Production, transformation and essential oils composition of leaves and stems of lemon verbena [*Aloysia triphylla* (L'Herit.) Britton] grown in Portugal

Gomes, P. C. S. [1]**; Oliveira, H. R. C.** [1]**; Vicente, A. M. S.** [2]**; Ferreira, M. F.** [1]

[1]Universidade do Minho, Departamento de Biologia, Escola de Ciências, Largo do Paço 4709 Braga Codex, Portugal. mfferreira@bio.uminho.pt .[2]Direção Regional de Agricultura de Entre Douro e Minho, Quinta de S. José, S. Pedro de Merelim, 4700-859 Braga, Portugal.

ABSTRACT: Production, transformation and essential oils composition of leaves and stems of lemon verbena [*Aloysia triphylla* (L'Herit.) Britton] grown in Portugal. More than seventy compounds, including 13 *n*-alkanes, were identified by GC and GC-MS in essential oils from leaves of lemon verbena [*Aloysia triphylla* (L'Herit.) Britton] plants harvested and analysed with intervals of around two months during a year. In essential oils from stems of corresponding samples, a lower number of compounds were detected, ranging from 46 to 52 depending of the sample. In both cases, geranial, neral and limonene were the main compounds accounting together for 69-72% and 53-69% of the essential oils from leaves and stems respectively. The variation of the essential oil composition in stems was broader than in leaves. In stems, the levels of geranial decreased from around 40% in Sep. to 27% in Nov., increasing thereafter, in inverse correlation with the levels of caryophyllene oxide and *AR*-curcumene. In stems, the level of α-terpineol increased from 1.3% in July to 3.4% in April of the following year while that of methyl citronelate increased from 0.5% in July to 2.9% in Nov. In leaves, the levels of α-terpineol and methyl citronelate kept without apparent variation. In stems, either the variation of the monoterpene hydrocarbons or the variation of the sesquiterpene hydrocarbons was inversed relatively to respective variations in leaves. Limonene and *E*-ß-ocimene were the compounds that mostly contributed for variation of monoterpene hydrocarbons while *E*-caryophyllene, germacrene D, α-zingiberene and *AR*-curcumene were those that mostly contributed for variation of sesquiterpene hydrocarbons.

Key words: vervain, lemon verbena, *Aloysia triphylla* (L'Herit.) Britton, Verbenaceae, essential oils, geranial, neral, limonene.

INTRODUCTION

Lemon verbena [*Aloysia triphylla* (L'Herit.) Britton, *Aloysia citriodora* (Cav.) Ort *Lippia citriodora* (Ort.) HBK, *L. triphylla* O. Kze., *Verbena citriodora* Cav., *V. triphylla* L'Herit.] (Verbenaceae) is traditionally used as folk remedy in treatments of asthma, spasms, cold, fever, flatulence, colic, diarrhoea, indigestion, insomnia, and anxiety (Van Hellemon, 1986, Newal *et al*. 1996, Graça *et al*., 1996) and as source of analgesic, anti-inflammatory and/or anti-pyretic remedies (Pascual *et al*. 2001). The sedative and anxiolytic activities of lemon verbena infusions were not confirmed in clinic trials (Pascual *et al*. 2001, and references therein). However, the analgesic activity was confirmed by pharmacological assays and the respective active compound was isolated by methanol extraction, followed by partitioning with ethyl acetate, and identified as being acteoside (verbascoside) (Nakamura *et al*., 1997). This compound was found as one of the habitual main polyphenolic constituents from the lemon verbena tea, together with some essential oil constituents (Carnat *et al.* 1999). The essential oils are used in the industry of parfumes (Van Hellemon, 1986) and the leaves are used for flavouring of beverages and as seasoning for food preparations (Pascual *et al.* 2001) namely in the United States of America were vervain is listed as Generally Regarded As Safe (GRAS) for human consumption in alcoholic beverages.

