



Anais da Academia Brasileira de Ciências

ISSN: 0001-3765

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Academia Brasileira de Ciências

Brasil

Nascimento, Jeferson C.; Barbosa, Luiz C.A.; Paula, Vanderlucia F.; David, Jorge M.; Fontana, Renato; Silva, Luiz A.M.; França, Robson S.

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Anais da Academia Brasileira de Ciências, vol. 83, núm. 3, septiembre, 2011, pp. 787-799

Academia Brasileira de Ciências

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Chemical composition and antimicrobial activity of essential oils of *Ocimum canum* Sims. and *Ocimum selloi* Benth.

JEFERSON C. NASCIMENTO¹, LUIZ C.A. BARBOSA², VANDERLUCIA F. PAULA¹, JORGE M. DAVID³, RENATO FONTANA⁴, LUIZ A.M. SILVA⁴ and ROBSON S. FRANÇA¹

¹Departamento de Química e Exatas, Universidade Estadual do Sudoeste da Bahia, UESB, Rua José Moreira Sobrinho s/n, 45206-190 Jequié, BA, Brasil

²Departamento de Química, Universidade Federal de Viçosa, UFV, Avenida Peter Henry Holfs s/n, 36571-000 Viçosa, MG, Brasil

³Instituto de Química, Universidade Federal da Bahia, UFBA, Rua Barão de Geremoabo s/n, 40170-290 Salvador, BA, Brasil

⁴Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, UESC, Km 16 Rodovia Ilhéus-Itabuna 45662-000 Ilhéus, BA, Brasil

Manuscript received on December 9, 2009; accepted for publication on January 1, 2011

ABSTRACT

This work describes the chemical composition and antimicrobial activity of the volatile oils of *Ocimum canum* and *Ocimum selloi*, both occurring in Jequié/BA, northeastern Brazil. The plants were collected in the winter/2005 and summer/2006, the oils extracted by steam distillation and further analyzed by GC-MS. A total of 30 and 31 compounds was identified from the oils of *O. selloi* and *O. canum*, respectively. It was observed that the oil content of *O. canum* showed variation during the seasons, while the oils of *O. selloi* did not. Methylchavicol and linalool were the main chemical components found in the aerial parts and leaves of *O. canum*. This finding permitted to characterize this specimen as a new chemotype of *O. canum*. Regarding the aerial parts of *O. selloi*, eugenol, 1,8-cineole, *trans*-caryophyllene and linalool were identified as their major components. All extracted oils from the aerial parts showed biological activity against gram-positive cocci – *Staphylococcus aureus* ATCC 25923 – but only the *O. canum* one showed activity against gram-negative bacilli – *Escherichia coli* ATCC 25922.

Key words: antimicrobial activity, essential oil, Lamiaceae, *Ocimum canum*, *Ocimum selloi*.

INTRODUCTION

The world trade for essential oils has been growing approximately 11% a year (Bizzo et al. 2009). In Brazil, the exports of essential oils have shown a significant increase in recent years, reaching US\$93,000,000 in 2008 (ALICE-Web System 2009). Among the species producing essential oils, those of the genus *Ocimum* have a small, but important contribution on the total exports.

The genus *Ocimum* L. (Lamiaceae) comprises about 160 species distributed in tropical and subtropi-

cal Africa, Asia and South America (Gupta 1994), many of which are used in folk medicine, as spices and also to control insects (Grayer et al. 1996). Moreover, there is an economic exploitation by large-scale extraction of essential oils. *Ocimum selloi* is popularly known as “elixir paregórico”¹ in the states of Rio de Janeiro and Espírito Santo, and as “anis” or “alfavaquinha” in Minas Gerais. These popular terms are related to its pharmacological properties and also to its chemical similarity to other species of *Ocimum*.

The volatile oils of *O. selloi* present antidiarrheal, antispasmodic and anti-inflammatory activities confirmed in preclinical testing (Vanderlinde et al. 1994).

Correspondence to: Jeferson C. Nascimento
E-mail: jefersonchag@gmail.com

¹ It is a folk name. This “elixir” does not contain opium.

Besides, there are many other biological activities reported for the volatile oils produced by this genus, such as antimicrobial (Prasad et al. 1986, Nakamura et al. 1999, Farago et al. 2004, Bassole et al. 2005), insecticide (De Paula et al. 2003, Paula et al. 2004), antioxidant (Ganiyu 2008) and analgesic (Franca et al. 2008).

The essential oils of *Ocimum* are composed by compounds such as estragole, eugenol, methyleugenol, citral, linalool, geraniol and thymol. These compounds are required as raw materials for the pharmaceutical, comestics and food industries, and as insecticides (Craiveiro and Queiroz 1993, Gupta 1994, Bizzo et al. 2009).

As a continuation of our studies on the composition of aromatic and medicinal plants cultivated in different parts of Brazil (Barbosa et al. 2005, Fonseca et al. 2006, Silva et al. 2007a), we describe in this paper the results of our investigation on the chemical composition and antimicrobial activities of the volatile oils produced by *O. canum* Sims and *O. selloi* Benth.

MATERIALS AND METHODS

PLANT MATERIAL

The species were collected in August 2005 and January 2006 at 7:00 a.m. in the Garden of Medicinal Plants of the Universidade Estadual do Sudoeste da Bahia (UESB). Specimens were prepared, identified and deposited in the Herbarium of the Universidade Estadual de Santa Cruz (Ilhéus-BA, Brazil) under 4385 and 4387 numbers for *O. canum* and *O. selloi*, respectively.

EXTRACTION AND CHEMICAL ANALYSES OF THE VOLATILE OILS

For each species, 100 g of fresh plant material from the aerial parts (leaves and fine stems) and from leaves, separately, were chopped and then subjected to a hydrodistillation in a Clevenger (1.5 h). The resulting oils were dried over anhydrous sodium sulphate (Merck), weighed and the reported yields calculated with respect to the fresh material weight. All distillations were repeated three times and the obtained oils were stored under nitrogen atmosphere and maintained at approximately 0°C, until their analysis.

