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Chemical composition of some Tunisian *Eucalyptus* essential oils as obtained by hydrodistillation using Clevenger type apparatus

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ABSTRACT

In this study we have determined the yield of the essential oil of 15 *Eucalyptus* species collected from Sidi Ismail Arboretum, in the Sahel of Tunisia. The average yield of leaf essential oil obtained by hydrodistillation varies according to species. It ranged between 3.367 % for *E. dumosa* and 1.167% for *E. bicolor*. The volatile oil compositions *of E. dumosa, E. cladocalyx, E. oleosa* and *E. transcontinentalis* were analysed qualitatively and quantitatively by GC/MS. Forty six different components were separated, and most of them identified. The main constituent was found to be 1-8 cineol (Eucalyptol). The extract possessed high yield of oxygenated monoterpens, followed by monoterpens hydrocarbons, then a wick level of sesquiterpens hydrocarbons.

Key words: *Myrtaceae, Eucalyptus* essential oil, GC/MS, 1-8cineol, oxygenated monoterpens, monoterpens hydrocarbons, Sesquiterpens monocarbons.

INTRODUCTION

The genus *Eucalyptus* includes more than 700 species and belongs to the family of *Mirtacea*, originally from Austria, it has spread worldwide, particularly in Africa, because of its easy adaptability and fast growth (Menut and *al.,* 1995)

In order to improve the forest production, great efforts of reforestation based on *Eucalyptus* were undergone in all of Tunisia (khouja and al., 2001).

The *Eucalyptus* essential oil is known to be used for its therapeutic virtue. The principal active ingredient is 1-8 cineol which has strong germicidal and disinfectant properties (Lassak and Mc Carthy, 1983). Because of its antimicrobial activity, *Eucalyptus* essential oil may be used in food manufactory not only as a flavoring agent, but also as a conservative one (Oussalah and *al*., 2006).

In the present work we have firstly determinate the yield of the essential oils of 15 Eucalyptus species implanted in the Sahel of Tunisia " Sidi Ismail Arboretum", secondly, we have studied the chemical composition of four Eucalyptus essential oils using the gas chromatography coupled with the mass spectrometry.

The main goal of this work is to determine the difference between species, and to find the major chemical components in these *Eucalyptus* essential oils.

MATERIAL AND METHODS

Plant Material

Samples of clean mature leaves were picked from three trees representing the same specie grown in "Sidi Ismail Arboretum" located between Msaken and Sfax, Tunisia, on January 2007. Leaves were harvested at a dry place for fifteen day. Samples used for hydrodistillation using Clevenger type apparatus were composed of leaves of fifteen different Eucalyptus species: *E. torquata, E. leuxophleba, E. incressata, E. intertexta, E. transcontinentalis, E. leucoxylon, E. stricklandy, E. dumosa, E. striaticalyx, E. oleosa, E. gilii, E. salubris, E. cladocalyx, E. bicolor* and *E. brevifolia.*

Essential oils extraction

Dried leaves of fifteen *Eucalyptus* species were subjected to hydro-distillation in a Clevenger-type apparatus for 3 h. The essential oils obtained were drying over anhydrous Na_2SO_4, and kept in a refrigerator for its further composition analysis by GC/MS.

Influence of crushing on the yield of essential oils extraction

To determine the variability of the yield of *Eucalyptus* essential oil extraction in accordance with crushing, leaves of *E. leucoxylon* were one time extremely crushed, medially crushed (10 mm of diameter), and no crushed. Each sample was then submitted to hydrodistillation (fig1).

Variability of the yield of essential oils in accordance with species

To investigate the influence of species on the yield of essential oil, leaves belonging to all species were medially crushed and submitted to hydrodistillation for 3 hours. To compare between species, a statistical test was carried out (SAS, version 1994).

Essential oils characterisation

Measurement of the Relative Density at 20°C (ISO 279:1998)

The relative density at 20°C is the ratio of volume of essential oil on the mass of the same volume of distilled water at 20°C. The density was measured by weighing 1 ml of essential oil in a tube, then 1 ml of distilled water.