This species, native in South America (Chile, Argentina, Uruguai, Peru), was introduced in Europe at the end of XVII century. Since then, it is cultivated in some countries of Southern Europe, namely Spain and Italy, and countries from North Africa, namely Morocco, as well as in India and Tunisia. In Portugal many rural people maintain in small farms or backyards lemon verbena shrubs for own consumption and recently it was included in Portuguese integrated programmes of research and exploitation of aromatic and medicinal plant species (PLANTAMEDI and HERBAROM) in order to meet the needs of the essential oil and extract industries and the need of finding new agrarian cultures as alternatives to the surplus traditional ones. In order to meet the Good Manufacturing Practices on Medicinal Plants, it is intended to submit the raw material and the essential oils to a rigorous quality control based on the respective chemical characterization. The lemon verbena drug has been characterized by the respective essential oils and flavonoids, chiefly 6-hydroxylated flavones (Bruneton, 1999). Flavonoids are good

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chemical markers and some of them have been described (Newal *et al.* 1996) and identified in lemon verbena raw material and derived products (Carnat *et al.*,1999, Skaltsa and Shammas, 1988, Carnat *et al*., 1995, Valentão, *et al*., 1999). However, the most suitable chemical markers may be based in the composition of the essential oils, namely when this species is cultivated and commercialized mainly to be used as aromatic in herbal infusions. In some Pharmacopoeias (*Pharmacopée Française*, 1989), it is recomended that the chemical characterization of lemon verbena is made by the TLC detection of citral (mixture of the isomers neral and geranial). However, neral and geranial are widespread in medicinal and aromatic plants and are among the major compounds in essential oils of different plant species. Therefore, these chemical markers are insufficient to guarantee the genuine *A. triphylla* origin of the lemon verbena raw material or respective essential oil. On the other hand, there is no defined standard quality profile of the *A. triphylla* essential oils. Different lists of essential oil compounds from *A. triphylla* plants of different origins have been reported (Carnat *et al.*, 1999, Bellakhdar and Idrissi, 1994, Özek *et al*., 1996) making difficult the definition of the respective standard of quality. At least in Portugal, the seeds of this species reveal low viability and consequently its propagation is essentially made by vegetative methods, namely by cuttings. This type of clonal propagation favours the maintenance of characteristics own of each cultivar and allows to realize the differences in composition of the essential oils from *A. triphylla* cultivars of different origin. However, to our knowledge the influence of the harvest time and environment factors on the composition of the lemon verbena essential oils were not yet evaluated.

Having in view the establishment of conditions for creation of a certificate of quality of our lemon verbena cultivar, we determined the composition of the respective essential oils obtained by hydrodistillation of separated fresh leaves and stems harvested five times over a year with intervals of around two months. This paper reports and discusses the results from that study in confrontation with the compositions of vervain essential oils reported by other authors.

MATERIAL AND METHOD

Plant cultivation and sampling. Cuttings obtained from lemon verbena stems of two mature plants growing in a land near Viana do Castelo were firstly rooted in 2L pots and then transferred, by December, to an experimental farm from DRAEDM, located at Arouca, and two private demonstration farms located in Covide and Mesio where plants were cultivated. For this study, the lemon verbena was cultivated in four 50.4 m2 ground plots with the plants separated 70 cm in the same line and 150 cm between lines. Around seven months after (July), branches of these young cultivated plants were randomly harvested in each plot and the respective leaves were separated from stems. The leaves and stems were independently subjected to hydrodistillation and the respective hydrodistillates were independently analysed. This procedure was repeated in the following months of September and December of the same year, and April and June of the following year.

Hydrodistillation and analysis procedure. The fresh biomass samples were submitted to hydrodistillation in a Clevenger type apparatus over 1 hr, using volumes of 1.0 ml of n-hexane for retention of the hydrodistillate components. The hydrodistillates from all samples were analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC analyses were performed using a Perkin Elmer Autosystem gas chromatograph equipped with a fused silica DB-5 column (30 m long x 0.25 ID, 0.25 μm film thickness composed by 5%-phenyl methylpolysiloxane, J & W Scientific. Temperature programme: 60-280º at 3º min^{-1} for column, 300º for injector and 320º for flame ionization detector (FID). H_2 was used as carrier gas at flow rate of 1.49 ml/min under a column head pressure of 12.5 psi. Injections (0.5 μL) were performed in a split/splitless injector, in the split mode, with the splitter opened at the 1:13 split ratio. Three replicates of each sample were processed in the same way. Percentage values from the listed compounds correspond to the values given in the GC report without correction factors.