GC analyses were accomplished with a GC-17A Series instrument (Shimadzu, Japan) equipped with a flame ionization detector (FID). Chromatographic con-

ditions were: fused silica capillary column (30 m × 0.22 mm) with a DB-5 bonded phase (0.25 µm film thickness); carrier gas, N₂ at a flow rate of 1.8 mL/min; injector temperature 220°C, detector temperature 240°C; column temperature was programmed to start at 40°C (isothermal for 2 min), with an increase of 3°C/min, to 240°C, isothermal at 240°C for 15 minutes; injection of 1.0 µL (1% w/v in CH₂Cl₂); split ratio 1:10; column pressure of 115 kPa.

The compounds were identified using a GC-MS unit (model GCMS-QP5050A, from Shimadzu, Japan) equipped with a DB-5 fused silica column (30 m × 0.22 mm i.d., film thickness 0.25 µm) and interfaced with an ion trap detector. Oven and injector temperatures were as described above; transfer line temperature, 240°C; ion trap temp., 220°C; carrier gas, He at a flow rate of 1.8 mL/min; split ratio 1:10; column pressure of 100 kPa; ionization energy, 70 eV; scan range, 29-450 u; scan time, 1s. The components were characterized by the comparison of their retention indexes (RI) relative to a standard alkane series (C₉-C₂₄), and also by the comparison of their mass spectrum with reference data from either the equipment database (Wiley 7 library) or literature (Adams 1995).

ANTIBACTERIAL ACTIVITY

The antimicrobial activity tests on the oils were performed by the method of diffusion in agar previously described (Bauer et al. 1966, Casteels et al. 1993, Fontana et al. 2004). The oils were tested against gram-positive and gram-negative species, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), respectively. A suspension of the tested microorganism (0.5 of MacFarland Scale) was spread on Petri plates with Mueller Hinton agar (Difco Laboratories). A 2 mm hole diameter was opened on the surface of inoculated plates to which were added 10 µL of an essential oil. The plates were incubated at 37°C for 24 hours. After this period of time, the diameters of the inhibition zones were measured using a caliper rule and expressed in millimeters.

RESULTS AND DISCUSSION

The essential oils of aerial parts (leaves and stems) of *O. canum* and *O. selloi* collected in the summer yielded

$0.12^2 \pm 0.002\%$ (w/w) and $0.16 \pm 0.003\%$ (w/w), respectively, while the oils obtained from the winter specimens yielded $0.06 \pm 0.010\%$ and $0.18 \pm 0.004\%$, respectively. The leaves of *O. canum*, when submitted to extraction, yielded $0.16 \pm 0.003\%$ of oil in the summer and $0.08 \pm 0.006\%$ in the winter, and the ones of *O. selloi* $0.18 \pm 0.004\%$ in the summer and $0.20 \pm 0.005\%$ in the winter.

The yield of volatile oils extracted from the aerial parts and leaves of *O. canum* in the summer is doubled compared to the winter, in agreement with the results obtained for *O. basilicum* L. (Silva et al. 2005). These results suggest that the seasonality may be one of the factors that should be considered when dealing with a possible economic exploitation. Other possible factors related to this variation can be rainfall, climate and leaf development (Gobbo-Neto and Lopes 2007). For the content of the essential oil of *O. selloi*, a significant variation on the yielding values for leaves and aerial parts in the two samples was not observed. Similar results were previously described by Moraes et al. (2002). Thus, these findings can suggest that, for this species, the seasonality is not a factor that could influence in the amount of essential oils produced.

The chemical composition of the essential oils of the aerial parts and leaves obtained in the winter of 2005 and in the summer of 2006 for *O. canum* is formed by methylchavicol and linalool, respectively, as its major constituents (Table I, Figures 2, 3, 6 and 10). *O. canum* is known to present different chemotypes with the volatile oils constituted mainly of linalool and limonene (Ngassoum et al. 2004, Ravid et al. 1997), methylcinnamate and caryophyllene (Murillo and Viña 2003, Martins et al. 1999), fenchone (Lawrence et al. 1980), eugenol (Ekundayo et al. 1989), 1,8-cineole (Bassole et al. 2005), citral (Choudhary et al. 1989), terpinen-4-ol (Sanda et al. 1998) and camphor (Chagonda et al. 2000). Therefore, according to the present study, it can be suggested that the plant under investigation may constitute a new chemotype methylchavicol and linalool. However, proposing the existence of a new chemotype has to be taken carefully since the chemical composition of the volatile oils can vary with the sea-

son, plant origin, plant age and soil composition, among other factors (Castro et al. 2004, Martins et al. 2006, 2007, Barbosa et al. 2007, 2008). In the present investigation, the influence of the harvest season on the composition of volatile oils is also observed. Specifically in the case of linalool content, a significant increase in aerial parts was observed in the winter compared to the summer.

Eugenol, 1,8-cineole, *trans*-caryophyllene and linalool were found to be major chemical constituents of essential oils of aerial parts from *O. selloi* (Table II, Figures 1, 4, 5, 6, 7, 8 and 9) in the two seasons. Therefore, it suggests that the access of *O. selloi* belongs to the eugenol chemotype (Farago et al. 2004, Paula et al. 2004). There are other chemotypes reported for this plant, such as estragole and methyleugenol (Martins et al. 1997, De Paula et al. 2003, Franca et al. 2008), *trans*-anethole (Moraes et al. 2002) and elemicine (David et al. 2006). It is noteworthy that, despite the literature describes the existence of an eugenol chemotype, as far as we know there are no GC nor GC-MS analyses reported for this variety. The results reported in the present investigation show small differences in the eugenol, 1,8-cineole, *trans*-caryophyllene and linalool contents present within the volatile oils from the aerial parts extracted during the winter 2005 and the summer 2006. Chromatograms of essential oils of *Ocimum selloi* and *O. canum* analyzed by GC/MS and mass spectra of major compounds identified in these oils, with the Retention time (Rt) of these compounds in the respective oils are presented in the Figures 1-10.

The results presented above should be analyzed carefully because, as previously discussed, individuals of the same species may contain many different volatile compounds. These variations are related to environmental factors such as temperature, soil type, moisture, climate, height and factors intrinsic to the plant, as its pathological condition and age (Barbosa et al. 2007, Martins et al. 2006, Silva et al. 2007b). The content of metabolites may also vary depending on the extraction method used (Charles and Simon 1990, Silva et al. 2004).

