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Chemical Profile of Essential Oils from the Leaves of *Eucalyptus nesophila* Acclimatised in Congo-Brazzaville. Statistical Approach

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Abstract: The steam distillation of leaves from the tree species *Eucalyptus nesophila,* native of Australia and acclimatised in Congo-Brazzaville, yielded essential oils with yields ranging from 0.1 to 0.5% over the year. To evaluate the variability of its chemical composition, 24 samples were studied. In these, 59 compounds were detected, of which 47 were identified. Two profiles were found, one rich in monoterpene hydrocarbons (α- and β-pinene), and the other rich in sesquiterpene alcohols (globulol, viridiflorol and epi-globulol). The essential oils studied contained mostly the same constituents in alternatively major and minor proportions, forming a relatively homogeneous population, the difference between the two types being essentially quantitative.

Key words: α-pinene, β-pinene, Congo-Brazzaville, chemical profile, epi-globulol, *Eucalyptus nesophila*, globulol, subgenus *Corymbia*, viridiflorol

INTRODUCTION

The species *Eucalyptus nesophila* acclimatised in Congo Brazzaville came originally from Australia: Western Australia (Kimberly), Northern Territory and North Queensland. In the classification of *Eucalyptus*, this species belongs to subgenus *Corymbia* section in Rufaria (Hill and Johson, 1995). *Eucalyptus nesophila* has adapted to a tropical climate with wet and dry seasons. Annual rainfall ranges from 1250 to 1500 mm and the dry season lasts 4-6 months. It grows on sandy soils with low relief in pure dominant stands or mixed with other species such as *Eucalyptus miniata* in open forest. It tolerates temperatures between 5 and 32ºC. Its durable dark red-brown wood is of moderate hardness, is resistant to mould and insects, though much less so to termites, and has some gum veins. It is used for construction throughout its native area.

Trees of 12 provenances were introduced in Congo - Brazzaville, precisely in the station Loandjili in Pointe Noire : (i) four between 1973-1974 (plots 73-11/12 and 74: one from Melville Island, Northern Territory (6675) and three from Cobourg Peninsula, Northern Territory zone 2 (1146-49; 1160-63 and 1173-77) and (ii) eight, grouping 36 separate lineages (plots 85-3 and 85-5), in 1985 (Gouma *et al*., 2002; Brézard, 1982).

In work on the non-timber commercial potential of the Pointe Noire forest, we undertook a systematic study to evaluate the potential for essential oil production from eucalyptus acclimatised in Congo-Brazzaville. This study, which concerns the provenance 1160/63 of *Eucalyptus nesophila*, follows on from that conducted previously on *Eucalyptus torelliana* (Silou *et al*., 2010).

MATERIALS AND METHODS

Plant material: The tree of *Eucalyptus nesophila* grows to a height of 25-30 m and a diameter of 90 cm, has a regularly shaped trunk and a well-balanced crown. Its bark is persistent, with small scales, scaly and almost tessellate. The young leaves are alternate, petiolate, wide oblong to oval and downy. The adult leaves are alternate, pedonculate, falcate and lanceolate to narrow lanceolate.

The 24 trees (20 years old) studied were located in the experimental station of the « Unité de Recherche sur la productivité des Plantations Industrielles (UR2PI, Pointe Noire, Congo-Brazzaville) ». Harvests were carried in 2006 and 2007, in September (dry season) and May (rainy season) to evaluate possible variations of yield and composition of leave essential oils. Leaves were harvested indiscriminately from adult trees. Essential oils were extracted and analysed. Samples collected in dry season are noted SS and that one collected in rainy season are noted SP.

Extraction of essential oils: The steam distillation set-up used consisted of a 2 L round bottomed steam generator surmounted by a 2 L glass reactor containing the plant

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material connected to a cooling system to condense the distillate. A volume of 250 mL of water was placed in the lower vessel and 50 g of plant matter in the upper reactor. The set-up was heated and the essential oil was carried off by the steam and condensed. The oil was then decanted. The extraction took 3 h and was repeated three times.

Determination of physico-chemicals parameters: The refractive index and specific rotation were determined by standard methods (AFNOR, 1996).

