Volatiles Leaf Oil Constituents of Eucalyptus camaldulensis Dehnh from Algeria

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Received: September 06th, 2015; Accepted: December 10th, 2015

Abstract: The chemical composition of the essential oil, extracted from *Eucalyptus camaldulensis* leaves originating from Tizi Ouzou region (north Algeria) has been studied. Analyses were made by GC/FID on two capillary columns, using a non-polar phase (DB1) and another polar one (Carbowax 20M). This essential oil was also analyzed by GC/MS on OV1 capillary columns. The main components identified after this investigation were: p-cymene (22.50%), spathulenol (22.05%), cryptone (16.79%) while 1.8-cineole was identified at a low concentration (1.23%).

Keywords: *Eucalyptus camaldulensis* Dehnh, Myrtaceae, Essential oil composition, p-Cymene, Spathulenol, Cryptone, 1.8-Cineole.

1. Introduction

The genus *Eucalyptus* (family Myrtaceae), comprises well-known plants of over 800 species. Although, most of the plants are natives to Australia and Tasmania (Isiaka et al 2003), its introduction in Algeria was in 1860, not less than 130 species have been planted in the country during the years from 60 to 70 but the pioneer species seems to be *E.camaldulensis* (La forêt algérienne 1996). This species is considered as one of the most widely planted trees in the world with 5000.00 ha planted (Muhammad et al 2010).

As other family members of Myrtaceae, leaves of eucalyptus are covered with oil glands. The abundant oil production is an important characteristic of this genus. This essential oil is used in certain medicinal, perfumery and flavour preparations, for their therapeutic, organoleptic and odorants properties (Curtis, 1990, Candy, 1977; Hmamouchi, 1997)

Sometimes the same species can exist in different chemotypes having different chemical compositions. However, examining the chemical composition appears therefore a crucial step

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(Rodriguez et al 1984).

The purpose of this study is first to determine the essential oil chemical composition of *E. camaldulensis* growing northern Algeria (Tizi Ouzou), second to compare it with those of the same species growing in Australia, Spain and Morocco and to see finally if they could be exploited as commercial sources for the production of essential oil in Algeria

2. Materials and methods

2.1. Plant Material and Extraction

The plant materiel of *Eucalyptus camaledulensis*, was collected in March, during the flowering stage at 10km from Tizi Ouzou city (north Algeria). It was identified by a plant taxonomist, voucher specimen deposited in the Herbarium of National Forestry Research Institute, Bainem; Algers (INRF) Mature leaves *E.camaldulensis* (150g) were air dried at room temperature in a dark place. The essential oils obtained by steam distillation, using a Clevenger type apparatus for 2 h30. After decanting and drying over anhydrous sodium sulfate, the essential oil was recovered in yield of 0.42% w/w. The sample was then kept in bottles covered with aluminum foil at 6°C.

2.2. Detection Method

The samples of essential oil were analyzed using gas chromatography with flame ionization detection (GC-FID) on two types of capillary column: DB1 and Carbowax20M. Then, gas chromatography coupled with mass spectrometry (GC-MS) on OV1 capillary column.

The GC-FID analyses were carried out using an a Chrompack chromatograph model 9002 gas chromatograph equipped first with DB1 a fused silica capillary column coated with a non-polar phase (30m L, 0.32 mm i.d, 0.25um film thickness). A Carbowax 20M coated with a polar stationary phase (30m L, 0.32 mm i.d, 0.25um film thickness) was then used to separate both samples. With both columns, Helium was used as the carrier gas at 1ml/min. While the oven temperature was programed as follow: 60C° (6min) to 240C° at a rate of 3C°/min, then isothermal at 240°C for 5min. Detector and injector were set at 280 °C and 250°C respectively. The retention indices (Ir) for all the compounds were determined according to Van den Dool and Kratz method using retention times of n-alkanes (C5-C22) that had been injected after the essential oils under the same chromatographic conditions (Van den Dool et al 1963).

