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Full Length Research Paper

Chemical composition, antioxidant and antifungal potential of *Melaleuca alternifolia* (tea tree) and *Eucalyptus globulus* essential oils against oral *Candida* species

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***Eucalyptus globulus* and *Melaleuca alternifolia* essential oils has been of interest to researchers because they are traditionally used for the treatment of fungal infections and especially candidiasis. The chemical composition of hydrodistilled essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS), and their antifungal activity was tested against 32 *Candida* strains including 15 species. The antioxidant activities (DPPH, reducing power, and superoxide anion radical-scavenging activity) were also investigated. Tea tree essential oil was particularly rich on terpinen-4-ol (40.44%), gamma terpinene (19.54%) and 1,8-cineole (95.61%) and alpha-pinene (1.5%) for the *E. globulus* oil. *E. globulus* oil was more efficient and had the best antifungal effect on oral *Candida albicans* and *Candida glabrata* strains comparing to the results obtained with Amphotericin B. Even at low concentrations, these oils drastically impair the maximum yield and growth rate of both *C. albicans* and *C. glabrata* on YPD medium. The Tea Tree essential oil displayed the highest DPPH scavenging ability with the lowest IC_{50} value (IC_{50}, 12.5 µg/ml), the greater reducing power and bleaching of β-carotene (EC_{50}, 24 μgml^{-1} and IC_{50}, 42 μgml^{-1}, respectively) as compared to *E. globulus* oil and BHT. These findings support the interest of *E. globulus* and *M. alternifolia* essential oils as an efficient oral hygiene tool (anti-*Candida* spp.) and as a source of antioxidant compounds.**

Key words: *Candida*, *Melaleuca alternifolia*, *Eucalyptus globulus*, gas chromatography-mass spectrometry, antioxidant, antifungal activities.

INTRODUCTION

Medicinal plants have been used as a source of remedies since ancient times and the ancient Egyptians were familiar with many medicinal herbs and were aware of their usefulness in treatment of various diseases (Abu-Shanab et al., 2004). In Tunisia, many plant extracts and essential oils have been shown to exert biological activity *in vitro* and *in vivo*, which justified research on traditional medicine focused on the characterization of antimicrobial activity of these plants (Snoussi et al., 2008; Hajlaoui et al., 2008, 2009, 2010; Noumi et al., 2010a,b).

The oil of *Melaleuca alternifolia* contains ~100 components, which are mostly monoterpenes, sesquiterpenes and related alcohols. The essential oil obtained by steam distillation from the leaves have long been used in aboriginal traditional medicine of Australia

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as remedies for wounds and cutaneous infections, to treat many pathological conditions such as empyema, ringworm, paronychia, tonsillitis, stomatitis and vaginal infections (Humphrey, 1930; Penfold and Morrison, 1937). Tea tree oil has been used medicinally in Australia, with uses relating primarily to its antimicrobial (Carson and Riley, 1993; Carson et al., 2002, Mondello et al., 2003), anti-inflammatory and antifungal especially anticandidal properties (Hammer et al., 1998, 2000). Tea tree oil efficiency was confirmed in the treatment of dandruff (Satchell et al., 2002) and oral candidiasis (Jandourek et al., 1998; Hammer et al., 2004). Data from an animal model also indicate that it may be effective in the treatment of vaginal candidiasis (Hammer et al., 2003).

The genus Eucalyptus, (family: *Myrtaceae*) is native to Australian region. The genus Eucalyptus comprises well-known plants of over 600 species of trees. *Eucalyptus globulus* is increasingly used in traditional medicine for various medical implications such as antibacterial, anti-inflammatory, and antipyretic effects. The plant is popular for this, it is cultivated in subtropical and Mediterranean regions more than other species. The essential oil of leaves of Eucalyptus species has been the object of several studies antibacterial, antioxidant, antihyperglycemic and antifungal activity (Derwich et al., 2009).

The aim of this study was to compare the antifungal activities of the essential oils of *E. globulus* and *M. alternifolia* against a range of *Candida* species associated with oral disorders, evaluating minimal inhibitory and minimal fungicidal concentrations, and kinetic parameters in an attempt to contribute to the use of these as alternative products for microbial control and as a natural source of antioxidant components.

MATERIALS AND METHODS

Plant material and essential oil

M. alternifolia (tea tree) essential oil (leaves) was purchased from Arkomédika (Laboratories Pharmaceutiques, BP 28-06511 Carros, France). *E. globulus* commercialized essential oil was kindly provided by the "Laboratoire de Pharmacognosie, Monastir (Tunisia).