GC and GC/MS analysis: GC analyses was performed on a Hewlett-Packard 6890 equipped with a split/splitness injector (280ºC, split ratio 1:10), using DB-5 column (30 m x 0.25 mm, film thickness: 0.25 mm). The temperature program was 50ºC (5 min) rising to 300ºC at rate of 5ºC/min. Injector and detector temperature was 280º. Helium was used as carrier gas at a flow rate 1 mL/min. The injection of the sample consisted of 1.0 L of oil diluted to 10% v/v with acetone.

GC/MS was performed on a Hewlett-Packard 5973/6890 system operating in EI mode (70 eV), equipped with a split/splitness injector (280°C, split ratio 1:20), using using DB-5 column (30 m x 0.25 mm, film thickness: 0.25 mm). The temperature program was 50ºC (5 min) rising to 300ºC at rate of 5ºC/min. Injector and detector temperature was 280º. Helium was used as carrier gas at a flow rate 1 mL/min.

The identification was carried out by calculating retention indices and comparing mass spectra with those in data banks (McLafferty and Stauffer, 1989; Adams, 1995).

Statistical treatment: Statistics were processed using the XLSTAT 2000.6 software, which is a Microsoft Excel add-in (www. xlstat.com). Principal Component Analysis (PCA) and Ascending Hierarchical Classification (AHC) were used to compress the data and so facilitate interpretation.

Principal Component Analysis (PCA) is a method for processing data contained in a table presenting individuals (rows) against quantitative variables (columns). It is used to reduce a large mass of data to a more manageable size by constructing a small number of new variables by linear combination of the starting variables. These new variables are called principal components. They are considered to condense the initial data as best as possible under certain conditions.

PCA is thus a method for compressing data. It allows simple graphical representations to visualise the links between variables and similarity between individuals: a correlation circle to highlight links between variables and the plane formed by the first two principal components (F1F2) to discern similarities between individuals.

Ascending hierarchical classification (AHC) is used to form homogeneous groups of objects (classes) on the basis of their description by a set of variables, or from a matrix describing the similarity or dissimilarity of objects.

It is an iterative classification method, based on a simple principle: Similarities between *N* objects are first calculated. The two that minimise a chosen aggregation criterion are then grouped, forming the first class. The similarity between this class and the remaining $N - 2$ objects is then calculated, and the first class is then grouped with the next object minimising the aggregation criterion. This grouping is continued until a final class is obtained that includes all the objects. In this way a binary classification tree or dendrogram is constructed. A partition can subsequently be chosen by truncating the tree at a given level as required.

At the same time as the representations, the XLSTAT 2006.2 software used calculates criteria to check the reliability of the procedure: percentage of the variability accounted for by the different principal components, correlation matrix for the variables, square cosine for the variables, etc.

RESULTS AND DISCUSSION

The essential oil contents of the 24 samples of provenance 1160/63 harvested in the dry and rainy seasons ranged between 0.1-0.35% and 0.17-0.50% respectively. Like the great majority of the species of this genus (Coppen, 2002; Webb *et al*., 1956), and in particular the subgenus *Corymbia*, *Eucalyptus nesophila* yielded only small amounts of essential oil. The observed contents were always lower than 1%, in both rainy and dry seasons.

The values of refractive index ($1.4845 < n < 1.4855$) and specific rotation ($+9.76° < \alpha < +9.9°$) obtained were consistent with those reported in the literature (Coppen, 2002; Webb *et al*., 1956).

Of the 59 constituents detected, 47 were identified (Table 1). Six major constituents stood out (Table 2): α-pinene, β-pinene, globulol, viridiflorol, guaiol and α-eudesmol. All these compounds, except for β-pinene, also occur as major constituents of essential oil of *Eucalyptus torelliana*, *Eucalyptus citriodora*, *Eucalyptus maculata*, acclimatised in Congo-Brazzaville (Loumouamou, 1998; Silou *et al.*, 2009). Botanists classify these species in the same subgenus *Corymbia*. This finding is an *a posteriori* chemotaxonomic validation of this classification. Moreover, qualitatively, chemical profile of oil from trees acclimatised in Congo-Brazzaville is identical to that of oils extracted from the same trees in their native habitat (Hill and Johson, 1995).