GC-MS analysis was performed on a gas chromatograph Shimadzu GC-17A equipped with Shimadzu QP-500 mass spectrometer, operating at 70 eV. MS source temperature: 250°C. The GC is carried out on a capillary column OV-1 (30m L, 0.32 mm i.d, 0.25um film thickness) equivalent to DB1. The carrier gas was helium with inlet pressure of 90kpa. Samples were injected with a split ratio of 1: 30. The temperature programming was identical with that of GC-FID analyses. Identification of components were first done by comparison of their retention indices with those reported in published works (NIST 2005; Davies, 1990), then confirmed by comparison of their mass spectra with those of standards Adams library (Adams, 2007).

3. Results and discussion

The average yield obtained from leaves is low than that obtained from the leaves of Australian *E. camaldulensis* species for both chemotypes (2 and 2.3% vs. 0.42%). The Moroccan and Algerian chemotypes give intermediate yields (0.84% and 0.70%) respectively (Farah et al 2002; Benayache et all 2001). Chromatographic analysis of the essential oils samples, allowed to identify about 33 components representing 86.98% of total oil; these compounds have previously been detected in *Eucalyptus* essential oil (Zrira et all 2004, Hmamouchi et al 1992).

The major constituents were: p-cymene (22.50%), spathulenol (22.05%), cryptone (16.79%), terpen-4-ol (5.18%), cuminaldehyde (3.29%) and α-terpineol (2.68%), while the 1.8-cineole which is the main known constituent of essential oil of *Eucalyptus* was identified with a low concentration (2.23%).

The oxygenated monoterpenes are relatively numerous, some are present only in average amounts like: terpin-4-ol (5.18%), cuminaldehyde (3.29%) and α-terpineol (2.68%), while others are present only in low concentration like: carveol (0.13%), trans-piperitone (0.56%) and linalool (0.81%).

From a quantitative point of view, the hydrocarbon monoterpenes are less abundant than oxygenated ones; their concentrations vary between 0.08 and 22.5% respectively for sabinene and p-cymene.

The γ-elemene and caryophyllene are only current sesquiterpenes with respectively 0.22% and 0.53%.

As illustrated in the histogram (Figure 2), the *E.camaldulensis* oil is constituted approximately by 39% of oxygenated monoterpenes, 34% of monoterpenic hydrocarbons, 26% of oxygenated sesquiterpenes and less than 0.9% of hydrocarbon sesquiterpenes

The composition of our essential oil, has many similarities with that of Spain chemotype, which was reported to be dominated with spathulenol (41.46%), p-cymene (21.92%) and cryptone (7.76%), whereas, the content of 1,8-cineole is 1.92 % (Verdeguer et al 2009).

Essential oil studied in this paper is significantly different to the Australian, Moroccan and Algerian chemotypes; respectively the first is dominated by the spathulenol (18.30%), the viridiflorol (14.80%) and the β-pinene of (10.60%) (Boland et al 1991).

Table 1. Chemical Composition of essential oil of *E.camaldulensis*

Compound	I (1)	I (2)	I (3)	I (4)	%	(6)	(7)
α-Thujene	922	926	1032	1030	0.18	90	Ir
α-Pinene	928	927	1038	1033	0.35	91	Ir
Sabinene	962	960	1140	1130	0.08	-	Ir et MS
β-Pinene	966	961	1128	1113	0.15	88	Ir
β-Myrcene	978	976	1151	1150	0.25	90	Ir
α-Phellandrene	995	1003	1178	1168	0.66	95	Ir
α-Terpinene	1002	1008	1185	1176	0.15	89	Ir
p-Cymene	1008	1014	1281	1270	22.5	95	Ir
β -Phellandrene	1014	1025	1223	1216	4.55	91	Ir
1,8 Cineole	1019	1027	1236	1230	2.23	93	Ir
γ-Terpinene	1040	1042	1254	1250	0.29	91	Ir
m-Cymenene	1074	1062	-	-	0.32	-	Ir et MS
trans-Sabinene hydrate	1079	1066	-	-	0.11	80	Ir et MS
Linalool	1083	1087	1557	1550	0.81	91	Ir
Thujone	1094	1114	-	-	0.26	87	Ir et MS
α -Campholenal	1115	1116	1513	1507	0.07	70	Ir et MS
trans.Pinocarveol	1120	1123	1677	1670	0.19	70	Ir et MS
p-Mentha-1,5-dien-8-ol	1129	1135	-	-	0.2	-	Ir et MS
trans-Sabinol	1135	1140	1684	1683	0.15	-	Ir et MS
Terpin-4-ol	1163	1163	1615	1613	5.18	90	Ir
Cryptone	1171	1169	1692	1695	16.79	86	Ir
α-Terpinéol	1192	1189	1707	1710	2.68	91	Ir
cis-Carveol	1200	1195	1855	1850	0.13	-	Ir et MS
Cuminaldehyde	1204	1202	1770	1766	3.29	95	Ir
trans-Carveol	1212	1208	1869	1873	0.18	82	Ir
Carvone	1220	1210	1716	1715	0.3	90	Ir
Piperitone	1238	1236	1746	1739	0.56	91	Ir
p-Cymene-7-ol	1288	1287	-	-	0.58	85	Ir et MS