Gas chromatography-mass spectrometry (GC-MS) analysis conditions

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC, equipped with a HP-5 MS capillary column (30 m· 0.25 mm i.d., 0.25 µm) and a HP 5972 mass selective detector. For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially kept at 50°C for 3 min, then gradually increased to 150°C at a 3° C/min rate, held for 10 min and finally raised to 250°C at 10°C/min. Diluted samples (1/100 in acetone, v/v) of 1 µl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC–MS system and literature data (Adams, 2001). The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2001).

Antioxidant activities

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion radical-scavenging activity

The effect of the tested essential oil on DPPH degradation was estimated according to the method described by Hajlaoui et al. (2010). The essential oil was diluted in pure methanol at different concentrations, and then 2 ml were added to 0.5 ml of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 mn. The absorbance of the resulting solution was then measured at 517 nm measured after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (µg/ml), the antiradical dose required to cause a 50% inhibition. A lower IC_{50} value corresponds to a higher antioxidant activity of essential oil. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1) \times 100]/A_0 \quad (1)$$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. Superoxide anion scavenging activity was assessed using the method described by Trabelsi et al. (2010). The reaction mixture contained 0.2 ml of essential oil has different concentration, 0.2 ml of 60 mM PMS stock solution, 0.2 ml of 677 mM NADH and 0.2 ml of 144 mM NBT, all in phosphate buffer (0.1 mol/l, pH 7.4). After incubation at ambient temperature for 5 min, the absorbance was read at 560 nm against a blank. Evaluating the antioxidant activity was based on IC_{50}. The IC_{50} index value was defined as the amount of antioxidant necessary to reduce the generation of superoxide radical anions by 50%. The IC_{50} values (three replicates per treatment) were expressed as µg/ml.

As for $DPPH^{\cdot}$, a lower IC_{50} value corresponds to a higher antioxidant activity of plant extract. The inhibition percentage of superoxide anion generation was calculated using the following formula:

$$\text{Superoxide quenching (\%)} = [(A_0 - A_1) \times 100]/A_0$$

Where A_0 and A_1 have the same meaning as in Equation (1).

Reducing power

The ability of the extracts to reduce Fe^{3+} was assayed by the method of Oyaizu (1986). Briefly, 1 ml of each essential oil were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. After incubation at 50°C for 25 mn, 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 650 x g for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% aqueous $FeCl_3$. The absorbance was measured at 700 nm.

The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power. EC_{50} value (mg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as positive control.

β-Carotene-linoleic acid model system (β-CLAMS)

The β-CLAMS method by the peroxides generated during the oxidation of linoleic acid at elevated temperature. In this study the β-CLAMS was modified for the 96-well micro-plate reader according the protocol described by Koleva et al. (2002). In brief, the β-carotene was dissolved in 2 ml of $CHCl_3$, to which 20 mg of linoleic acid and 200 mg of tween 40 were added. $CHCl_3$ was removed using rotary evaporator. Oxygenated water (100 ml) was added, and the flask was shaken vigorously until all material dissolved. This test mixture was prepared fresh and using immediately. To each well, 250 µl of the reagent mixture and 35 µl sample or standard solution were added. The plate was incubated at 45°C. Readings were taken at 490 nm using visible/UV microplate kinetics reader (EL x 808, Bio-Tek instruments). Readings of all samples were performed immediately (t = 0 mn) and after 120 mn of incubation. The antioxidant activity (AA) of the extracts was evaluated in term of *β*-carotene blanching using the following formula:

$$AA\ (\%) = [(A_0-A_1)/A_0]*100$$

Where A_0 is the absorbance of the control at 0 min, and A_1 is the absorbance of the sample at 120 mn. The results are expressed as IC_{50} values (µg/ml). All samples were prepared and analyzed in triplicate.

Antifungal activity

A total of 32 *Candida* strains including 15 species (*Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida famata*, *Candida kefyr*, *Candida sake*, *Candida holmii*, *Candida lusitaniae*, *Candida intermedia*, *Candida atlantica*, *Candida maritima*, *Pichia guillermondii* and *Pichia jardinii*) were used in this study. Clinical isolates were taken from the oral cavity of patients by using a swabbing method.

A sterile cotton swab (Nippon Menbo, Tokyo, Japan) was immediately cultured into Sabouraud Chloramphenicol agar (Bio-rad, France) to obtain isolated colonies. All isolates were incubated at 30°C for 24 to 48 h and yeast-like colonies were isolated and identified by the ID 32C (bio-Mérieux, Marcy-l'Étoile, France) assimilation kit. The ATCC *Candida* species were used as reference strains.

Disc diffusion method

The anti-*Candida* spp. activity was achieved by the agar-well diffusion method according the protocol described by Hajlaoui et al. (2010).