The amplitudes of min-max variations in the contents of these constituents (Table 3) reveal a non-negligible quantitative variation. We note, for example, (i) for

Table 1: List of constituents detected in the 24 samples studied

1	α-thujene	17	α-terpineol	33	globulol
2	α-pinene	18	α-gurjunene	34	viridiflorol
3	α-fenchene	19	β-caryophyllene	35	guaiol
4	camphene	20	aromadendrene	36	epi-globulol
5	β-pinene	21	α-humulene	37	5-epi-7-epi-α-eudesmol
6	myrcene	22	allo-aromadendrene	38	humulene epoxide II
7	para-cymene	23	γ-gurjunene	39	eremoligenol
8	limonene	24	viridiflorene	40	γ-eudesmol
9	1,8-cineole	25	α-selinene	41	epi-α-cadinol
10	γ-terpinene	26	γ-cadinene	42	β-eudesmol
11	terpinolene	27	δ-cadinene	43	α-eudesmol
12	citronellal	28	trans-calamenene	44	α-cadinol
13	endo-fenchol	29	elemol	45	selin-11-en-4-α-ol
14	trans-sabinol	30	ledol	46	bulnesol
15	borneol	31	spathulenol	47	(Z,Z)-farnesol
16	terpinen-4-ol	32	caryophyllene oxide		

Table 2: Main major constituents in essential oils of *E. nesophila* (individual content >4% in at least one sample and compounds present in more than half the essential oils studied

Major constituents	Dry season content (%)	Rainy season content (%)
α-pinene	0.06-14.7	4.07-63.14
β-pinene	0.03-12.26	0.45-30.39
globulol	6.24-34.62	0.05-39.02
viridiflorol	0.05-70	3.57-52.16
guaiol	0.00-15.57	-
epi-globulol	0.00-5.12	-

Table 3: Structure of classes

Class	1	2
Objects	15	9
Intra-class variance	250.7	699.7
Minimum distance from barycentre	7.6	5.0
Mean distance from barycentre	14.8	22.1
Maximum distance from barycentre	22.6	44,2
	SS 1	SS 2
	SS 3	SS 8
	SS4*	SS9
	SS 5	SP 10
	SS 6	SP 11
	SS7	SP12*
	SP 1	SP 13
	SP 2	SP 14
	SP 3	SP 15
	SP 4	
	SP 5	
	SP 6	
	SP 7	
	SP 8	
	SP 9	

*: Central objects, SS: Dry season, SP: Rainy season

pinenes, a marked increase in the contents of the pinenes from dry season to rainy season, (ii) roughly constant levels of globulol irrespective of season, (iii) a fall in viridiflorol levels and (iv) the disappearance of guaiol and α-eudesmol from dry season to rainy season.

Principal Component Analysis (PCA) and Ascending Hierarchical Classification (AHC) lend these results greater legibility by compression of data and progressive grouping of most similar individuals.

The PCA representation of variables and individuals on the principal plane F1F2 constructed with the 47

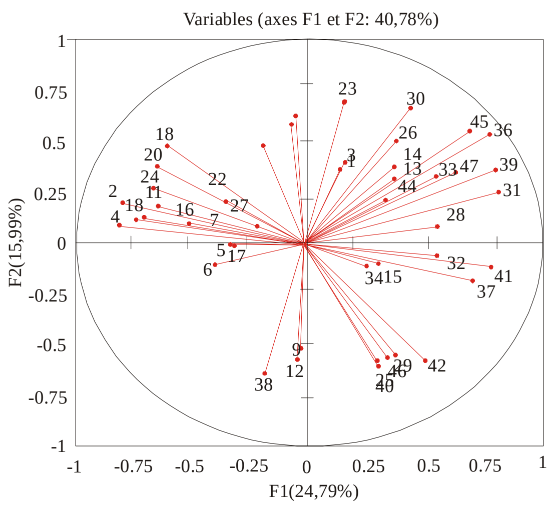
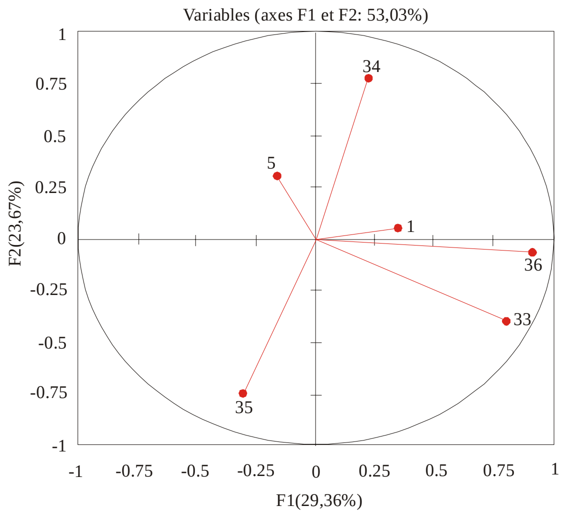