$d_{20} = (m_2 - m_0) / (m_1 - m_0)$

m_0: weigh of the tube (g)

m_1: weigh of the tube + essential oil (g)

m_2: weigh of the tube + distilled water (g)

Measurement of the Acid Index (ISO 1242:1999)

The acid index is the number of milligrams of Potassium hydroxide able to neutralise free acids present in one gram of essential oil. For that purpose, 2 g ± 0.005 of essential oils were weighed and diluted in 5 ml ethanol 95%. Then, 5 drops of phenolphthalein were added to the mixture, and neutralised with potassium hydroxide solution 0.1 N. Acid index was calculated:

$$I_a = (5.61*V)/ m$$

m: mass of essential oil (g)

V: Volume of the potassium hydroxide solution (ml)

Gas chromatography–mass spectrometry analysis

The GC–MS analyses were carried out using a Hewlett Packard 6890 SERIES GC system plus mass spectrometry Hewlett Packard 5973 equipped with a HP 5MS 30 m × 0.25 mm × 0.52 µm film thickness capillary column. Helium was used as carrier gas. The initial temperature of the column was 60 °C during 8 min, and then it was heated to 180 °C at a rate of 4 °C/min then held at that temperature for 10 min. Finally, it rise to 220°C at a rate of 5 °C/min and held at that temperature for 5 min. Split ratio, 1:10. 1 µl of each sample, dissolved in ethanol (1:10 v/v), was injected. The percentage composition of the oils was computed by the normalization method from the GC peak areas (Jamoussi and *al.*, 2005). Just essential oils obtained from E. *transcontinentalis, E. oleosa, E. dumosa* and *E. cladocalyx* were analysed by GC-MS.

Identification of components

The components of the oils were identified by comparison of their mass spectra with those obtained from authentic samples and Wiley.275L mass spectral database. They were also confirmed by comparison of their retention indices (RI) and retention times (RT).

RESULTS AND DISCUSSION

Influence of crushing on the yield of essential oil extraction

Results obtained after hydrodistillation showed that samples medially crushed (10 mm of diameter) present the highest yield of extraction (fig2). In fact, crushing is a technical practice which enlarges the contact area between solid medium (leaves) and the boiled water. In addition to that, crushing betters the extraction speed, and also the yield of hydrodistillation. However, an extreme crushing may disturb the steam circulation, and thus slowing down the extraction speed (Fellah and *al.*, 2006).

Variability of the yield of essential oil in accordance with species

A statistical analysis was investigated after the essential oil extraction of 15 *Eucalyptus* species to demonstrate that the yield of extraction depends on species (fig 3). All leave samples were medially crushed, and the hydrodisyillation was about 3 hours. Results showed that the average yield varies according to species. It is 3.63% for *E. dumosa,* which is the most generative of essential oil and 1.16% for *E. bicolor* which seems to be the less generative of essential oil.

Table 1: SNK grouping of different yield (SAS, 1994)

SNK grouping	Mean yield	N	*Eucalyptus* species
A	3.637	3	*E. dumosa*
BA	3.363	3	*E. salubris*
BC	3.060	1	*E. cladocalyx*
BC	3.005	2	*E. leucoxylon*
BC	2.970	1	*E. gilii*
C			
C	2.787	3	*E. striaticalyx*
D	2.387	3	*E. leuxophleba*
E	1.990	3	*E. stricklandy*
E	1.880	2	*E. intertexta*
E	1.867	3	*E. torquata*
E	1.750	2	*E. oleosa*
E	1.740	3	*E. incressata*
E	1.720	1	*E. transcontinentalis*
E	1.605	2	*E. brevifolia*
F	1.163	3	*E. bicolor*

N : observation number, mean yield : (g E.O/100g dry leaves)

Table 2: Physicochemical characteristics of essential oils

Eucalyptus essential oil	Relative density (20°C)	Acid index (mg KOH/ g E.O)
E. transcontinentalis	0.93	1.40
E. dumosa	0.93	0.71
E. oleosa	0.92	3.58
E. cladocalyx	0.91	2.19

E.O.: Essential Oil

Statistical analysis using Student Newman and Keuls Test, showed the presence of six statistical group ($p<0.05$) (Table 1).

Comparing our results to those obtained by other researchers, it was determinate that the average yield varies according species, location and season. As example, species studied by Elaissi, 2005, were *E. salubris, E. transcontinentalis, E. oleosa* and *E. striaticalyx.* These species were located in Hajeb Layoun arboretum positioned in the middle of Tunisia. Essential oils were extracted on January and June 2005.

Essential oil characterisation

It was noticed that *E. dumosa, E. cladocalyx, E. transcontinentalis* and *E. oleosa* belongs to three different groups concerning the yield of essential oil extraction. For that reason, only these four species were subject of the rest of this study.