All *Candida* strains were inoculated into Sabouraud chloramphenicol agar and incubated for 18 h at 37°C. The yeast cultures were harvested and than suspended in sterile saline (0.8% NaCl) and the cell density was adjusted to 10^7 cells/ml (OD_{540}= 0.5). For the antifungal activity of the plants oils used in this study, three sterile 6 mm paper discs (Whatman paper N°3), impregnated with 30 mg of essential oil (10 µl/disc) were placed on the inoculated surface. Plates were then incubated at 37°C for 18 to 24 h. The ATCC strains were used as a quality control strains. The diameter of the zones of inhibition around each disc were examined after 24 h, measured and recorded as the mean diameter (mm) of complete growth-inhibition. As a positive control, 10 µg of amphotericin B (Fungizone, BioBasic INC) was used.

Tests were done in triplicate and results given as mean average (Table 2).

Microdilution method for the determination of the (minimal inhibition concentration) MIC and (minimal fungicidal concentration) MFC

The MIC and the MFC values were determined for all *Candida* strains according the protocol described by Hajlaoui et al. (2010). The inoculums of the yeast strains were prepared from 12 h Sabouraud dextrose broth cultures and suspensions were adjusted to an optical density of 0.5 at 540 nm. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth (1% NaCl) and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of each plants extract was added into the first wells. Then, 100 µl from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195 µl of nutrient broth (1% NaCl) without essential oil and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. The plates were incubated at 37°C for 24 h. The plants extract tested in this study was screened two times against each strain. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MFC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth. All tests were performed in triplicate.

Effect of the essential oils on the kinetic growth of *Candida* strains on YPD broth

The effect of *M. alternifolia* and *E. globulus* essential oils on the kinetic growth of *C. albicans* (15_B) and *C. glabrata* (15_T) strains was tested. Cultures were grown on YPD broth for 18 to 24 h at 37°C. The enrichment cultures were used to inoculate the sterile glass bottles containing 30 ml of new YPD broth and the initial OD_{600} was adjusted at 1 (10^7 to 10^8 cfu/ml). These bottles were then prepared at different concentration with the two tested essential oils (1/2 MIC, MIC and MFC) and incubated on rotatory shaker (150 rpm) at 37°C. At regular time intervals, fungal growth was evaluated by measuring absorbance at 600 nm using the spectrophotometer after 0, 1, 2, 3, 6, 9, 12 and 24 h of incubation. All values were conducted in triplicate and average values were calculated using the SPSS 13.0 statistics package for Windows. Morphology of the fungal cells was observed under a binocular light microscope.

RESULTS AND DISCUSSION

Essential oil composition

Thirty five components were identified in the essential oil of Tea tree and only thirteen in the essential oil of *E. globulus*. The main compounds of the oils are given in Table 1, where the components were listed according to their elution on the Innowax column. Tea tree essential oils was particularly rich on: terpinen-4-ol (40.44%), γ-terpinene (19.54%), α-terpinene (7.69%), 1,8-cineole (5.20%), para-cymene (4.74%), α-terpineol (3.31%), α-terpinolene (3,09%), α-pinene (2.67%), alloaroma-dendrene (1.47%), Δ-Cadinene (1.47%), ledene (1.20%) α-thujene (0.90%), myrcene (0.75%), β-pinene (0.73%), aromadendrene (0.52%).

Our results are in accordance with previous works dealing about the chemical composition of both *M. alternifolia* and *E. globulus* essential oils. In fact, six

Table 1. The main components identified in the essential oils of *M. alternifolia and E. globulus* used in this study.

Compounds identified	*(KI) HP-5	Percentage	Identification
***M. alternifolia* essential oil (Total identified components 97.19%)**			
terpinen-4-ol	1186	40.44	MS, KI
γ-terpinene	1062	19.54	MS, KI
α-terpinene	1019	7.69	MS, KI
1,8-cineole	1033	5.20	MS, KI
para-cymene	1026	4.74	MS, KI
α-terpineol	1195	3.31	MS, KI
α-terpinolene	1089	3.09	MS, KI
α-pinene	935	2.67	MS, KI
***E. globulus* essential oil (Total identified components 99.19%)**			
1-8,cineole	1040	95.61	MS, KI
α-pinene	935	1.50	MS, KI
myrcene	991	0.53	MS, KI
β-pinene	979	0.40	MS, KI
α-terpineol	1192	0.28	MS, KI

*: (KI) HP-5: Kovats index.