Carvacrol	1298	1297	2150	2159	0.48	84	Ir
Caryophyllene	1400	1405	1615	1617	0.53	88	Ir
γ--Elemene	1481	1475	1640	1642	0.22	-	Ir et MS
Spathulenol	1573	1576	2138	2140	22.05	89	Ir
Viridiflorol	1601	1599	2120	2112	0.51	87	Ir

(1): Calculated retention indices using retention times of n-alkanes (C5-C22) on DB1 (2): Reference retention indices on DB1 (NIST 2005) (3): Calculated retention indices using retention times of n-alkanes (C5-C22) on Carbowax20M (4): Reference retention indices on Carbowax20M (Davies, N.W. 1990) (5): percentage of essential oil sample compounds (6): Identification safety coefficient given by spectral data bank (7): Identification method

The second is characterized by a relatively high content of 1.8-cineole (50.6%) against low level of spathulenol (4.9%) (A. Farah et al 2002); the last is composed by 1.8-cineole (38.6%), p-cymene (15.2%) and spathulenol (4%) (S. Benayache et al 2001).

This variation of essential oil content of *E.camaldulensis* from different countries might be attributed to the diverse agroclimatic conditions of those regions. (A. Muhammad et al 2010)

4. Conclusions

In this paper, 30 major and minor constituents of *E.camaldulensis* essential oils growing at Tizi-Ouzou were identified, the obtained results show that the main components were: p-cymene, spathulenol and cryptone. Oxygenated monoterpenes constitute the major fraction of the essential oil followed by hydrocarbon monoterpenes and the oxygenated sesquiterpenes. This result suggests the existence of the antimicrobial and bactericidal properties of this essential oil which must be confirmed by other investigations and to evaluate its potential