Sensorial characterisation

Essential oils obtained were liquid; the colour was clear yellow for *E. cladocalyx* and *E. dumosa*, however it was dark yellow for *E. oleosa* and *E. transcontinentalis*. The odour was fresh, characteristic and persistent.

Chemical composition of four *Eucalyptus* essential oils

The main components of the analysed *Eucalyptus* oils were determinate by GC-MS analysis. Table 3 contains results of the retention

Table 3 a: Chemical composition of *E. cladocalyx*, *E. dumosa*, *E. oleosa* and *E. transcontinentalis* essential oils

R.I	Chemical components	% chemical composition in *E. cladocalyx*	% chemical compositionin *E. dumosa*	% chemical compositionin *E. oleosa*	% chemical compositionin *E.transcontinentalis*
926	Tricyclène	-	-	0.66	-
931	α thujène	5.53	-	11.42	-
939	α pinène	2.92	5.72	0.57	5.71
1026	p-cimène	-	2.25	10.91	1.59
1033	1-8cinéole	71.19	72.45	22.94	70.41
1040	z-β ocimène	0.93	-	-	-
1058	γ-terpinène	-	0.51	-	-
1126	α-campholénal	2.44	4.45	3.66	5.09
1139	Trans-pinocarvéol	-	-	0.86	1.32
1147	Camphre	0.45	0.79	-	-
1151	Bornéol	-	0.37	-	-
1154	Trans-sabinol	-	-	1.41	-
1163	Pinocarvacrone	-	0.58	1.58	-
1168	Myrténal	-	0.81	-	-
1183	p-cimène-8-ol	-	-	-	0.39
1217	Trans carvéol	-	-	0.71	-
1242	Carvone	-	-	-	1.23
1252	Pipéritone	-	-	-	0.86
1255	Géraniol	-	-	-	0.82
1257	Acétate de linalyl	-	-	-	3.27
1275	Acétate de bornyl	-	0.71	-	1.057
1298	Carvacrole	-	-	-	1.17
1310	Thymol	-	-	2.04	-
1330	Acétate de terpényl 4	-	1.97	-	-

index (RI) of each component, which was calculated using the followed formula:
RI = 100y + 100 [tr (x) – tr (y)/ tr(z) – tr (y)] (Dunlop and *al*., 1995)
tr (x) : retention time of the wanted components x
tr (y): retention time of standard n-alcane preceding the wanted component x
tr (z): retention time of the standard n-alcane following the wanted component x.

The chemical composition of essential oil is affected by several factors such as species, geographical location, harvest time, part plant used and method of isolation. In the essential oil extracted from immature leaves of four eucalyptus species: *E cladocalyx, E transcontinentalis, E dumosa* and *E. oleosa*, 46 compounds were identified corresponding to more than 90% of the total oil. The four essential oils analysed consists for their major part on oxygeneted monoterpenes such as 1-8 cineole, α-campholénal. The percentages are as follows around 80%, 78%, 73% and 30% for *E. transcontinentalis, E. dumosa*, *E. cladocalyx and E. oleosa.*

Monoterpenes hydrocarbons were also present such as α thujene, α pinen and *p*-cymene, but in lower percentage 23% for *E .oleosa,* and 8% for each one of following species: *E. dumosa, E. cladocalyx* and *E. transcontinentalis*. The last group is sesquiterpenes hydrocarbons, for instance alloaromadendrene. Figures are respectively about

Table 3b: Chemical composition of *E. cladocalyx*, *E. dumosa*, *E. oleosa* and *E. transcontinentalis* essential oils

R.I	Chemical components	% chemical composition in *E.cladocalyx*	% chemical compositionin *E. dumosa*	% chemical compositionin *E. oleosa*	% chemical compositionin *E.transcontinentalis*
1350	AcétateD'α-terpényl	-	0.37	0.75	-
1354	α-cubébène	-	-	0.74	-
1371	α-copaène	2.49	-	-	-
1387	β-élémène	0.42	-	0.79	-
1391	Thumbergol	-	-	0.92	-
1394	Longifolène	-	-	23	-
1409	β-cubébène	-	0.56	6.03	-
1418	β-caryophyllène	-	0.59	1.7	-
1423	Â-gurjunène	-	3.52	0.85	-
1432	α-cédrène	-	1.17	1.05	-
1439	Aromadendrène	-	0.47	0.52	-
1447	α-Himachalène	-	-	0.63	-
1450	Á-Humulène	-	-	1.31	-
1460	Alloaromadendrène	-	-	3.48	-
1476	γ-Murolène	-	1.68	0.52	-
1532	Elemol	0.93	-	-	-
1542	Á-calacorène	0.58	-	-	-
1578	Oxyde de β-caryophyllène	6.74	-	-	-
1589	Cédrol	1.66	-	-	-
1603	Hexadicane	0.39	-	-	-
1625	T cardinol	0.37	-	-	-
1639	α-cardinol	0.63	-	-	-
	Total of identified components (%)	97.7	99	99.1	93