chemotypes of *M. alternifolia* essential oil have been described including terpinen-4-ol, terpinolene chémotype and four 1-8 cineole chemotypes (Williams, 1998; Homer et al., 2000). Tea tree oil (TTO) is composed of terpene hydro-carbons, mainly mono-terpenes, sesquiterpenes, and their associated alcohols. Early reports on the composition of TTO described 12, 21, and 48 components (Carson et al., 2006). In addition, the seminal work done by Brophy and collaborators examined over 800 TTO samples by GC and GC/MS and reported approximately 1010 components and their range of concentrations as follow: terpinen-4-ol (40.1%), gamma terpinene (23.0%), alpha terpinene (10.4%), 1,8-cineole (5.1%), terpinolene (3.1%), para-cymene (2.9%), alpha-pinene (2,6%), alpha terpineol (2.4%), aromadendrene (1.5%), delta Cadinene (1.3%), limonene (1%), sabinene (0.2%), globulol (0.2%) and viridoflorol (0.1%).

1,8-cineole (95.61%) and alpha-pinene (1.5%) were the main components of *E. globulus* essential oil tested in the present work. In fact, multiple studies have been reported on the chemical composition of the essential oils of Eucalyptus species belonging to different regions in the world. The chemical compositions of the leaf oils of Eucalyptus from various parts of the world have been reported and the 1.8-Cineole was identified as the major component in from samples growing in Taiwan, Uruguay, Algeria, Burundi, Congo, Mozambique, Greece, Australia, Tunisia, Italy, Nigeria, Turkey and Morocco (Boland et al., 1991; Dethier et al., 1994; Derwich et al., 2009).

Antifungal activity

Early data on the susceptibility of fungi to tea tree and *E. globulus* essential oils were largely limited to *Candida albicans*, which was a commonly chosen model test organism. We investigated in the present study the antifungal activity of *M. alternifolia* and *E. globulus* essential oils against several *Candida* species including those isolated from Tunisian patients suffering from oral candidiasis. Antifungal effects are reported as inhibition zones using the disc diffusion method and *in vitro* activity as MIC and MFC values (Table 3).

The two plant essential oils showed significant antifungal activity against all *Candida* strains tested. Overall, the best antifungal activity was against *C. albicans* ATCC 90028 for *M. alternifolia* (19.33 mm) and against *C. glabrata* ATCC 90030 for *E. globulus* oil (22.33 mm). Essential oil of *E. globulus* was more efficient and had the best antifungal effect for oral *C. albicans* strain (15_B) (IZ= 19.33 mm) comparing to the results obtained with Amphotericin B (IZ= 11 mm) and also for *C. glabrata* ATCC 90030 strain (IZ= 22.33 mm) comparing to Amphotericin B results (IZ= 14.33 mm). Table 3 summarizes the MIC and MFC of the two plants essential oils. The lowest values of MIC were seen against two *C. glabrata* isolates with *E. globulus* oil (strains 15_T and ATCC 90030; MIC: 0.078 mg/ml), followed by 0.156 mg/ml for *C. albicans* isolates (strains $15B_B$ and ATCC 90028). The MFC values were similar for all *Candida* tested strains (10 mg/ml). As to the he standard antifungal drug used in this work, Amphotericin B was more active against all oral and reference *Candida* strains (MIC range: 0.012 to 0.39 mg/ml; MFC range: 0.195 to 1.562 mg/mg) comparing the two essential oils.

The medicinal properties of tea tree oil were first reported by Penfold in the 1920s. Contemporary data clearly show that the broad-spectrum activity of TTO

Table 2. Antifungal activity of *M. alternifolia* and *E. globulus* oils against *Candida* strains.