The essential oil of the aerial parts of *O. selloi* showed an 8 mm diameter of inhibition zone against

² Mean of three replications \pm SD.

TABLE I
Chemical constituents of essential oil of *Ocimum canum* Sims.

Compound	RI Cal.	RI Lit.	Percentage of area on			
			Aerial parts		Leaves	
			August 2005	February 2006	August 2005	February 2006
α -pinene	931	939	Tr	0.20 \pm 0.02	—	tr
Camphene	944	953	Tr	tr	—	tr
β -pinene	972	980	Tr	0.32 \pm 0.07	tr	tr
β -myrcene	989	991	tr	0.18 \pm 0.05	tr	tr
α -terpinene	1011	1018	tr	tr	tr	tr
1,8-cineole	1029	1033	3.47* \pm 4.39	4.98 \pm 0.37	2.45 \pm 0.61	2.51 \pm 2.01
γ -terpinene	1056	1062	tr	0.21 \pm 0.04	tr	0.11 \pm 0.03
Fenchone	1082	1087	tr	tr	tr	0.10 \pm 0.20
Linalool	1098	1098	33.07 \pm 2.08	26.74 \pm 1.85	30.81 \pm 2.45	23.93 \pm 4.00
Camphor	1138	1143	0.19 \pm 0.05	0.41 \pm 0.05	0.19 \pm 0.08	0.36 \pm 0.04
Borneol	1164	1165	0.20 \pm 0.01	0.17 \pm 0.02	0.18 \pm 0.04	0.13 \pm 0.02
<i>trans</i> - β -terpineol	1163	1163	0.20 \pm 0.01	0.17 \pm 0.02	0.18 \pm 0.04	0.13 \pm 0.02
terpinen-4-ol	1174	1177	1.56 \pm 0.01	1.64 \pm 0.09	1.53 \pm 0.18	1.75 \pm 0.30
α -terpineol	1189	1189	0.93 \pm 0.14	0.78 \pm 0.13	0.82 \pm 0.13	0.88 \pm 0.22
methylchavicol	1195	1195	46.38 \pm 7.50	52.71 \pm 8.59	56.78 \pm 4.94	63.34 \pm 5.35
<i>endo</i> fenchyl acetate	1215	1220	0.25 \pm 0.01	0.21 \pm 0.03	tr	0.19 \pm 0.05
bornyl acetate	1280	1285	0.99 \pm 0.05	0.64 \pm 0.18	0.54 \pm 0.14	0.56 \pm 0.20
Eugenol	1356	1356	2.28 \pm 1.98	2.60 \pm 2.04	0.68 \pm 0.05	0.23 \pm 0.10
β -elemene	1388	1391	0.35 \pm 0.13	0.29 \pm 0.26	tr	0.13 \pm 0.03
<i>trans</i> -caryophyllene	1414	1418	0.79 \pm 0.75	0.50 \pm 0.38	0.26 \pm 0.02	0.12 \pm 0.01
<i>trans</i>-α-bergamotene	1432	1436	2.86 \pm 0.37	1.70 \pm 0.27	1.77 \pm 0.40	1.61 \pm 0.45
α -humulene	1448	1454	tr	tr	tr	tr
germacrene D	1474	1480	0.90 \pm 0.09	0.52 \pm 0.14	0.44 \pm 0.18	0.44 \pm 0.14
bicyclogermacrene	1491	1494	0.60 \pm 0.42	0.60 \pm 0.42	0.27 \pm 0.08	0.18 \pm 0.05
germacrene A	1499	1503	0.51 \pm 0.33	0.54 \pm 0.29	0.25 \pm 0.12	0.23 \pm 0.07
γ -cadinene	1507	1513	0.69 \pm 0.24	0.38 \pm 0.10	0.52 \pm 0.15	0.44 \pm 0.15
δ -cadinene	1516	1524	0.78 \pm 0.11	tr	—	0.13 \pm 0.04
germacrene B	1550	1556	tr	tr	tr	—
Spathulenol	1571	1576	tr	tr	tr	—
Cedrol	1606	1596	0.35 \pm 0.06	0.22 \pm 0.09	0.27 \pm 0.13	0.24 \pm 0.08
Cadinol	1635	1640	2.49 ^a \pm 0.34	1.18 ^a \pm 0.53	1.99 \pm 0.86	1.59 \pm 0.56
Total			96.68	98.04	99.93	99.33

*All values reported as an average of three replicates \pm SD; tr – trace compound (less than 0.10%); RI Cal. – retention indices calculated; RI Lit. = Adams 1995.

Staphylococcus aureus, and it did not show inhibition against *Escherichia coli*. The oil of *O. canum* showed 9 mm of inhibition for both microorganisms. For the negative control it was not observe any inhibition halo. As stated, the oil of *O. canum* presented, by the method of agar diffusion capacity, a 9 mm inhibition zone and,

thus, it may be considered active against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). These findings are consistent with those previously reported (Bassole et al. 2005, Janssen et al. 1989). It was also possible to observe that the oil of *O. selloi* showed antibacterial activity against *S. aureus* (ATCC 25923).

TABLE II
Chemical constituents of the essential oil of *Ocimum selloi* Benth.