Fig. 1: Representation with 47 variables (constituents) by PCA for the first two principal components F1F2

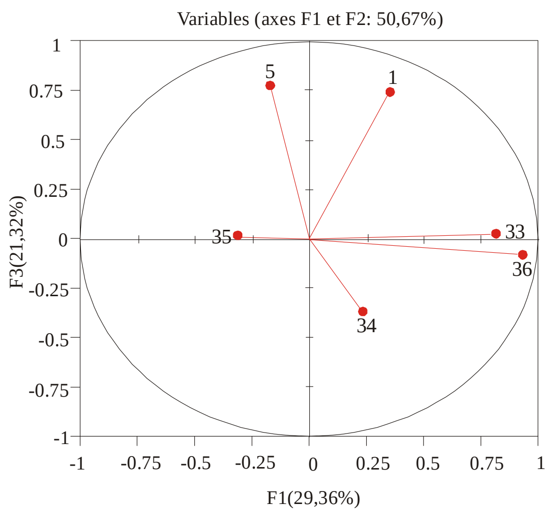
identified constituents which account for only 40.79% of the variability does not significantly improve this legibility (Fig. 1).

The same treatment reduced to the first six major constituents (α-pinene, β-pinene, globulol, viridiflorol, guaiol and α-eudesmol) and after elimination of closely correlated variables, gives the same representation of the variables, while improving the cumulated rate of variability accounted for (74.35%) (Fig. 2).

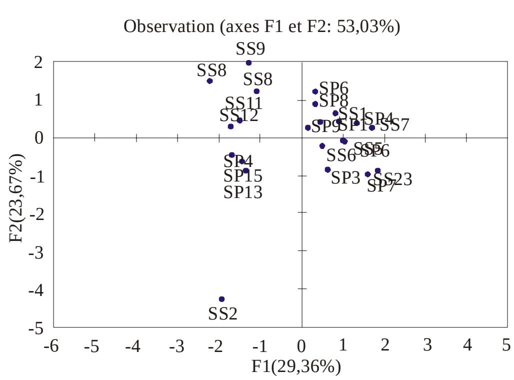
This figure shows (i) that on the first principal plane F1F2 accounting for 53.03% of the variability, sesquiterpene alcohols are strongly represented (globulol, viridiflorol, guaiol and epi-globulol), while monoterpenes are poorly represented (α-pinene and β-pinene); the globulols are anti-correlated with α-pinene and β-pinene; viridiflorol and guaiol are mutually anticorrelated but are independent of the globulols and pinenes, and (ii) that on the second principal plane F1F3, which accounts for 50.67% of the variability, the pinenes, which are strongly represented on the F3 axis, are anticorrelated with



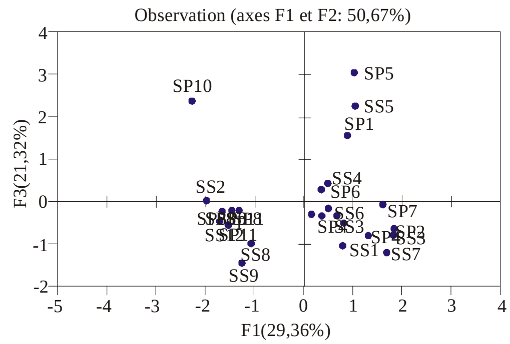
(a)



(b)



(c)



(d)

Fig. 2: Representation of variables and individuals by PCA on principal planes F1F2, F1F3 (cumulated variability 74.35%): (a, b): correlation of variables; (c, d): representation of individuals

viridiflorol, while globulol, still well represented and mutually closely correlated on this plane, are anticorrelated with guaiol.

The representation of individuals, on either the principal plane F1F2, or the F1F3 plane, shows two main groups of unequal size, separated by the axis F1, and one isolated individual in each case.