GC-MS analyses were performed with a Perkin-Elmer 8500 gas chromatograph equipped with a fused silica DB-5 as that of GC, connected with a Finnigan MAT Ion Trap Detector (ITD; software version 4.1) operating in electron impact (EI) mode at 70eV. Injector, interface and ion-source temp. were 300º, 260º and 220º respectively. The oven temp. program and injection conditions were as above described for GC. Helium was used as carrier gas with a column head pressure of 12.5 psi.

The compounds were identified with basis of the respective mass spectra and retention times in confrontation with those in computer libraries of terpenes and by comparison of their DB-5 retention indexes with those in the literature (14). The identification of compounds available in the market was confirmed by GC-MS injection of the respective standards. The retention indexes of the compounds were determined relatively to n-alkanes of a complete series, from the n-octane to the n-tetratriacontane, eluted in the same conditions as the essential oil samples.

RESULT AND DISCUSSION

The growth of the first lemon verbena thorns occurred by the end of March, 1999, around fifteen weeks after the begining of the cultivation of the respective rooted cuttings. Sixteen weeks later, the first pruning of the young plants, performed at around 15 cm over the ground, gave a fresh biomass yield per plant of 333g, corresponding to a dry weight of 75g/plant.

The separated hydrodistillation of leaves and stems from the harvested fresh biomass samples, constituted by lemon verbena branches of ~30 cm long and destined to essential oils study, gave essential oil yields of 0.8% (w/w) for leaves and 0.08% (w/w) for stems, in a basis of biomass dry weight. The essential oil yield of *A. triphylla* leaves from this study is within the range of values reported for this species by different authors: 0.18% (12), 0.2% (15), 0.2-0.4% (3), 0.2-1.0% (6), 1.1-1.2% (13). The lower essential oil yield obtained from stems was expected due to its woody tissue.