Compound*	RI Cal.	RI Lit.	Percentage of area on	
			Aerial parts	
			August 2005	February 2006
α -pinene	931	939	0.25* \pm 0.20	0.26 \pm 0.20
camphene	944	953	tr	tr
sabinene	970	976	0.25 \pm 0.13	0.22 \pm 0.16
β -pinene	972	980	1.02 \pm 0.55	1.01 \pm 0.50
β -myrcene	989	991	0.40 \pm 0.15	0.34 \pm 0.20
1,8-cineole	1029	1033	18.68 \pm 6.02	21.02 \pm 8.61
(Z)- β -ocimene	1039	1040	4.52 \pm 0.31	4.00 \pm 2.08
(E)- β -ocimene	1048	1050	0.34 \pm 0.04	0.27 \pm 0.23
linalool	1098	1098	6.10 \pm 1.82	6.83 \pm 0.15
borneol	1162	1165	0.40 \pm 0.08	0.39 \pm 0.05
trans- β -terpineol	1163	1163	0.40 \pm 0.08	0.39 \pm 0.05
terpinen-4-ol	1176	1177	tr	0.18 \pm 0.05
α -terpineol	1189	1189	1.17 \pm 0.18	1.28 \pm 0.25
methylchavicol	1193	1195	0.29 \pm 0.23	2.12 \pm 1.48
δ -elemene	1133	1139	tr	0.18 \pm 0.05
eugenol	1356	1356	38.95 \pm 2.82	37.27 \pm 5.37
β -elemene	1388	1391	1.88 \pm 0.36	1.89 \pm 0.75
methyleugenol	1401	1401	tr	—
trans-caryophyllene	1414	1418	8.31 \pm 1.17	7.00 \pm 0.87
trans- α -bergamotene	1432	1436	tr	0.25 \pm 0.07
α -humulene	1448	1454	1.67 \pm 0.16	1.48 \pm 0.27
alloaromadendrene	1453	1461	—	0.39 \pm 0.15
(E)- β -farnesene	1454	1458	0.41 \pm 0.12	—
germacrene D	1475	1480	0.22 \pm 0.03	0.28 \pm 0.17
bicyclogermacrene	1491	1494	4.25 \pm 0.65	4.49 \pm 0.96
germacrene A	1499	1503	3.27 \pm 1.06	3.15 \pm 0.58
germacrene B	1550	1556	3.70 \pm 3.00	0.57 \pm 0.15
spathulenol	1571	1576	1.11 \pm 0.31	0.73 \pm 0.63
caryophyllene oxide	1575	1581	0.57 \pm 0.21	0.79 \pm 0.55
isospathulenol	1632	—	0.21 \pm 0.02	—
β -eudesmol	1644	1649	tr	—
Total			98.37	96.79

*All values reported as an average of three replicates \pm SD; tr – trace compound (less than 0.10%); RI Cal. – retention indices calculated; RI Lit. = Adams 1995.

However, it did not show activity against *E. coli* (ATCC 25922). These results are in agreement with that previously observed (Farago et al. 2004), which reported a small antimicrobial activity of the essential oils of eugenol variety for both microorganisms. The observed activity against *S. aureus* can be attributed to the large amount of eugenol present in the oil, as reported by Nakamura et al. (1999).

ACKNOWLEDGMENTS

We are grateful to Universidade Estadual do Sudoeste da Bahia (UESB) for a graduate scholarship (R.S. França), and for the financial support of this project. We also acknowledge to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for research fellowships (JMD and LCAB) and financial support.

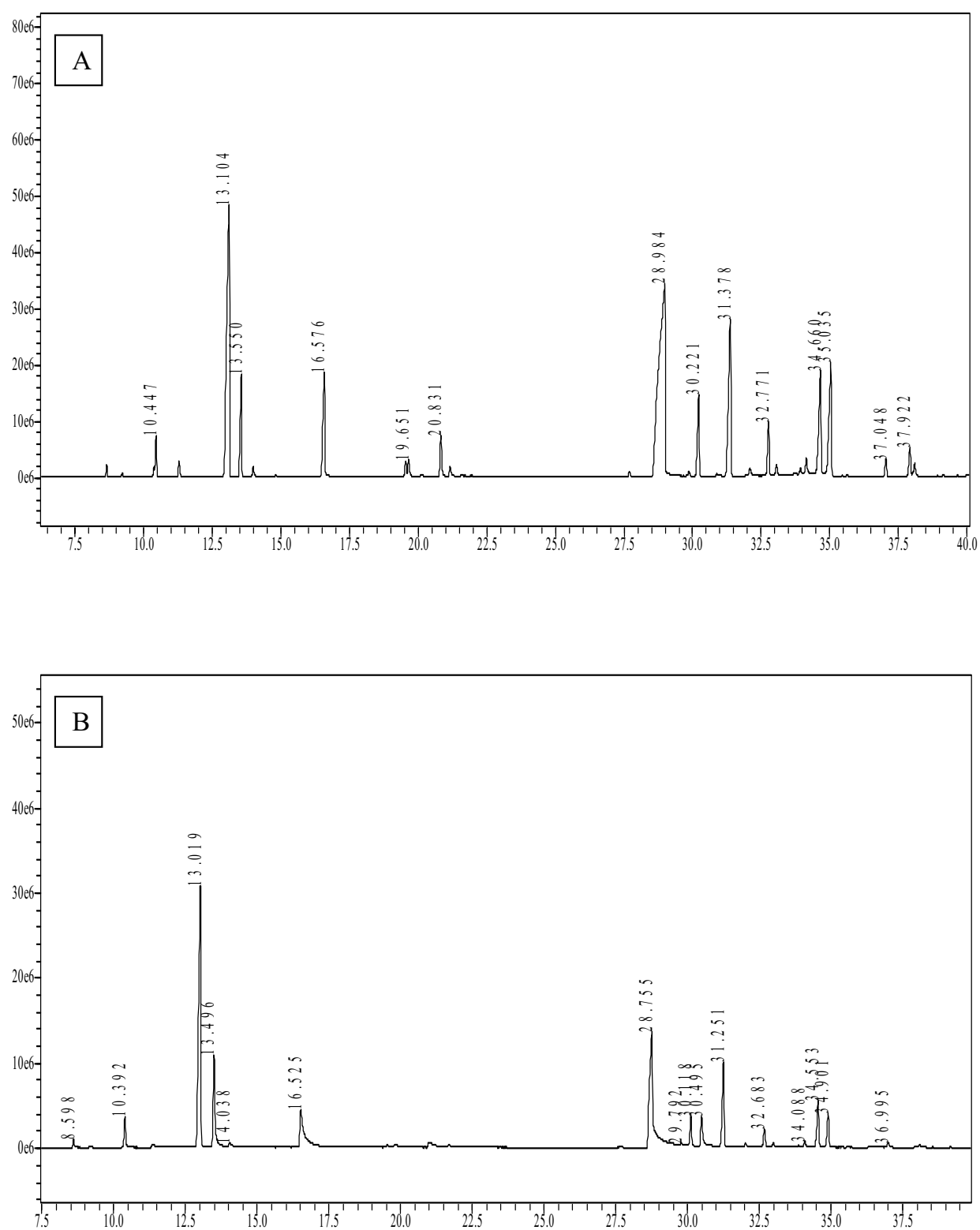


Fig. 1 – Chromatograms of essential oils of the aerial parts from *Ocimum selloi* (APOS) collected in the (A) winter (August/2005) and (B) summer (February/2006).