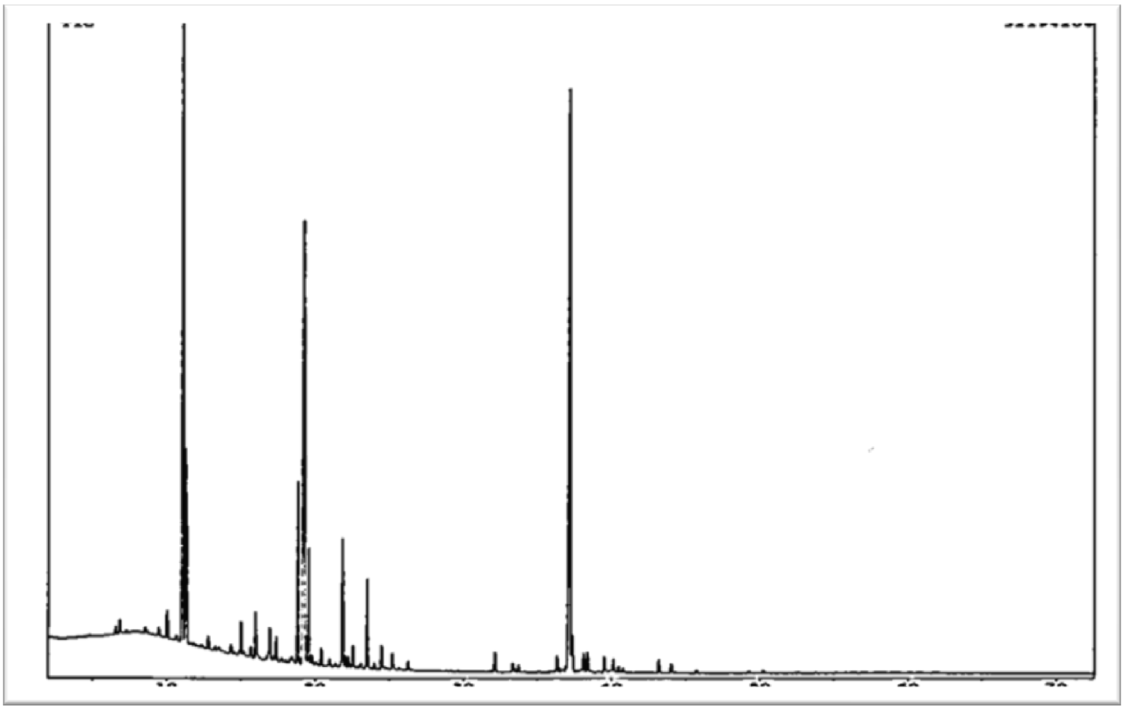

Figure 1. Chromatogram of essential oil of *E.camaldulensis* on OV-1

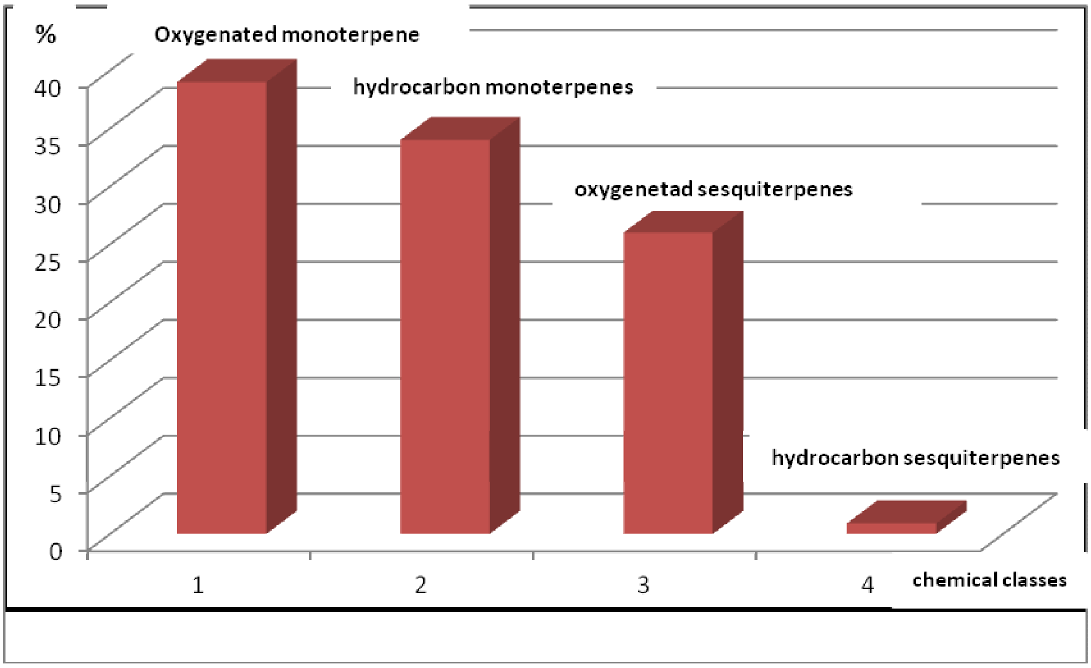

Figure 2.Histogram of the chemical classes of *E.camaldulensis* essential oil

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Arabian Journal of Medicinal and Aromatic Plants

www.ajmap.org ISSN 2458-5920