Ascending hierarchical classification, which automatically classifies individuals by progressively grouping most similar individuals, leads to the same conclusion. The two unequal classes appear more distinctly (Fig. 3).

Class 1 contains 15 objects with sample SS4 as central object, i.e. that most representative of its class. This is an essential oil composed mainly of sesquiterpene alcohols (viridiflorol, globulol and epi-globulol). The 15 essential oils all belong to this class.

Class 2 contains nine objects with as central object SP12, made up mostly of pinenes (>50%) (α-pinene and β-pinene). Three atypical samples among the nine objects placed automatically in this class do not have the characteristic profile of this class.

A major rainy season type (SP12) and a major dry season type (SS4) might have been expected, but we find a random distribution of the samples from the two seasons in the two classes, with Class 2 containing two-thirds of the objects. Thus there is no correlation between the composition of the oils and the harvesting season.

CONCLUSION

We can assert that the oils studied form a relatively homogeneous population, with the same overall qualitative composition. The quantitative variation of the constituents gives two chemical profiles, accounting for the inversion of the proportions of these constituents in different oils. Table 4 groups the oils of the samples in

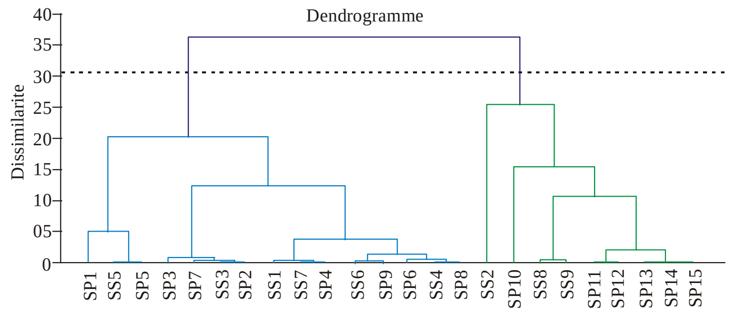


Fig. 3: Repartition of individuals by ascending hierarchical classification

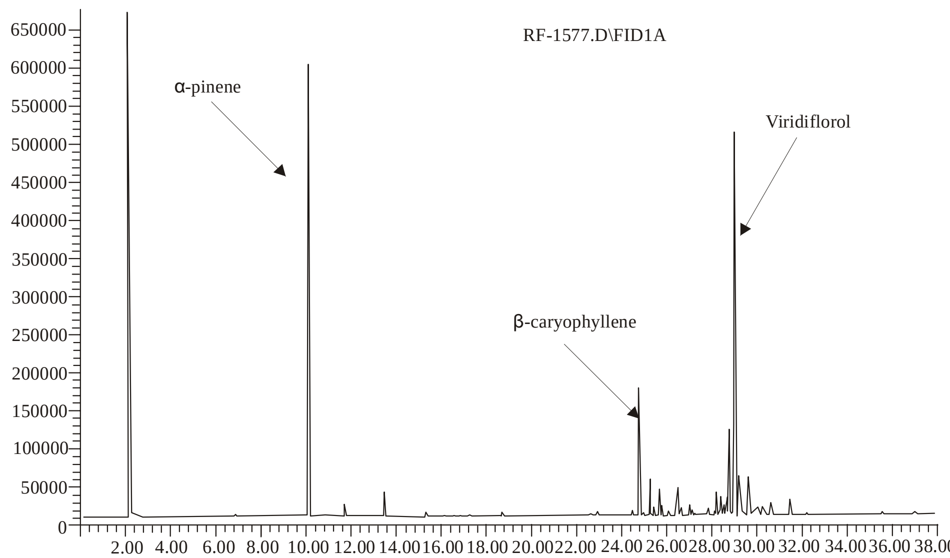


Fig. 4: Chromatographic profil of essential oil from *E. nesophila* rich in α-pinene

Table 4: Levels of main major constituents of essential oils in Class 1 (15 objects of Class 2)