No crushed sample

Medially crushed sample
(10mm of diameter)

Extremely crushed sample
(2 mm of diameter)

Fig. 1: Diameter of leave samples

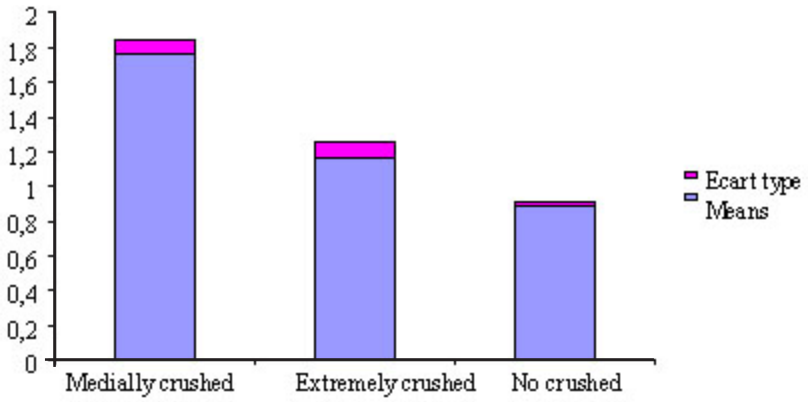
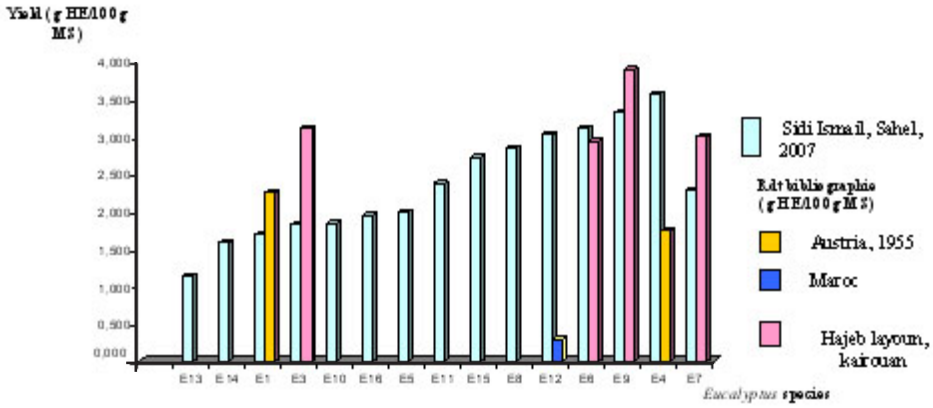


Fig. 2: Variability of extraction yield of *Eucalyptus leucoxylon* in accordance with crushing



E13 : *E. bicolor* E14: *E. brevifolia* E1: *E. leucoxylon* E3: *E. transcontinentalis* E10: *E. torquata*
E16 : *E. incressata* E5 : *E. stricklandy* E11 : *E. leuxophléba* E15 : *E. intertexta* E8 : *E. gilii*
E12 : *E. cladocalyx* E6 : *E. striaticalyx* E9 : *E. salubris* E4 : *E. dumosa* E7 : *E. oleosa*

Fig. 3: Comparison of yield means of 15 species picked on January 2007 from Sidi Ismail Arboretum, with some other species