Strains	Inhibition zone in diameter (mm ± SD). MIC and MFC (mg/ml)								
	M. alternifolia			*E. globulus*			AmB		
	IZ	MIC	MFC	IZ	MIC	MFC	IZ	MIC	MFC
				C. albicans					
ATCC 90028	19.33±0.57	0.312	>10	17.66±0.57	0.156	10	11	0.097	0.781
ATCC 2091	21±1	0.0097	>10	20±0	0.0097	5	14.66±0.57	0.024	0.781
17	12.66±0.57	0.0097	>10	19.66±0.57	0.0097	5	10.66±1.52	0.097	0.781
H_8	12±0	0.0097	10	17±1	0.039	2.5	11.66±0.57	0.097	0.195
H_5	14.66±0.57	0.0195	10	12±0	0.156	2.5	9.66±0.57	0.39	0.195
15_B	15.33±0.57	0.625	10	19.33±0.57	0.156	10	11±1	0.012	0.781
H_3	15.66±0.57	1.25	10	21.66±0.57	0.156	5	9.66±0.57	0.012	0.097
16	16.33±0.57	0.0195	>10	24.33±0.57	0.0097	10	11.66±0.57	0.024	0.04
4	18.66±0.57	0.0097	10	18.33±0.57	0.0097	10	11±1	0.006	0.048
I_2	19.66±0.57	5	10	26.33±1.15	0.0097	1.25	11.33±0.57	0.012	0.024
1_4	19±1	0.0097	10	20.66±0.57	0.0097	5	11±1	0.048	0.781
H_2	20.33±0.57	0.156	>10	24.33±0.57	0.0195	5	10.33±0.57	0.097	0.195
65	21.33±0.57	0.0097	10	17.66±0.57	0.0097	5	10.66±1.15	0.097	1.562
1_1	21.66±1.154	0.0097	5	21±1	0.0097	10	7± 0	0.048	1.562
21	24.33±0.57	0.0097	>10	16±1	0.0097	10	11±1	0.097	0.195
7	24±0	0.0097	10	17.66±0.57	0.0097	10	9.66±0.57	0.048	0.39
10	25.66±0.57	0.0097	>10	19.33±0.57	2.5	10	11± 0	0.048	1.562
				C. parapsilosis					
I_3	14.66±0.57	5	>10	15.33±0.57	0.0195	10	11±1	0.097	0.195
				C. kefyr					
CECT 1017	19.33±1.15	0.0097	5	19.33±0.57	0.0097	5	10.66±1.15	0.195	0.39
35	25.33±0.57	0.0097	10	20.66±0.57	0.0195	10	9.66±0.57	0.39	1.562
				C. glabrata					
ATCC 90030	14.33±0.57	0.625	10	22.33±0.57	0.078	10	14.33 ± 0.57	0.195	1.562
15_T	12±0	0.0097	10	12.66±1.15	0.0097	10	10.66 ± 0.57	0.195	0.39
I_1	11.66±0.57	0.0195	2.5	16.33±0.57	0.0097	>10	10.33±1.15	0.39	0.195
				Others					
C. dubliniensis CECT 11455	15±0	0.0097	5	20.33±0.57	0.0097	5	11.33±0.57	0.012	0.195
C. lusitaniae CECT 1145	15.33±0.57	0.0097	>10	18.66±1.15	0.0097	2.5	12.33±1.15	0.097	0.195

Table 2. Contd.

C. sake CECT 1044	16.33±0.57	0.0097	10	17.33±1.15	0.097	2.5	12±1	0.097	0.39
Pichia jadinii CECT 1060	17.66±0.57	0.0097	10	16.33±0.57	0.0097	5	11.66±1.15	0.006	0.195
C. famata CECT 11957	20.66±0.57	0.0097	>10	21.33±0.57	0.0097	5	12.33±0.57	0.195	0.39
C. intermedia CECT 11869	20±0	0.0097	>10	19±0	0.0097	5	12±0	0.012	0.195
Pichia guilliermondii CECT 1456	20±0	0.0097	10	16.33±1.15	0.0097	5	12.33±0.57	0.097	0.195
C. atlantica CECT 1435	21±1	0.0097	10	20.66±0.57	0.0097	5	11±0	0.195	0.39
C. maritima CECT 1435	24.66±0.57	0.0097	10	23.66±0.57	0.0097	2.5	12.66±1.15	0.097	0.195

Table 3. Antioxidant activities of *M. alternifolia* and *E. globulus* essential oils compared to BHT ones: DPPH, superoxide radicals and *β*-Carotene bleaching test. Reducing power was expressed as EC_{50} values (µg/ml).

	Essential oils		BHT
	M. alternifolia	***E. globulus***	
DPPH IC_{50} ($\mu g.ml^{-1}$)	12.5	57	11.5
$O_2^{\cdot-}$ IC_{50} ($\mu g.ml^{-1}$)	26.6	14	1.5
RP EC_{50} ($\mu g.ml^{-1}$)	24	48	75
β-carotenes IC_{50} ($\mu g.ml^{-1}$)	42	48	75

DPPH radical–scavenging activity is expressed as IC_{50} values (µg/ml); RP: reducing power was expressed as EC_{50} values (µg/ml); β-Carotenes bleaching test is expressed as IC_{50} values ($\mu g.ml^{-1}$); $O_2^{\cdot-}$: Superoxide anion radical-scavenging activity is expressed as IC_{50} values (µg/ml).

includes antibacterial, antifungal, antiviral, and antiprotozoal activities. Of all these properties, antimicrobial activity has received the most attention. For this, TTO is employed for its antimicrobial property and is incorporated as the active ingredient in many tropical formulations used to treat cutaneous infections. In fact, our results agree with previous works dealing about the high susceptibility of a wide range of yeasts, dermatophytes, and other filamentous fungi (Carson et al., 2006).

