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Antibacterial activity and chemical composition of 20 *Eucalyptus* species' essential oils

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ABSTRACT

The essential oils of twenty *Eucalyptus* species harvested from Zerniza and Souinet arboreta (North West and North of Tunisia), were screened for their antibacterial activities by the agar disc diffusion method. Eighteen major compounds, identified by GC and GC/MS, have been retained for the study of the chemical and biological activity variability. The main ones were 1,8-cineole followed by α-pinene, *p*-cymene, borneol, cryptone, spathulenol, viridiflorol and limonene. The chemical principal components analysis identified 10 chemotypes, however that of the inhibition zone diameter (izd) of growth bacteria separated 5 groups of *Eucalyptus* oils, characterised by their antibacterial inhibition ability. The most sensitive strain was the Gram positive *Staphylococcus aureus* with that of *E.* odorata oil (16.0 ± 1.0 mm izd), while the most resistant bacteria was *Pseudomonas aeruginosa*. Some correlation between the amount of 1,8-cineole, *p*-cymene, α-pinene, or of cryptone and the antibacterial activity were observed.

1. Introduction

The *Eucalyptus*, a native genus from Australia belongs to Myrtaceae family and comprises about 800 species (Ogunwande, Olawore, Adeleke, & Konig, 2003). More than 300 species of this genus contain volatile oil in their leaves. Less than 20 of these have ever been exploited commercially for the production of essential oils rich in 1,8-cineole (more than 70%) by pharmaceutical and cosmetic industries (Pino, Marbot, Quert, & Garcia, 2002). Leaf extracts of *Eucalyptus* have also been approved as food additives (Takahashi, Kokubo, & Sakaino, 2004). In fact, for many years, essential oils have involved interest as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infections and as a natural food preservative (Schuenzel & Harrison, 2002; Tepe, Daferera, Sökmen, Polissiou, & Sökmen, 2004). A number of studies have demonstrated the antimicrobial properties of *Eucalyptus* species essential oils against a wide range of micro organisms. The most studied were those from *E. globulus*, *E. camaldulensis*, *E. tereticornis* and *E. citriodora* (Cimanga et al., 2002). Only a few studies investigated their activity against, pathogenic and food spoilage bacteria (Moreira, Ponce, del Valle, & Roura, 2005). In 1957, Tunisia introduced 117 species of *Eucalyptus*; they were essentially used as fire wood, for the production of mine wood and in the fight against erosion (Khouja, Khaldi & Rejeb, 2001).

In our previous investigation we have studied the leaves essential oil chemical composition of 56 species of *Eucalyptus* developed in Tunisia, harvested from Hajeb Layoun (Elaissi et al., 2007; Elaissi et al., 2010a), Souinet (Elaissi et al., 2010b), Korbous (Elaissi et al., 2010c), Djebel Abderrahman (Elaissi et al., 2011a), and Zerniza arboreta (Elaissi et al., 2011b).

In this paper the essentials oils of 20 *Eucalyptus* species leaves harvested from Souinet and Zerniza arboreta which chemical composition has been studied by Elaissi et al. (2010b, 2011b), were screened for their antibacterial activity against four food spoilage bacteria models. A correlation between eighteen major compounds and their antibacterial ability was investigated.

2. Materials and methods

2.1. Plant materials

Samples of clean mature leaves of 20 species of the genus *Eucalyptus* L'HÉR. were picked, from three trees, in June 2006 for *E. bicostata* Maiden, Blakely & Simmonds, *E. cinerea* F. Muell. ex Benth., *E. exerta* F. Muell., *E. gigantea* Hook. f., *E. gunnii* HooK. f., *E. macarthurii* Deane & Maiden., *E. macrorrhyncha* F. Muell., *E. maidenii* F. Muell.,

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E. odorata Behr., *E. pauciflora* Sieber ex Sprengel., *E. sideroxylon* A. Cunn. ex Woolls, *E. tereticornis* Sm. and *E. viminalis* Labill, which were acclimated in Souinet arboreta (Ain Draaham region, North of Tunisia), and in June 2007 for *E. botryoides var. botryoides* Sm., *E. cladocalyx* F. Muell., *E. citriodora* Hook., *E. diversicolor* F. Muell., *E. fasciculosa* F. Muell., *E. grandis* W. Hill and *E. ovata* Labill, which were acclimated in Zerniza arboreta (Sejnene region, North West of Tunisia). For *E. botryoides* var. *botryoides* leaves were collected from trees having two origins: Morocco and Italy (Vilmorin). Botanical voucher specimens have been deposited in the Pharmacognosy Laborotary Herbarium in the Faculty of Pharmacy, Monastir, Tunisia, under the following references: 0119, 0120, 0121, 0122, 0123, 0124, 0125, 0126, 0127, 0128, 0129, 0130, 0131, 0143, 0144, 0145, 0146, 0147, 0148, 0149 and 0150.

2.2. Extraction of essential oils

Extraction was carried out by hydro distillation for 4 h, using a standard apparatus recommended in the European Pharmacopoeia. We repeated this 3 times for each sample of 100 g of boorishly crushed dried leaves and for each species. The oil collected from each plant was dehydrated with Na_2SO_4 and stored at 4 °C, until analysis and biological activities testing.

2.3. Chemical analysis

Quantitative and qualitative data of all the essential oils were determined in triplicate by GC and GC/MS respectively.