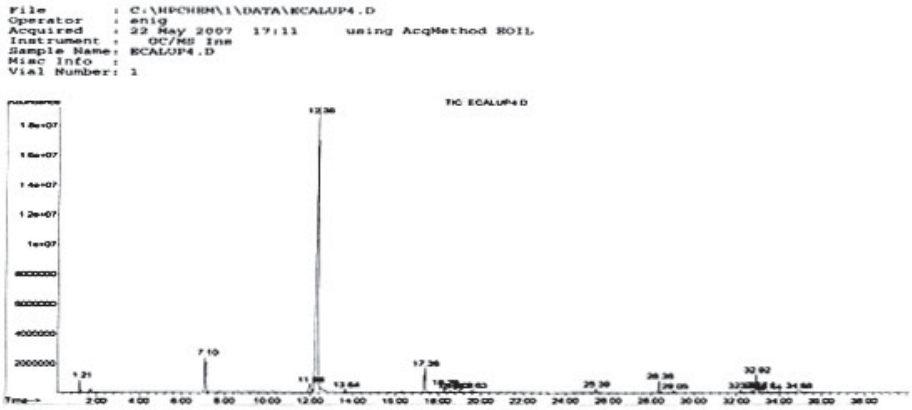


Fig. 4 : *E. dumosa* chromatogram

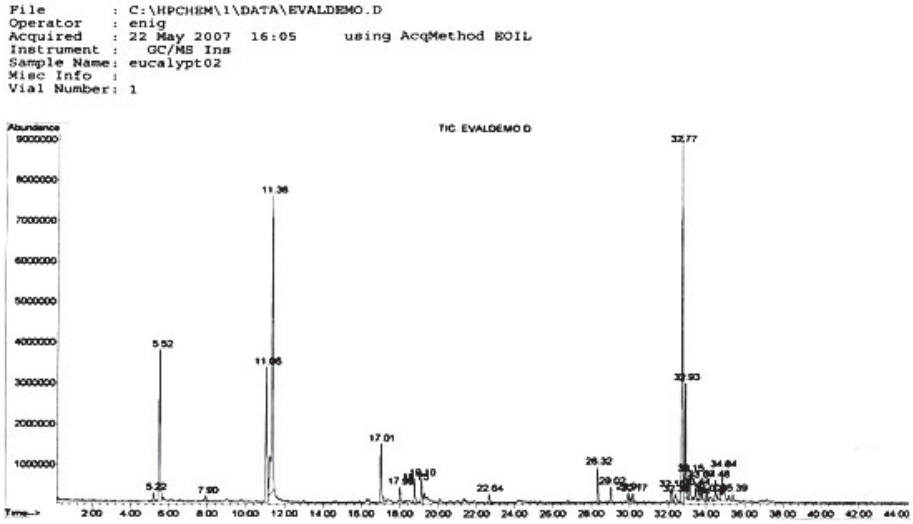


Fig. 5 : *E. oleosa* chromatogram

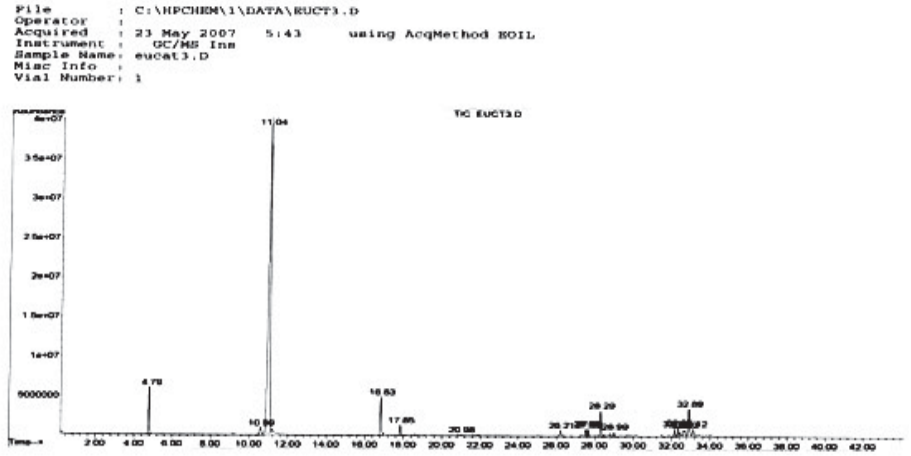


Fig. 6: *E. transcontinentalis* chromatogram

5%, 3.5% and 3.68% for *E. dumosa, E. transcontinentalis* and *E. dumosa.* We didn't identify any sesquiterpenes hydrocarbons on *E. cladocalyx* essential oil.

Results (Table 3a, 3b) showed that there is a qualitative and quantitative difference between the four *Eucalyptus* essential oils tested. Some components were present in certain essential oils samples and absent in others. As example, we cite thymol, a monoterpen recognized by its antimicrobial activity (Lambert *et al*., 2001). This component was only ingredient of *E. oleosa* essential oil; it was present in 2.04%.