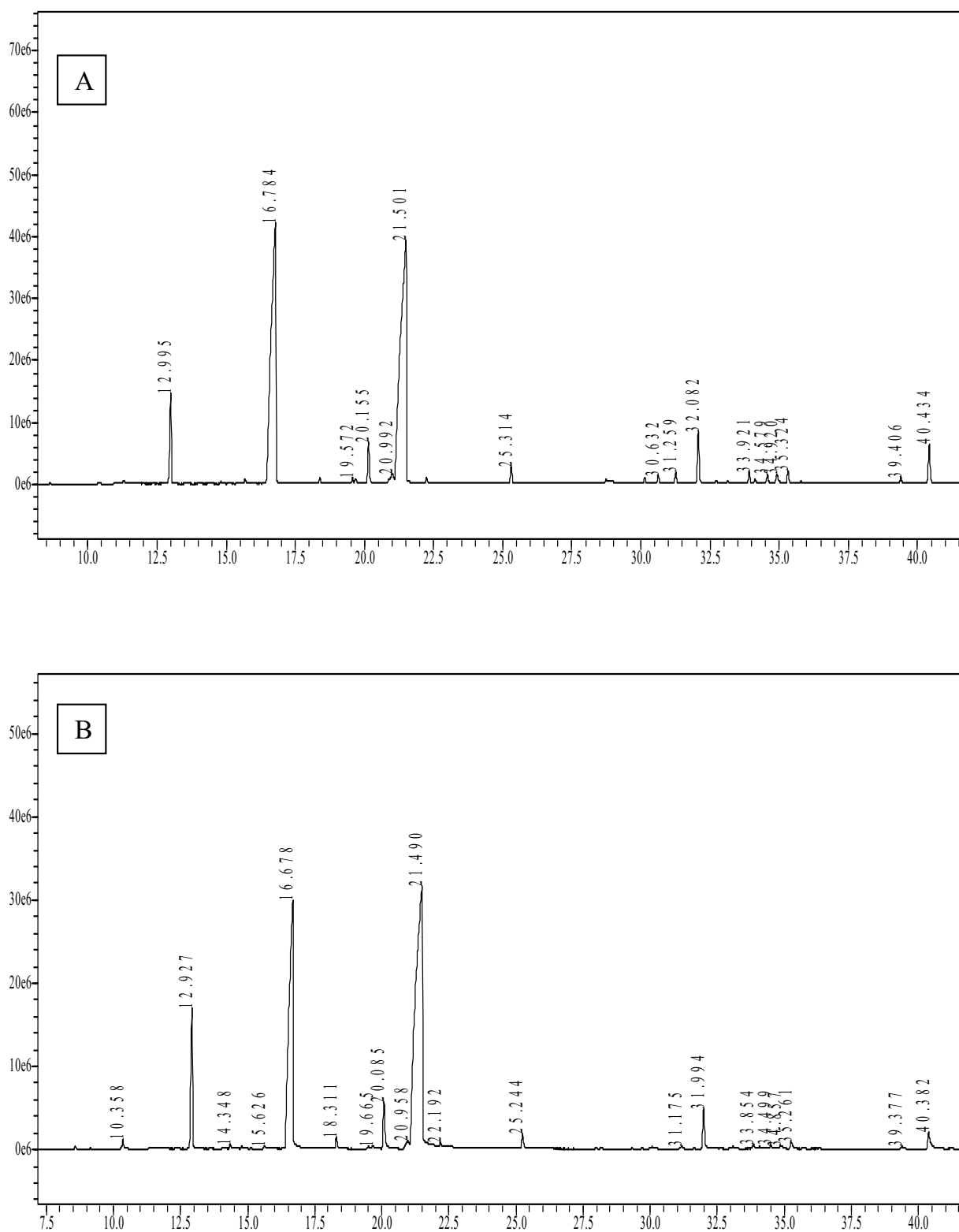


Fig. 2 – Chromatograms of essential oils of leaves from *Ocimum canum* (LOC) collected in the (A) winter (August/2005) and (B) summer (February/2006).