2.3.1. GC analysis

GC was carried out using HP 6890 chromatography apparatus equipped with FID and SPB20 column (30 m × 0.32 mm i.d., film thickness 0.25 μm). Analytical conditions were: injector and detector temperature was maintained at 250 and 280 °C, respectively; oven temperature programmed to rise from 35 to 250 °C at 5 °C/min, isothermal temperature 35 °C for 1 min and 250 °C for 3 min; carrier gas was Nitrogen with a flow rate of 1.2 ml/min. Injected volume was 1 μl sample of 10% solution of oil in purified hexane. Relative concentration was calculated using the software HP Chemstation, which allows assimilation of the percentages of the peak areas to the percentages of the various constituents. Retention indices were obtained by running a series of aliphatic hydrocarbons (C9-C28) increasing number order of carbon atoms on the SPB20 column.

2.3.2. GC/MS analysis

All the essential oils were carried out on Hewlett–Packard (HP) 5890 series II gas chromatography equipped with a polar column Carbowax (30 m × 0.32 mm i.d., film thickness 0.25 μm) and 5972 mass selective detectors. Helium was used as the carrier gas. The mass spectrometer operating conditions were: ionisation voltage, 70 eV, ion source 230 °C. The GC–MS parameters were identical to those for the GC analysis.

2.3.3. Compound Identification

The identification of the compounds was based on a comparison of retention indices (determined relatively to the retention time of aliphatic hydrocarbons (C9-C28)), of the mass spectra with those of authentic compounds by means of *NBS75K.L.* and *Wiley 275* databases and with the literature data (Willey & Sons, 1998).

2.4. Antibacterial testing

The antibacterial activity of the different essential oils was evaluated by the paper-disk agar diffusion method against the two Gram-negative model bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and the two Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212). Microorganisms were obtained from the culture collection of the Laboratory of transmissible diseases and biologically active substances, Faculty of Pharmacy, Monastir, Tunisia. Organisms were maintained on Muller-Hinton agar (MH) (BIORAD). Inocula were prepared by diluting overnight (24 h at 37 °C) cultures in Muller Hinton Broth medium to approximately 10^6 CFU/ml. Absorbent disks (Whatman disc No. 3, 6 mm diameter) were impregnated with 10 μl of oil and than placed on the

Table 1
Antibacterial activity of the 20 *Eucalyptus* essential oils.

Microorganisms No.	Essential oils	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 227853	*Enterococcus faecalis* ATCC 292112	*Staphylococcus aureus* ATCC 25932
1.	*E. bicostata*	*9.0 ± 0.0	7.7 ± 0.6	11.0 ± 2.6	15.0 ± 0.0
2.	*E. botryoides origin* Morocco	10.7 ± 1.5	6.8 ± 1.0	8.3 ± 0.6	14.3 ± 0.6
3.	*E. botryoides* originVilmorin	11.0 ± 1.0	7.0 ± 0.0	10.7 ± 2.1	13.0 ± 1.0
4.	*E. cinerea*	10.0 ± 0.0	9.0 ± 0.0	8.3 ± 0.6	12.3 ± 0.6
5.	*E. citriodora*	10.0 ± 0.0	8.3 ± 1.2	7.7 ± 0.6	7.7 ± 0.6
6.	*E. cladocalyx*	9.0 ± 0.0	6.7 ± 0.6	9.3 ± 2.3	6.0 ± 0.0
7.	*E. diversicolor*	10.0 ± 0.0	0.0	8.7 ± 2.1	13.7 ± 1.2
8.	*E. exserta*	0.0	0.0	6.0 ± 0.0	8.0 ± 1.4
9.	*E. fasciculosa*	8.3 ± 1.2	0.0	7.0 ± 1.0	10.0 ± 0.0
10.	*E. gigantea*	6.7 ± 0.6	8.3 ± 0.6	9.3 ± 1.2	10.0 ± 0.0
11.	*E. grandis*	8.0 ± 0.0	0.0	7.7 ± 0.6	10.7 ± 0.6
12.	*E. gunnii*	7.0 ± 0.0	9.0 ± 0.0	9.0 ± 1.4	7.5 ± 0.7
13.	*E. macarthurii*	8.0 ± 1.0	8.0 ± 0.0	9.3 ± 2.3	10.7 ± 0.6
14.	*E. macrorrhyncha*	6.3 ± 0.6	7.7 ± 0.6	7.0 ± 1.0	7.7 ± 0.6
15.	*E. maidenii*	9.0 ± 0.0	7.3 ± 0.6	11.7 ± 0.6	9.3 ± 1.5
16.	*E. odorata*	10.0 ± 0.0	7.7 ± 0.6	10.3 ± 1.5	16.0 ± 1.0
17.	*E. ovata*	10.7 ± 1.2	6.7 ± 1.2	7.0 ± 0.0	8.0 ± 2.0
18.	*E. pauciflora*	7.0 ± 0.0	7.5 ± 0.7	8.0 ± 0.0	7.0 ± 1.4
19.	*E. sideroxylon*	8.7 ± 1.5	7.7 ± 0.6	7.3 ± 0.6	9.3 ± 0.6
20.	*E. tereticornis*	8.0 ± 1.4	7.0 ± 0.0	9.5 ± 0.7	11.5 ± 2.1
21.	*E. viminalis*	9.0 ± 0.0	8.0 ± 0.0	9.5 ± 0.7	11.0 ± 0.0
22.	Gentamicine	20.0 ± 1.0	14.3 ± 6.6	13.0 ± 1.0	29.3 ± 1.2

* Values are means ± Standard Deviation (SD) of triplicate determinations.