Myrtenal was only present in *E. dumosa* essential oil. The major components were α pinen, *p* cymen, 1-8 cineol, α campholenal, longifolen, alloaromadendren, campher, borneol and carvacrol.

Results showed that α pinen percentage was about 0.57%, 2.92% and 5.7% respectively for *E. oleosa*, *E. cladocalyx*, *E. transcontinentalis* and *E. dumosa*.

E. oleosa essential oils presented the most important percentage of *p*-cymen (10.9%), followed by *E. dumosa* (2.25%), then *E. transcontinentalis* (1.6%). This component (*p*-cymen), is the biological precursor of carvacrol, it is hydrophobic and causes swelling of the cytoplasmic membrane to a greater extent than does carvacrol.

P-cymen is active when combined with carvacrol (Ultee *et al*., 2002).

In addition to that, the study of the chemical composition showed that 1-8 cineol was the major component. The percentage was about 72.5%, 71.2%, 70.4% and 23% respectively for *E. dumosa, E. cladocalyx, E. transcontinentalis* and *E. oleosa.*

This component was abundant in all species and those results agree with (Giamakis and *al*., 2001) whom funded that the percentage of 1-8 cineol in Eucalyptus essential oil is about 20 till 90%.

It was concluded that the four *Eucalyptus* essential oils were rich in oxygenated monoterpen as carvon, α campholenal, 1-8 cineol, transpinocarvon, myrtenal, borneol, transcraveol, piperitoin and thymol. That class enclosed different active components which suppose an antimicrobial power of our essential oils (Juven *et al*., 1994; Dorman and Deans, 2000; Ultee *et al*., 2000; Lambert *et al*., 2001).

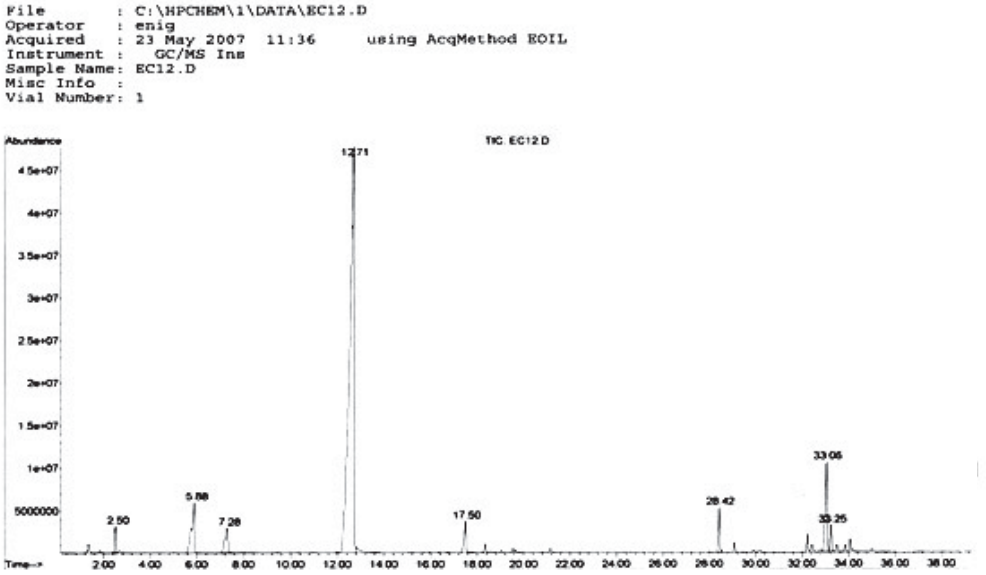


Fig. 7 : *E. cladocalyx* chromatogram

CONCLUSION

Yields of different essential oils from dried leaves of some selected *Eucalyptus* species varied greatly from one species to another. 46 constituents were identified in four *Eucalyptus* essential oils (*E. dumosa, E. cladocalyx, E. transcontinentalis* and *E. oleosa*).

1-8 cineol, α pinen, α-campholnal were prevalent constituents. It was found that *Eucalyptus oleosa* essential oil possess specific active chemical components such as thymol, p-cymen recognized as antimicrobial components. These properties of *Eucalyptus* essential oil tested could be used in several fields especially food alimentary as preservative.

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