	α-pinene	β-pinene	β-caryophyllene	globulol	viridiflorol	epi-globulol
SS1	4.13	-	3.52	9.98	46.04	4.31
SS3		-	2.76	34.62	28.97	3.78
SP3	26.15	-	-	24.64	18.19	2.59
SS4	14.67	6.63	4.01	10.78	34.08	3.39
SS5	14.51	12.26	-	21.52	23.51	2.77
SS6	5.54	2.14	2.4	11.92	24.54	3.57
SS7	-	-	-	20.07	46.56	5.12
SP1	27.27		6.25	6.1	32.98	3.13
SP2	6.88	-	3.54	32.18	26.17	4.23
SP3	26.1		-	24.6	18.1	-
SP4	4.07	3.11	-	17.43	43.62	4.78
SP5	15.25	16.37	2.41	23.62	19.07	2.44
SP6	5.85	4.68	5.04	5.77	52.16	3.44
SP7	18.03	-	2.4	32.82	22.20	3.24
SP8	9.81	7.47	5.25	8.75	43.70	-
SP8	3.1	-	-	6.24	54.7	-
SP9	5.66	2.29	5.29	39.02	13.58	4.11

Class 1. They are made up of sesquiterpene alcohols, in particular globulol, viridiflorol and epi-globulol (Fig. 5). In the essential oils grouped in Table 5 pinenes predominate (35-63%) over sesquiterpene alcohols, which are essentially reduced to viridiflorol (9-29%); Fig. 4 represent this profile. A similar profile was observed on essential oil of *Eucalyptus nesophila* growing in Australia by Brophy and Southwell (2002), but with the major compound as globulol (68%); α-pinene is the most important hydrocarbon (17%).

Table 5: Levels of main major constituents of essential oils in Class 2 (6 objects out of 9: Class 2)

	α-pinene	β-pinene	β-caryophyllene	Globulol	viridiflorol	Others
SS2*	6.8			9.5		Elemol : 16.5 ; guaiol : 15.5
SS8						
SS9*	3.2	-	-	-	70	-
SP10	15.86	30.39	2.6		29.11	
SP11	32.89	2.29	5.36	2.65	32.28	
SP12	49.06	1.92	2.95		26.62	Viridiflorene : 2.19
SP13	63.14	-		5.81	3.57	
SP14	50.32	-	3.22	3.41	9.86	
SP15	51.03	-	6.52	7.43	9.51	Viridiflorene : 6.67

*: Misclassified objects

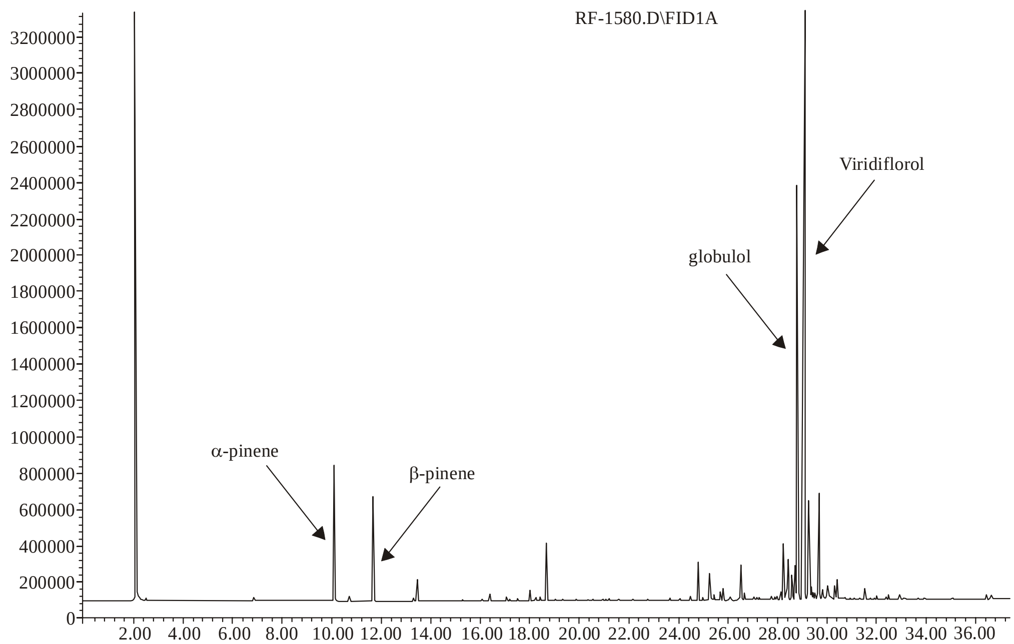


Fig. 5: Chromatographic profil of essential oil from *E. nesophila* rich in sesquiterpene alcohols (globulol and viridiflorol)

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