The antifungal activity of TTO is due to its lypophilic nature, which facilitates skin penetration. In this context, it has been clinically evaluated for the treatment of several superficial fungal infections, including onychomycosis (Syed et al., 1999), tinea (Tong et al., 1992) and refractory oral candidiasis (Jandourek et al., 1998). In 1998, Hammer and colleagues tested *in vitro* the antifungal activity of 24 essential oils against fourteen *Candida* spp. isolates and founded that *E. globulus* essential oil inhibit the growth of *C. albicans* ATCC 10231 at MIC=1% (v,v) and from 0.12 to 0.5% for all *Candida* species tested. In 2005, Tampieri and colleagues founded that 1,8-cineole (81.4%) and limonene (7.01%) were the main components of *E. globulus* essential oil and that these two components have the same fungistatic activity at >1000 and 1000 ppm respectively. The highest antifungal activity was observed in three active principles including (trans-cinnamaldehyde, 1-decanol and β-phellandrene) with MIC= 50 ppm even after 48 h or 7 days of application. In the same year (2005), Devkatte and colleagues studied the *in vitro* efficacities of 38 plant essential oils against four isolates of *C. albicans*. Twenty three of them caused a 1-30 mm zone of inhibition (ZOI), seventeen oils caused a 10-20 mm ZOI and six

showed a 1-9 mm ZOI. The *E. globulus* oil caused 6.3 to 10 mm ZOI and tea tree caused 11 to 24 mm ZOI. Seven oils were found to be the most effective with MICs values ranging from 0.03 to 0.15% concentration and tea tree cause fungicidal effect at 0.25% concentration comparatively to 1.5 - 2.5% for *E. globulus* oil.

Growth kinetics of *C. albicans* (15_B) and *C. glabrata* (15_T) on YPD medium in the presence of increasing concentrations of *E. globulus* and *M. alternifolia* essential oils (Figure 1) showed that, even at low concentrations, these oils drastically impair the maximum yield and growth rate of both fungi. In fact, as can be shown in Figure 1, a concentration as low as 0.078 mg/ml (Eucalyptus oil) and 0.3125 mg/ml (tea tree) inhibits the growth of both *C. albicans* and *C. glabrata* strains. At high concentrations (MFC): 10 mg/ml (respectively for Eucalyptus and tea tree plant oils), the growth of *C. albicans* and *C. glabrata* strains was inhibited signalling the fungicidal effect of these oils within the first hours of the experiment. A comparison of the curves obtained with different concentrations of *E. globulus* and *M. alternifolia* oils confirms the highest efficiency of the second plant oil on these two *Candida* strains. Both investigated *C. albicans* and *C. glabrata* strains were susceptible to tea tree plant oil at MIC values of 0.625 µg/ml, respectively. All of the untreated *Candida* cells were round or oval in shape and their number was significantly reduced depending on the concentration of tea tree oil added (Figure 2).

In fact, tea tree oil and components appear to affect membrane properties and integrity in a manner consistent with other lypophilic, membrane-active agents such as the terpenes, thymol (Shapiro and Guggenheim, 1995) and geraniol (Hisajima et al., 2008).

Mondello et al. (2003) showed that TTO inhibited the growth of all isolates tested inclusive those resistant to fluconazole and Itraconazole and that the MICs values ranged from 0.15 to 0.5%. MIC_{90}s were 0.25 and 0.5% for azole-susceptible and –resistant *C. albicans* strains respectively, 0.125% for *C. krusei* and *C. glabrata*, and 0.06% for *C. neoformans* and *C. parapsilosis*. All azole-resistant isolates of *C. albicans* were killed within 30 mn by 1% TTO and within 60 mn by 0.25% TTO at pH 7. At pH 5, the decrease in viable count was less rapid, nonetheless, a 100% killing within 30 mn by TTO 1% was achieved.

Also, TTO inhibits the formation of germ tubes, or mycelial conversion, in *C. albicans*. Hammer and colleagues have shown that germ tube formation was completely inhibited in the presence of 0.25 and 0.125% TTO. Recently, we reported that *M. alternifolia* essential oil has an antimycelial activity against *C. albicans* isolates higher than *E. globulus* essential oil. In fact, only 1/2 MIC (0.312 mg/ml) of *M. alternifolia* was able to inhibit totally mycelium in *C. albicans* isolate while 2 MIC (0.312 mg/ml) of the second essential oil was necessary to inhibit germ tube formation in the same strain (Noumi et al., 2010a).