The GC-MS analysis of the hydrodistillates from leaves revealed a complex mixture of compounds, 73 of which were identified (Table 1). This number includes 13 *n*-alkanes which correspond to the last peaks of the respectives chromatograms. A lower number of compounds (46 to 52) were found and identified in hydrodistillates from stems, all of them included in the compound lists of essential oils from leaves (Table 1). Depending of the harvest time, the sum of the GC peak areas of the identified compounds corresponded to 95-98% and 93-98% of the sum of GC peak areas of all compounds from essential oils of leaves and stems respectively. With the exception of *cis*-thujone and germacrene A (in this case, sample of Sept.) the lemon verbena essential oil compounds, not detected in stems, kept at trace amounts (<0.05%) in the essential oils from almost all samples of the respective leaves. Different compositions have been described in reports from previous studies on essential oils from lemon verbena, at least in what concerns the lower to medium represented compounds. The same is true in what respects the total number of lemon verbena essential oil constituents. In a review on the genus *Aloysia* the essential oil from *A. triphylla* was characterized by the presence of citral A (37.15%), citral B (22.34%), 1,8-cineole (15.43%), geraniol (10.6%), as main constituents, besides other eleven minor compounds which included neral and geranial (Terblanché and Kornelius, 1996). According to some authors, the essential oils from *A. triphylla* leaves of commercial origin (Chile) contain, as main constituents, geranial (23.5%), neral (17.6%), limonene (12.8%), caryophyllene oxide (6.3%), cineole (5.7%), citronellol (5.3%) and spathulenol (4.0%), besides other eight minor ones (Carnat *et al*., 1999). In the essential oils from air-dried plants (small stems, leaves, and flowers) of *A. triphylla* of Moroccan origin and cultivated near Agadir, a total of 61 compounds were identified (Özek *et al*., 1996). The major essential oil compounds from those Moroccan lemon verbena plants were 1,8-cineole (12.4%), geranial (9.9%), 3-methyl-5-hepten-2-one (7.4%), neral (6.9%), caryophyllene oxide (5.5%), spathulenol (5.2%), α-curcumene (4.6%), limonene (3.7%), α-terpineol (2.3%), and geranyl acetate (1.6%). A higher number of compounds (69) were identified in essential oils obtained, by hydrodistillation, of dried leaves and leafy branches of *A. triphylla* plants grown in Turkey (Özek *et al*., 1996). In this case, the major compounds were limonene (14.8-18.6%), geranial (11.9-19.1%), 1,8-cineole (6.8-10.1%), neral (6.0-8.1%), *AR*-curcumene (4.9-5.7%), caryophylene oxide (3.1-4.9%), spathulenol (3.9-4.3%), *E*-caryophyllene (3.5-4.3%), sabinene (2.8-2.5%), and α-terpineol (2.0-2.0%). Comparing the list of the essential oil composition from the vervain plants grown in Morocco with that from the lemon verbena plants grown in Turkey, only 34 compounds are common to both lists. Despite we have searched for the constituents previously reported, only 33 compounds identified by us (Table 1) are common to the composition determined for Moroccan lemon verbena plants (Bellakhdar and Idrissi, 1996), 45 are common to the composition determined for those grown in Turkey (Özek *et al*., 1996), and only 29 compounds are common to both composition lists reported by the referred authors. On the other hand, from the 15 compounds identified in the essential oils from dried lemon verbena leaves of commercial plants from Chile (Carnat *et al*., 1999) we found only 10, lacking the alcohols citronellol, nerol, geraniol, and *cis*-nerolidol, and the ester α-terpinyl acetate, reported by these authors. The composition of the essential oils of all five vervain samples from our study reveals the highest levels of geranial and neral with regard to data published in the literature (Carnat *et al*., 1999, Bellakhdar and Idrissi, 1994, Özek *et al*., 1996 and references therein). On the other hand, in previous reports no mention has been done to the presence of *n*-alkanes. In virtue of all differences of composition here described, it seems clear that the composition of the essential oils of *A. triphylla* is subject to more variation than has been generally accepted. This point of view had been already pointed out by other authors (Bellakhdar and Idrissi, 1994).

TABLE 1. Percentage composition of essential oils from leaves and stems from cultivated plants of *Aloysia triphylla* grown in DRAEDM experimental farm located at Arouca and harvested five times over a year.