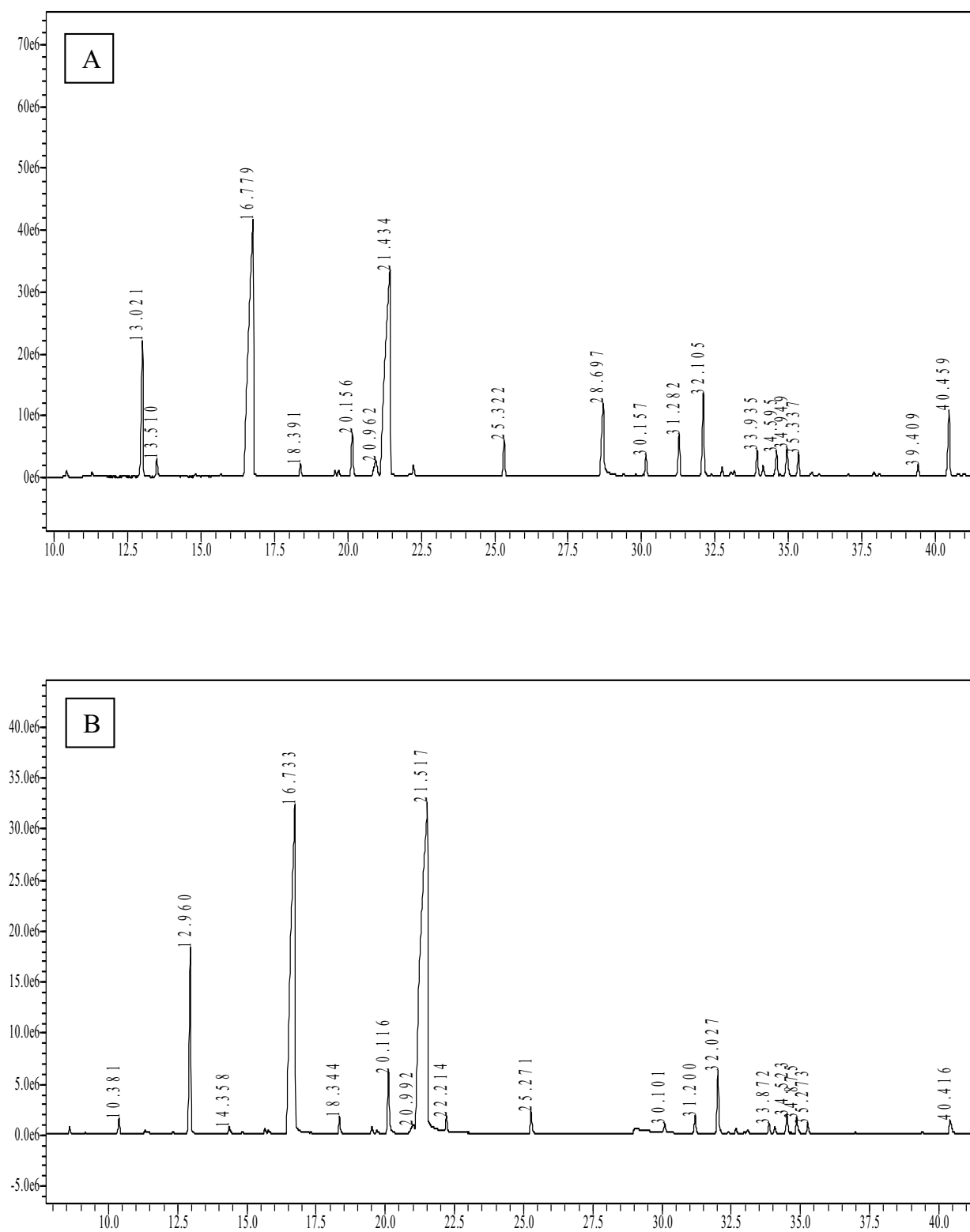


Fig. 3 – Chromatograms of essential oils of the aerial parts from *Ocimum canum* (APOC) collected in the (A) winter (August/2005) and (B) summer (February/2006).

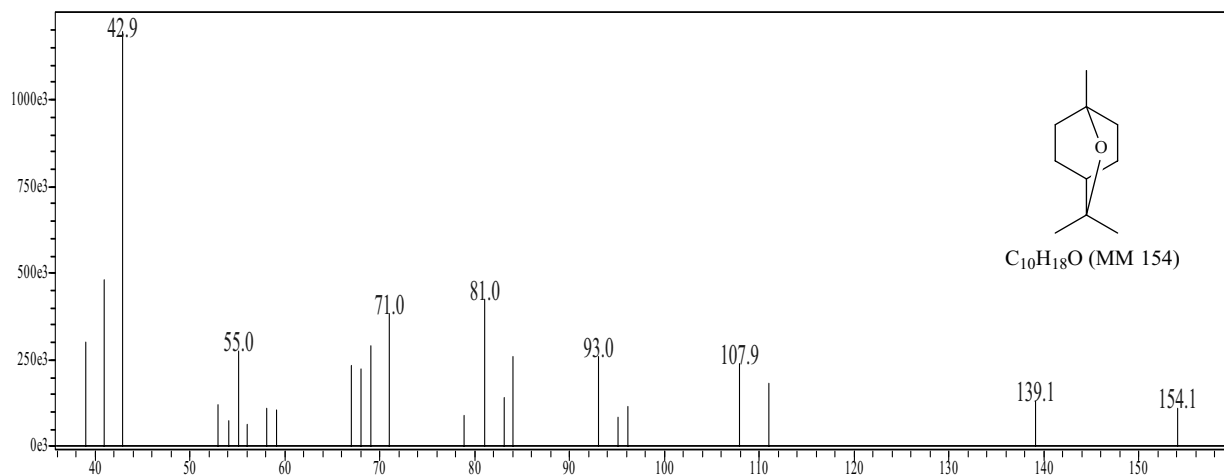


Fig. 4 – Mass spectrum of 1,8-cineole ($R_t = 13.10, 12.93$ and 12.96 min, in APOS, LOC and APOC, respectively).

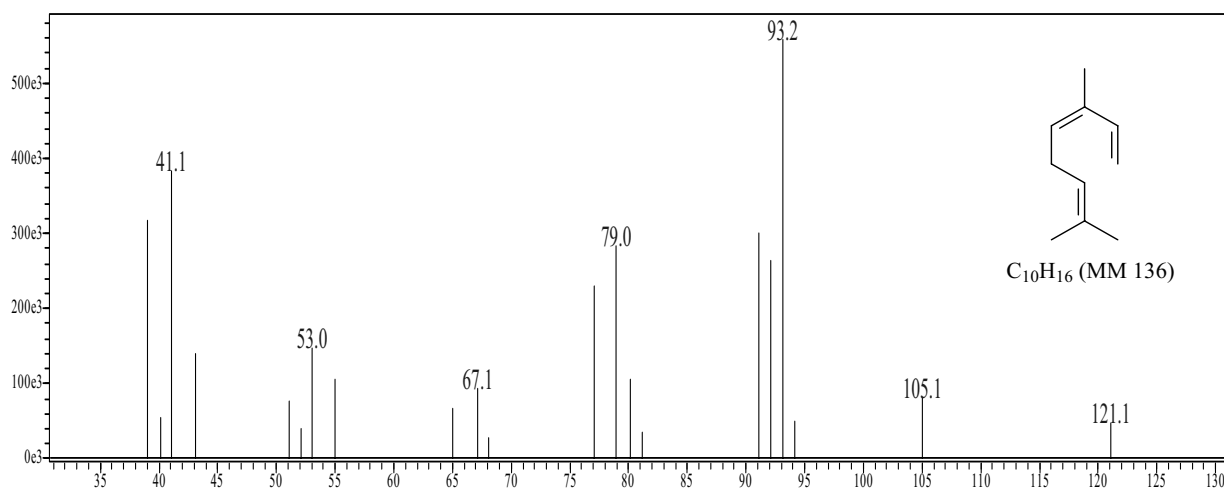


Fig. 5 – Mass spectrum of (*Z*)- β -ocimene ($R_t = 13.55$ min, in APOS).