Antioxidant activities

Table 3 illustrates scavenging of the DPPH radical by *M. alternifolia* and *E. globulus* essential oils. The scavenging effect of essential oil and standard (BHT) on the DPPH radical expressed as IC50 values were 12.5 µg/ml for *M. alternifolia* oil and 11.5 µg/ml for BHT. The results obtained with the PMS-NADH-NBT system demonstrated that the inhibiting capacities of superoxide were very interesting for *E. globulus* essential oil (IC_{50}=14 µg/ml) comparing to the results obtained for *M. alternifolia* oil (IC_{50}=26.6 µg/ml), but these results are inferior as compared to BHT value obtained with the same test (IC_{50}=1.5 µg/ml).

Another reaction pathway in electron donation is the reduction of an oxidized antioxidant molecule to regenerate the ‘‘active” reduced antioxidant. As showed in Table 2, the reducing power of *M. alternifolia* essential oil, expressed as CE_{50}, was clearly more important than the reducing power of *E. globulus* (24 and 48 µg/ml, respectively) and that of positive control BHT (75 µg/ml). The results obtained with β-carotene bleaching test demonstrate that the two essential oils have approximately the same IC_{50} values (42 and 48 µg/ml respectively for *M. alternifolia* and *E. globulus* essential oils). These results are clearly more important than positive control BHT (IC_{50}=75 µg/ml). In 2010, Mishra and colleagues tested the phytochemical analysis and antioxidant activities of the essential oil extracted from eucalyptus leaves. These authors founded that the free radical scavenging activity of the different concentrations of the leaf oil (10, 20, 40, 60 and 80% (v/v) in DMSO) of *E. globulus* increased in a concentration dependent fashion. In DPPH method, the oil in 80% (v/v) concentration exhibited 79.55 ± 0.82%. In nitric oxide radical scavenging assay method, it was found that 80% (v/v) concentration exhibited 81.54 ± 0.94% inhibition.

Conclusion

Our results showed that tea tree essential oil exhibited important antioxidant activities comparatively to the Eucalyptus essential oil. In addition, there was a little inter-species variation in susceptibility and all *Candida* spp. tested were uniformly susceptible. Although essential oil values where high when compared with those of Amphotericin B, but these results were in interest as we were dealing with an essential oil and not a pure product. The present study together with previous analysis supports the antibacterial properties of *M. alternifolia* and *E. globulus* essential oils and suggests them as antibacterial additives. Additional clinical trials of these oils have to be performed if they are to be used for

