citriodora* oil.

The Gram negative bacterium, *P. aeruginosa*, was found to be the most resistant to *Eucalyptus* species by many researches [3, 4, 40]. *P. aeruginosa* is known to have a high level of intrinsic resistance against many antimicrobials and antibiotics due to a very restrictive outer membrane barrier, being highly resistant even to synthetic drugs [41].

Few studies have reported the antifungal potential of the *Eucalyptus* essential oil. Gilles *et al.* [4] reported that the Essential oils from the three *Eucalyptus* species *E. staigeriana, E. dives* and *E. oilda* showed high antimicrobial activity against the yeast *C. albicans*. The highest antimicrobial activity was observed for *E. staigeriana* oil with an inhibition zone diameter of 26.7 mm. The lowest activity against the yeast was recorded for the essential oil of *E. olida*^{NSW} at 12.6 mm while the essential oil of *E. dives* and *E. oilda*^{VIC} had similar activities at 15.4 and 15.6 mm, respectively. Tyagi & Malik [9] tested the *Eucalyptus globulus* essential oil against *Candida albicans*. The oil showed a good potential against *Candida albicans* with a diameter of inhibition zone measured as 34 mm.

The antibacterial activity of many essential oils, and in particular *Eucalyptus* species, is related to the presence of some favorable classes of compounds such as alcohols, aldehydes, alkenes, esters. Furthermore, the antimicrobial activity of the *Eucalyptus citriodora* Hook. Essential oil could be due to the two major compounds, Citronellal and Citronellol. The antimicrobial properties of *Eucalyptus citriodora* essential oil and its constituents have been assessed and reviewed by few researchers. Ramzani *et al.* [13] studied the fungicidal effect of the monoterpene, Citronellal, against *Rhizoctonia solani, Helminthosporium oryzae* and reported that Citronellal alone was found to be more effective than eucalypt oils. Lee *et al.* [16] studied the antifungal activity and have shown that the inhibition rates of Citronellol against *Phytophthora cactorum* were close to 100 %. Low, Rawal and Griffin [42] showed that the *E. citriodora* exerted its antimicrobial activity through the synergistic action of Citronellal and Citronellol. In contrast, the *Eucalyptus citriodora* essential oil containing a major account of 1, 8 cineole (54 %) has a weak antibacterial activity [3]. In addition, the minor compounds could also contribute to the antimicrobial activity of the oils. The inhibitory potential of the essential oils may be attributable both to their major compounds and to the minor ones. They may act together synergistically to contribute to the toxicity of the oil. [43, 44].

3.4.2. Disc volatilization method

Table 3 shows the results of the zone of inhibition due to the *Eucalyptus citriodora* essential oil vapour. As seen in the earlier assays using EO in solid agar, it also

increased with increasing concentration of the essential oil. As shown in the disc diffusion assay, the zone of inhibition due to the oil vapour was bigger for Gram positive bacteria and yeasts than for Gram negative bacteria. The Gram negative bacteria *Salmonella enteritidis*, *Salmonella Typhi Murium*, *Salmonella Gallinarum Pullorum*, and *Salmonella Seftenberg* which were sensitive to the essential oil in the liquid phase, were found to be resistant in the vapour phase. *P.aeruginosa* was the most resistant as shown in the results with the previous using volatile oil in liquid phase. *E. coli* was very sensitive to the oil (14 ± 0.00 mm diameter of inhibition zone) (Table 3). The Gram positive bacteria, *Staph epidermidis* showed the most important zone of inhibition (27 ± 1.41 mm). The two yeasts *Candida albicans* and *S. cerevisiae* showed inhibition zone diameters of 35 ± 0.0 mm, 12 ± 4.24 mm respectively.