Compound	R. I.	July 20, 1999		Sept. 14, 1999		Nov. 17, 1999		April 17, 2000		Jun. 7, 2000	
		Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
2-Hexenal	846	tr	-	tr	-	tr	-	tr	-	tr	-
α-Thujene	922	tr	-	tr	-	tr	-	tr	-	0.1	-
α-Pinene	929	0.2	0.1	0.2	0.1	0.3	0.4	0.3	0.2	0.5	0.3
β-Citronellene	938	tr	-	tr	0.4	tr	-	tr	-	tr	-
Sabinene	968	1.1	0.2	0.8	0.8	0.7	0.4	0.7	0.5	1.1	0.6
β- Pinene	973	0.5	0.4	0.6	1.6	0.9	1.5	0.7	0.5	0.5	2.2
6-Methyl-5-hepten-2-one	981	0.3	0.3	0.3	0.3	0.7	0.4	0.4	0.3	0.4	0.3
Myrcene	986	0.3	0.1	0.2	0.2	0.2	0.4	0.2	0.2	0.2	0.6
3-Octanol	994	tr	0.2	tr	0.3	tr	1.4	tr	0.1	tr	0.9
α-Terpinene	1014	tr	-	tr	-	tr	-	tr	-	tr	-
p-Cymene	1020	tr	-	tr	-	tr	-	tr	-	tr	-
Limonene	1022	9.6	3.3	5.6	5.4	7.3	3.2	7.3	4.1	9.8	4.9
1,8-Cineole	1031	0.1	tr	0.1	0.2	0.2	0.4	0.2	0.1	0.1	0.3
E-β-Ocimene	1042	2.0	0.7	2.6	2.4	2.7	1.5	3.1	1.8	1.5	0.9
y-Terpinene	1052	tr	-	tr	-	tr	-	tr	-	tr	-
cis-Sabinene hydrate	1062	0.3	0.2	0.2	0.1	0.2	0.4	0.1	0.2	0.2	tr
Terpinolene	1082	tr	-	tr	-	tr	-	tr	-	tr	-
Eucarvone (?)	1091	0.1	tr	tr	-	0.1	tr	tr	-	0.1	tr
Linalool	1095	0.3	0.7	0.3	1.1	0.3	0.4	0.3	0.5	0.3	0.6
cis-Thujone	1102	0.2	-	0.1	-	0.4	-	0.2	-	0.4	-
cis-Limonene oxide	1127	0.2	0.1	0.1	tr	0.3	tr	0.2	0.1	0.3	0.3
trans-Limonene oxide	1132	0.7	0.5	0.5	0.3	1.0	0.8	0.7	0.4	1.1	0.9
Citronellal	1147	0.5	0.4	0.6	0.3	0.9	0.4	0.6	0.4	0.7	0.6
4-Terpineol	1172	tr	-	tr	-	0.1	-	tr	-	tr	-
α-Terpineol	1189	0.3	1.3	0.1	1.8	0.2	3.0	0.3	3.4	-	1.5
Neral	1242	25.8	26.6	26.2	24.4	27.4	23.0	25.7	26.2	25.7	24.6
Methyl citronellate	1254	0.2	0.5	0.1	1.6	0.2	2.9	0.4	2.4	0.2	0.6
Geranial	1273	36.3	37.3	38.5	39.6	35.0	26.6	36.2	38.2	33.7	30.3
ð-Elemene	1334	0.3	0.2	0.8	0.5	0.4	0.1	0.9	0.5	0.6	0.3
Phenyl ethyl propanonate	1348	tr	-	tr	-	tr	-	tr	-	tr	-
Eugenol	1354	0.1	0.6	0.1	0.2	0.1	tr	tr	tr	tr	-
Neryl acetate	1361	0.2	0.2	0.2	0.1	0.3	0.4	0.2	0.1	0.3	0.3
α-Copaene	1371	tr	0.2	tr	-	tr	0.4	tr	-	tr	0.3
Geranyl acetate + β-Bourbonene	1380+1381	0.9	1.2	1.1	1.4	1.3	1.3	1.3	1.8	1.2	0.9
Methyl eugenol	1401	tr	-	tr	-	tr	-	tr	-	tr	-
α-Cedrene	1410	0.1	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.2	0.3
E-Caryophyllene	1415	2.3	2.5	2.1	1.3	1.8	2.1	1.5	1.1	2.5	2.3
β-Gurjunene	1424	0.1	tr	0.1	tr	0.1	tr	0.1	tr	0.1	tr
Aromadendrene	1439	tr	-	tr	-	tr	-	tr	-	tr	-
α-Humulene	1448	0.2	0.2	0.1	0.1	0.1	tr	0.1	tr	0.2	tr
E-β-Farnesene	1452	0.2	0.3	0.3	0.2	0.3	0.4	0.2	0.2	0.3	0.3
allo-Aromadendrene	1455	tr	-	tr	-	tr	-	tr	-	tr	-
Geranyl *n*-propanonate	1471	tr	tr	tr	tr	tr	0.3	tr	tr	0.1	0.1
Germacrene D	1475	2.7	3.2	3.9	2.6	2.0	1.7	3.1	2.4	2.8	2.0
AR-Curcumene	1479	1.0	2.3	0.6	1.3	1.0	6.1	0.8	1.4	1.7	3.5
α-Zingiberene	1490	2.8	3.1	4.4	3.7	3.0	1.9	5.7	3.4	3.7	2.3
Germacrene A (?)	1492	tr	-	1.1	-	tr	-	tr	-	tr	-
α-Muurolene	1495	tr	-	tr	-	0.1	-	tr	-	tr	-
β-Bisabolene	1504	tr	0.1	tr	0.1	tr	tr	tr	tr	0.5	0.3
y-Cadinene	1507	1.0	1.9	1.0	1.4	0.6	1.5	0.9	1.4	1.1	2.0
ð-Cadinene	1528	0.2	0.4	0.1	0.3	0.1	tr	0.1	0.3	0.1	0.3
(*1s,trans*)-Calamenene	1528	tr	-	tr	-	tr	-	tr	-	tr	-
E-Nerolidol	1564	tr	-	tr	-	tr	-	tr	-	tr	-
4-β-Hydroxygermacra-1(10),5-Diene	1574	1.1	0.9	1.1	0.7	1.3	1.5	1.2	1.1	1.0	0.6
Spathulenol	1578	0.1	0.1	0.1	0.1	3.3	0.1	0.8	-	2.2	-
Caryophyllene oxide	1582	2.5	2.7	1.6	1.3	3.3	5.1	0.8	1.6	2.2	2.6
epi-á-Cadinol + *epi*-á-Muurolol	1638+1639	0.2	0.3	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.3
α-Cadinol	1653	0.3	0.2	0.2	0.3	0.3	0.8	0.2	0.4	0.2	0.6
n-Heptadecane	1700	tr	tr	tr	-	tr	tr	tr	-	tr	tr
n-Octadecane	1800	tr	tr	tr	-	tr	-	tr	-	tr	-
n-Nonadecane	1900	tr	0.5	tr	0.5	tr	0.4	tr	0.3	tr	0.9
n-Eicosane	2000	tr	-	tr	-	tr	-	tr	0.2	tr	tr
n-Heneicosane	2100	tr	0.1	0.1	-	0.1	-	0.1	-	0.1	0.2
n-Docosane	2200	tr	-	tr	-	tr	tr	tr	-	tr	-
n-Tricosane	2300	tr	-	tr	-	tr	-	tr	-	tr	0.1
n-Tetracosane	2400	tr	-	tr	-	tr	0.1	tr	-	tr	-
n-Pentacosane	2500	tr	0.1	tr	0.3	tr	1.6	tr	0.1	-	-
n-Hexacosane	2600	tr	0.1	0.1	tr	tr	0.6	0.1	0.2	tr	0.2
n-Octacosane	2800	0.1	1.3	0.1	0.5	0.1	2.5	0.1	0.4	0.1	1.5
n-Nonacosane	2900	tr	0.3	tr	tr	tr	0.2	tr	tr	tr	0.2
n-Triacontane	3000	tr	0.2	tr	tr	tr	0.4	tr	-	tr	tr