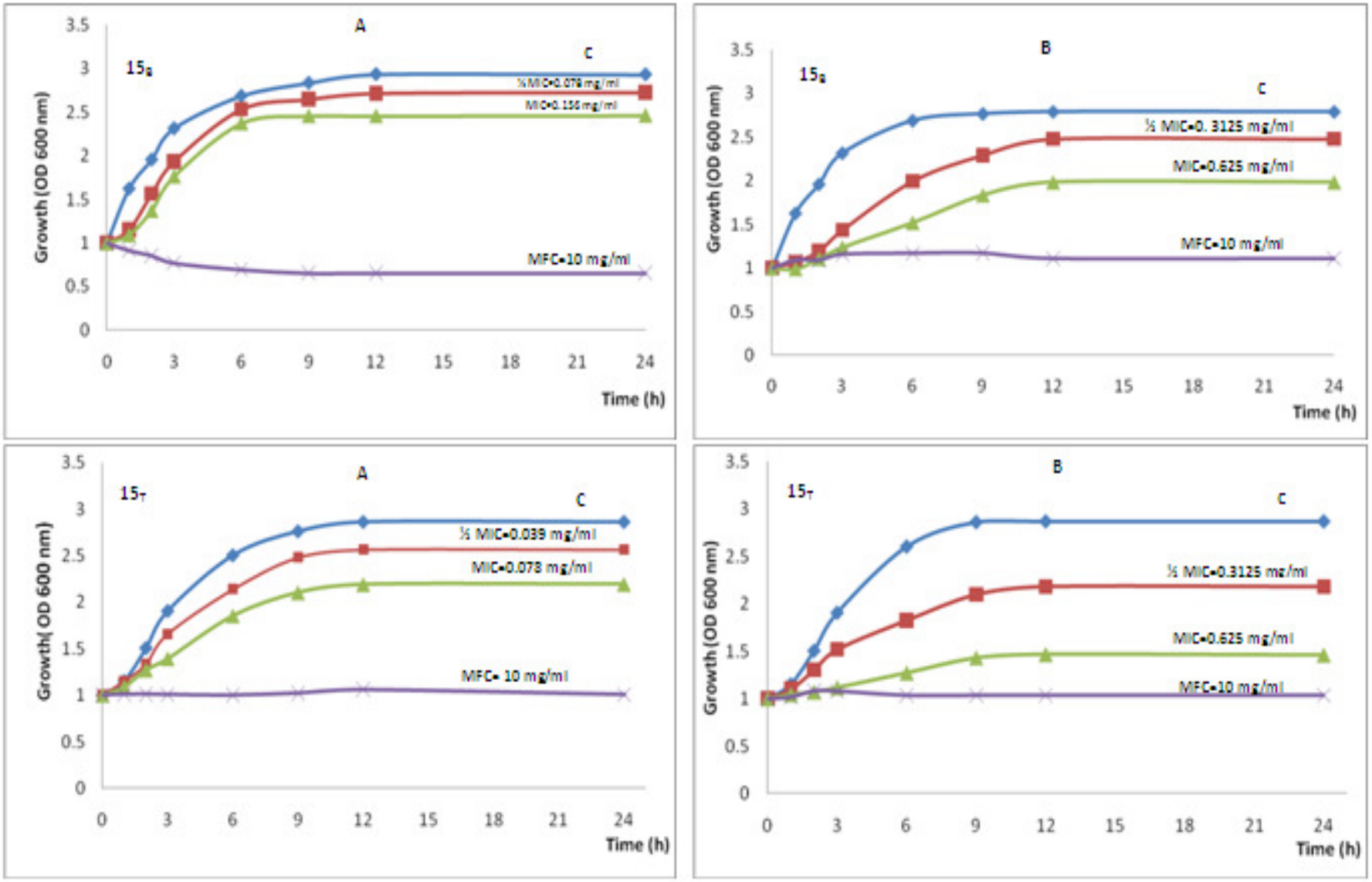

Figure 1. Growth kinetics of *C. albicans* (15B) and *C. glabrata* (15T) on YPD medium in absence (C ♦) and presence of respectively ½ MIC (■), MIC (▲) and MFC (x) (mg ml-1) of *E. globulus* (A) and *M. alternifolia* (B) essential oils. The essential oils were added to each experimental culture in zero time. Data represent the mean value of three measures of the optical density at 600 nm.

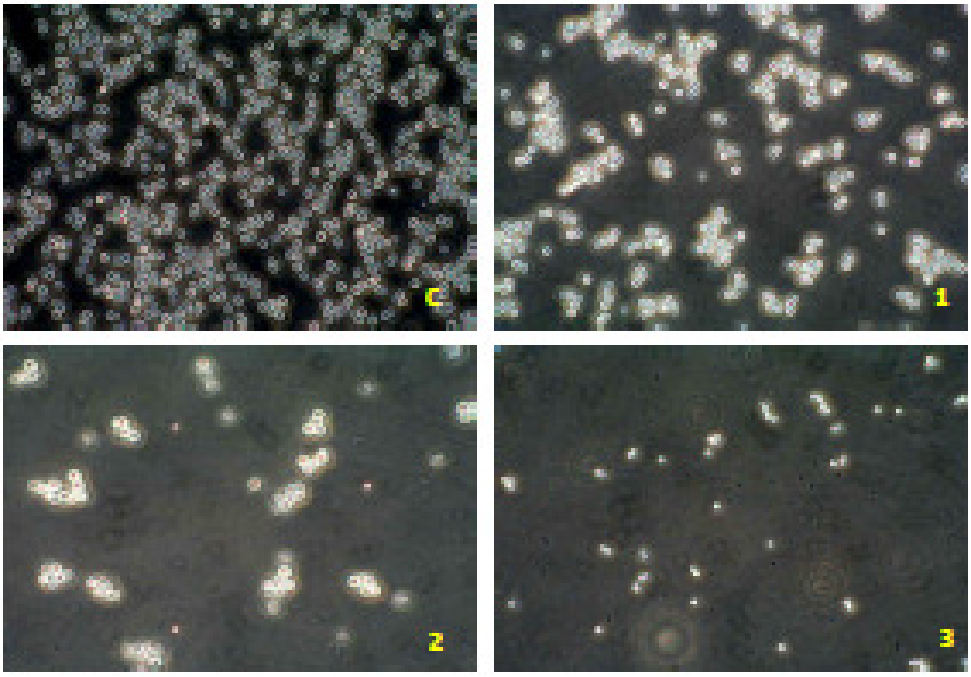

Figure 2. Microscopic examination showing the effect of *E. globulus* essential oil on the growth of *C. albicans* (strain 15B) tested at ½ MIC (1), MIC (2) and MFC (3) concentrations (Magnification 400×). The first photo (C) represents *C. albicans* (strain 15B) growing without essential oil in YPD after 24 h at 37°C.

medicinal purposes.

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