Few researches have studied the antimicrobial activity of the *Eucalyptus* essential oil in the vapour phase. Tyagi & Malik [9] studied the activity of *E. globulus* essential oil and reported that the higher potential of this essential oil was observed in the vapour phase against bacteria and fungi strains. Ghalem & Mohamed [45] studied the sensibility of *S. aureus* and *E. coli* to *E. camaldulensis* and *E. globulus* essential oils in the vapour phase. In our study, Superior antimicrobial activity of the oil was observed in the liquid phase. This result is in disagreement with the study reported by Vilela *et al.* [46] who studied the essential oil activity from leaves of *E. globulus*, as well as its major compound 1,8 - cineole, against the storage fungi *A. flavus* and *Aspergillus parasiticus* by contact and headspace volatile exposure assays and found that the inhibition of the oil in the vapour phase was more important than in the liquid phase. However, in the direct contact assays, the activity depends upon the diffusibility and solubility of the EO compounds into the agar while the antimicrobial activity of the vapour assay depends upon the volatility of each compound.

3.4.3. Minimum inhibitory concentration (MIC)

The MIC of *Eucalyptus citriodora* essential oil was determined using the agar dilution method against bacteria (*Corynebacterium striatum*, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticcus*, *Escherichia coli* ATCC 25922, *Salmonella enteritidis*, *Salmonella Typhi Murium*, *Salmonella seftenberg*, *Enterococcus faecalis*, and *Salmonella Gallinarum Pullorum*), and two yeasts (*Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*). The results in Table 3 showed that the oil exhibited a concentration-dependent inhibition of growth.

The obtained data indicated that the MIC varied from 20 to 0.6 µl/ml for bacteria. The MIC of Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus epidermidis* (0.6 µl/ml) < *Staphylococcus aureus* ATCC 25923, *Corynebacterium striatum*, *Staphylococcus saprophyticcus* (1.25 µl/ml)) was smaller than for the Gram negative bacteria *Salmonella enteritidis*, *Salmonella Typhi Murium*, *Salmonella Gallinarum Pullorum*, *Salmonella seftenberg*, *Enterococcus faecalis* (>20 µl/ml), and *E. coli* (5 µl/ml). The MIC of the two yeasts (*C. albicans* ATCC 10231 and *Saccharomyces cerevisiae*) had a value of 0.125 µl/ml. The results from both the disc diffusion method and MIC determination indicated that the Gram positive bacteria and the two yeasts were the most sensitive strains tested, (showing the largest inhibition zones and the lowest MIC values). The Gram positive bacteria were more sensitive than the Gram negative bacteria. This may be due to the different nature of the Gram negative cell envelope (made up of lipopolysaccharide), which makes the access to the membrane more restricted in Gram negative bacteria. In the present study the highest MIC was exhibited by *S. typhimurium*, *Salmonella Gallinarum Pullorum*, *Salmonella Seftenberg*, and *Salmonella enteritidis*. Similar results were shown by Luqman *et al.* [15] who reported that the MIC of *S. typhimurium* and *E.coli* was greather than 10 mg/ml and the MIC of *Staphylococcus aureus* equal to 10 mg/ml, while the MIC of *C. albicans (Al)* and *C. albicans MTCC* was equal to 5 and 2.5 mg/ml respectively. On the other hand, Tyagi, A. & Malik [9] reported that the MIC value for the *Eucalyptus globulus* was 4.5 mg/ml and 2.25 mg/ml for *E.coli* and *Staphylococcus aureus*, respectively. For the yeasts, they reported that the MIC of *C. albicans* (2.25 mg/ml) was higher than for *S. cerevisiae* (1.13 mg/ml).

4. Conclusion

It can be concluded that *Eucalyptus citriodora* essential oil which is part of Algerian Flora, is of a particular importance to food hygiene and appears to be a potential and natural way to help in antibacterial, antifungal and a moderate antioxidant at limited amounts. Our results demonstrated that the Algerian *Eucalyptus citriodora* essential oil exhibited moderate antioxidant

activity, while it exhibited variable degrees of antimicrobial activity against bacteria and yeasts. The Gram positive bacteria *Staphylococcus epidermidis* was the most sensitive while, the Gram negative bacterium *Pseudomonas aeruginosa* was the most resistant.

Further *in vivo* investigation will be performed to confirm this antimicrobial potential of the oil and of the monoterpenes present as major compounds in the oil. *Eucalyptus citriodora* essential oil may be used as a new antimicrobial agent recommended by the pharmaceutical industries, therefore a natural replacement for harmful synthesized chemicals.

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