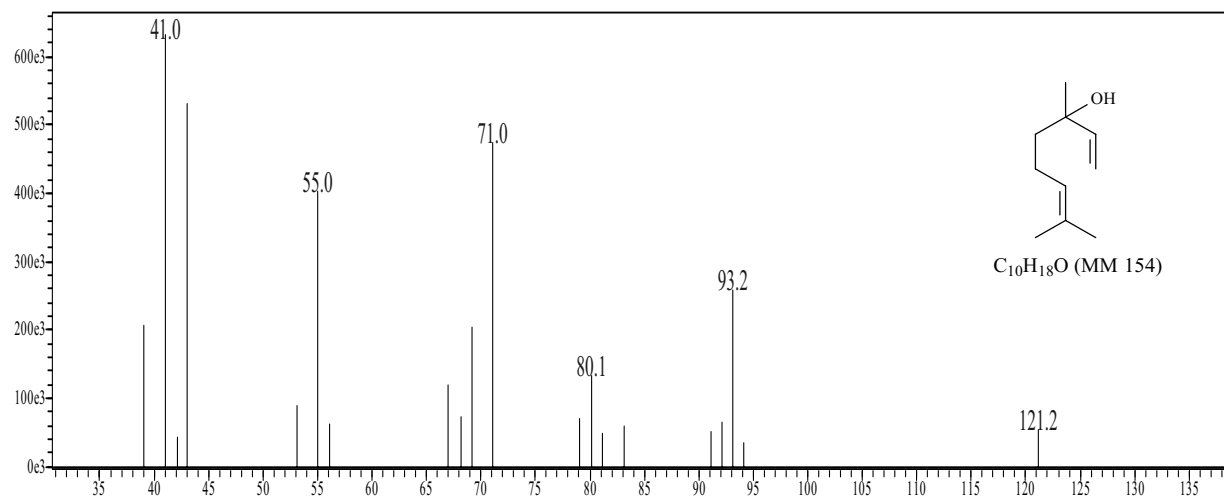
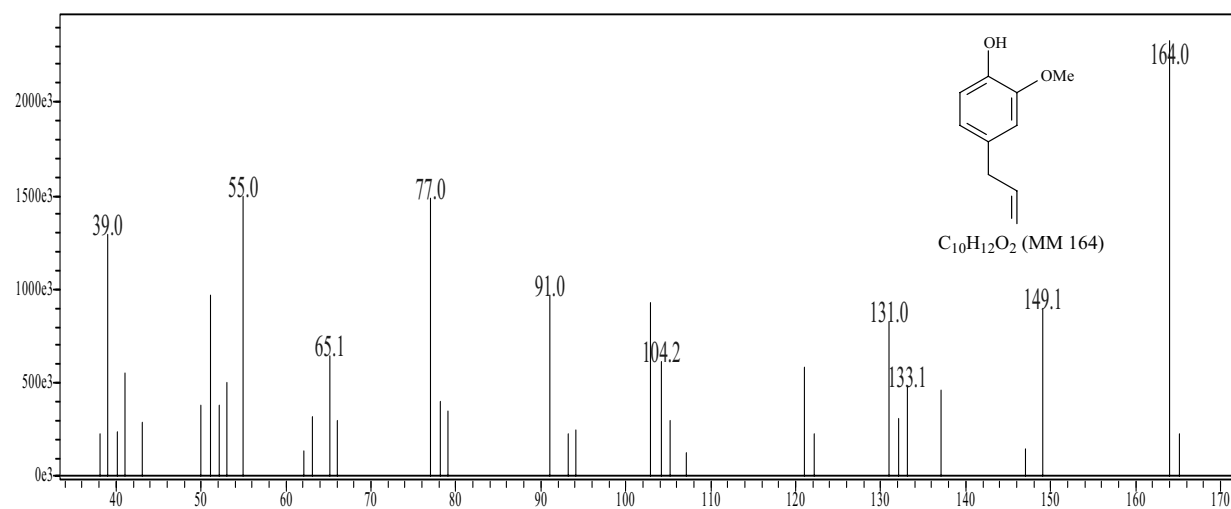
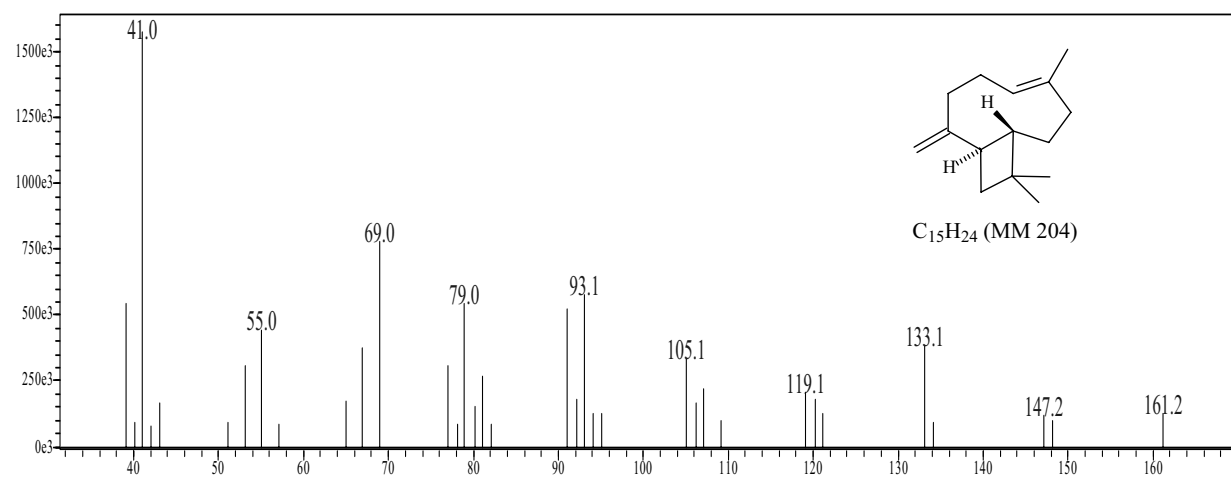
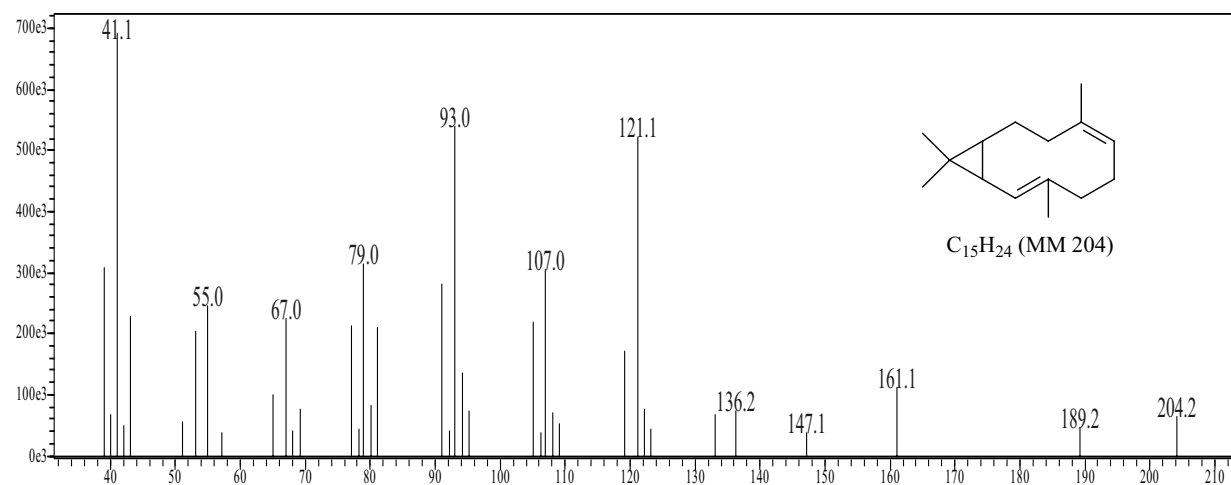