Most of the compounds from the lemon verbena essential oil can be distributed by five main compound groups: monoterpene hydrocarbons, oxygen-containing monoterpenes, sesquiterpene hydrocarbons, oxygen-containing sesquiterpenes and *n*-alkanes (Table 2). The greatest change in composition of essential oils occurred in samples of stems harvested in November 1999. The level of oxygen-containing monoterpenes in those samples decreased 15-18% and the sub-group of aldehydes 22-23%. The compound which most contributed for that variation was geranial whose level decreased from 39.6%, in Sept., to 26.6%, in November (Table 1). The levels of the oxygen-containing monoterpene group in essential oil from leaves kept without significant change over the year (64-68%) as well as its main sub-groups namely, aldehydes (60-65%), alcohols (0.5-1.0%), and esters (0.9-1.3%) (Table 2). The decrease in the levels of oxygen-containing monoterpenes in essential oils from stems recorded in Nov. 1999, relatively to Sept. 1999 and April 2000, was associated with increased levels of oxygen-containing sesquiterpenes and sesquiterpene hydrocarbons (Table 2). The compounds that most contributed for the variations in the levels of these groups were caryophyllene oxide and *AR*-curcumene respectively (Table 1). In December 1999, around half of the the lemon verbena cultivated plants were contaminated by fungi namely, *Cylindrocarpon* spp. and *Verticillium* spp., with visible alteration of the stem skin, which showed multiple fissures. In virtue of that contamination, it is possible that the changes in essential oill composition from the stems of the samples harvested in November was affected by fungi infection, not yet visible at that time, but eventually present in an initial phase. All the contaminated plants were pulled out and the remaining ones were kept apparently healthy over the following harvests. However, this study revealed other differential changes in composition of the essential oils from leaves and stems, which apparently are not related with fungi contamination. Those are the cases of the sesquiterpene hydrocarbons and monoterpene hydrocarbons whose level variations in stems were inversed relatively to respective variations in leaves. *E*-caryophyllene, germacrene D, α-zingiberene and *AR*-curcumene were the compounds that mostly contributed for variation of sesquiterpene hydrocarbons. In leaves, the level of monoterpene hydrocarbons decreased 38% from July to Sept. 1999 rising thereafter, inversely to what ocurred in the stems. The compounds that mostly contributed for variation of monoterpene hydrocarbons were limonene and *E*-β-ocimene. On the other hand, the level of the alcohols sub-group in stems rose from July 1999 to April 2000 decreasing thereafter. Similar change occurred in stems with the sub-group of esters whose level increased from July 1999 to Nov. 1999, decreasing since then up to June 2000. The compounds which most contributed for variations in the levels of monoterpenols and monoterpene esters were α-terpineol, and methyl citronelate respectively (Table 1). The levels of these two sub-groups, in the leaves, kept basically without change (Table 2).