Fig. 6 – Mass spectrum of linalool ($R_t = 16.57, 16.78$ and 16.73 min, in APOS, LOC and APOC, respectively).

Fig. 7 – Mass spectrum of eugenol ($R_t = 28.98$ min, in APOS).Fig. 8 – Mass spectrum of *trans*-caryophyllene ($R_t = 31.37$ min, in APOS).Fig. 9 – Mass spectrum of bicyclogermacrene ($R_t = 34.66$ min, in APOS).

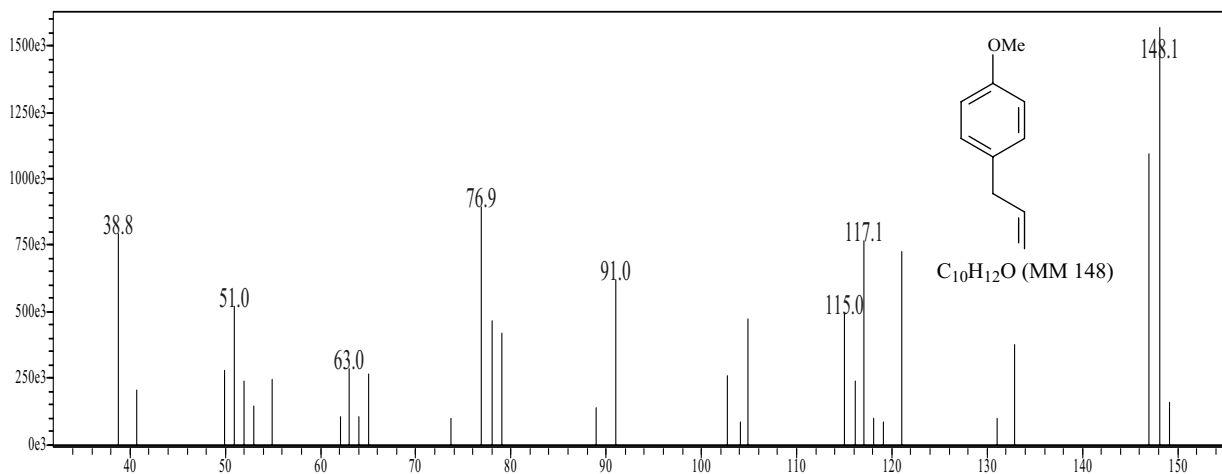


Fig. 10 – Mass spectrum of Methylchavicol (Rt = 21.50 and 21.52 min, in LOC and APOC, respectively).

RESUMO

Este trabalho descreve a composição química e a atividade antimicrobiana dos óleos voláteis de *Ocimum canum* e *Ocimum selloi*, que ocorrem em Jequié/BA, nordeste do Brasil. As plantas foram colhidas no inverno de 2005 e verão de 2006 e os óleos extraídos por destilação a vapor foram posteriormente analisados por GC-MS. Um total de 30 e 31 compostos foi identificado a partir dos óleos de *O. selloi* e *O. canum*, respectivamente. Foi observado que o teor de óleo de *O. canum* apresentou variação durante as estações do ano, enquanto o óleo de *O. selloi* não. Metilchavicol e linalol foram os principais componentes químicos encontrados na parte aérea e folhas de *O. canum*. Esta descoberta permitiu caracterizar este espécime como um novo quimiotipo de *O. canum*. Com relação às partes aéreas de *O. selloi*, eugenol, 1,8-cineol, trans-cariofileno e linalol foram identificadas como os seus principais componentes. Todos os óleos extraídos das partes aéreas apresentaram atividade biológica contra cocos gram-positivo – *Staphylococcus aureus* ATCC 25923 – mas apenas aquele de *O. canum* apresentou atividade contra bacilo gram-negativo – *Escherichia coli* ATCC 25922.

Palavras-chave: atividade antimicrobiana, óleo essencial, Lamiaceae, *Ocimum canum*, *Ocimum selloi*.

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