TABLE 2. Percentage composition of the main essential oil compound groups from leaves and stems from cultivated plants of *Aloysia triphylla* grown in DRAEDM experimental farm located at Arouca and harvested five times over a year.

	July 20, 1999		Sept. 14, 1999		Nov. 17, 1999		April 17, 2000		Jun. 7, 2000	
Compound group	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Monoterpene hydrocarbons	13.7	4.8	8.5	10.9	12.1	7.4	12.3	7.3	13.7	9.5
Oxygen-containing monoterpenes:	65.8	69.0	67.7	70.4	67.4	59.7	65.9	72.9	64.0	60.6
- oxides	1.3	0.6	0.8	0.5	2.0	1.2	1.4	0.6	2.2	1.5
- alcohols	1.0	2.8	0.7	3.2	0.9	3.8	0.7	4.1	0.5	2.1
- aldehydes	62.6	64.3	65.3	64.3	63.3	50.0	62.5	64.8	60.1	55.5
- esters	0.9	1.3	0.9	2.4	1.2	4.7	1.3	3.4	1.2	1.5
Sesquiterpene hydrocarbons	12.4	16.1	16.2	13.0	10.9	16.7	15.3	12.8	15.4	14.9
Oxygen-containing sesquiterpenes	3.1	3.3	2.0	1.8	7.0	6.2	1.9	2.2	4.7	3.5
n-Alkanes	0.1	2.6	0.3	1.3	0.2	5.6	0.3	1.2	0.2	3.1
Others	4.9	4.2	5.3	2.6	2.4	4.4	4.3	3.6	2.0	8.4

Despite the variations in the levels of some compounds and compound groups revealed in this study, the differences in composition relatively to data previously published by the several authors kept basically the same (Table 1) excluding the seasonal factors as source of the main variability. As some authors already suggested (Bellakhdar and Idrissi, 1994), it is possible that the main source of variability in the essential oil composition of *A. triphylla* is in keeping with the origin of cultivar. This hypothesis, is supported by data on essential oils from the same cultivar established in another site located around 250 km far from Arouca whose composition profile is simillar to those of Table 1 (data not shown). The confrontation of our results with those reported in the literature allows to realize the absence of a general standard of quality defined and accepted for the essential oil of *A. triphylla*. However, the definition of a standard of quality reflecting the origin of the cultivar, based in the percentage composition of the most

representative constituents of the essential oils, would be useful in the commercialization of lemon verbena with the respective certificate of origin.

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