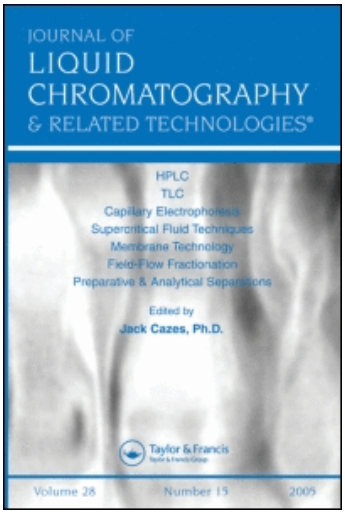

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TLC Separation and Identification of the Essential Oil Constituents from *Aloysia gratissima*

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Abstract: The present work had as objective the isolation of the five compounds by thin-layer Chromatography (TLC) from the essential oil of the *Aloysia gratissima*. For this, a number of systems of eluents were used for its separation, indicating that through the system acetone/hexane in proportions (v/v) 1:30 it was possible to isolate guaiol, elemol, pinocanphone (*trans*-3-pinanone), *cis*-pinocarvyl, and acorenone. The isolation of the compound *acorenone* from the other compounds was possible with the mixture of solvents hexane/dichloromethane in proportions (v/v) (1:1,3).

Keywords: *Aloysia gratissima*, Eluents, Essential oil, Guaiol, Isolation, TLC

INTRODUCTION

Essential oils represent a small fraction of a plant's composition that confers the characteristic for which aromatic plants are used in the pharmaceutical, food, and fragrance industries. Essential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols,

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oxides, lactones, acetalse, ethers, and esters). Both hydrocarbons and oxygenated compounds are responsible for the characteristic odors and flavors.[1]

Terpenoids are found in almost all plant species. Their physiological, metabolic, and structural roles include, among others, those of light-harvesting pigments in photosynthesis or the regulatory activities of the many terpenoid plant hormones. In addition, a large number of structurally diverse plant terpenoids are known or assumed to have specialized functions associated with interactions of sessile plants with other organisms in the context of reproduction, defense, or symbiosis.

These interactions involve specialized plant terpenoids, for example, in the form of attractants, repellents, anti-feedants, toxins, or antibiotics. The chemical diversity of terpenoids is probably a reflection of their many biological activities in nature, which have made them a widely used resource for traditional and modern human exploitation.[2]

The essential oil composition from *Aloysia gratissima* leaves has been identified previously.[3] TLC is a great technique for raw vegetal extracts analysis, permitting the optimization of the solvent system for a given separation problem. Continuing our investigations on aromatic plant volatile composition, this paper presents the isolation of the 5 compounds from *Aloysia gratissima* essential oil which belong to the terpenoid class. This plant plays an important role in Brazilian folk medicine: its branches' and twigs' infusion are used as analgesic and aphrodisiac, beyond alleviating stomach pains and being recommended for pains in the bladder.[4–7] In this work, we were able to prove the existence of some previously identified compounds,[3] separating each of them and also suggesting a system of eluents that permit its separation.

EXPERIMENTAL

Plant Material and Oil Isolation

Aerial parts of *Aloysia gratissima* were collected from São Carlos (Brazil) during their flowering period in September 2002. The plant's identification (No. 006697) was determined by Maria Inês Salgueiro Lima and has been deposited at the herbarium of Universidade Federal de São Carlos (UFSCar). The leaves were dried in a room with controlled humidity for ten days (50% relative humidity) at 30°C until reaching constant weight and was then subjected to hydrodistillation using a Clevenger-type apparatus for 4 h. The yield of the oil was found to be 2.3% (v/w) and it was stored at −18°C until analysis.

Gas Chromatography

The GC analysis was carried out using a Hewlett-Packard 5890 instrument equipped with a flame ionization detector (FID) and a HP-5 column, (25 m × 0.22 mm, film thickness 0.30 μm). The operating conditions were as follows: injector and detector temperatures were 300ºC. Hydrogen was used as carrier gas at a flow rate of 1 mL/min. The column temperature was kept at 80ºC for 2 min, heated to 150ºC with 7ºC/min rate and then heated to 300ºC with 12ºC/min rate and kept constant at 300ºC for 20 min. Split ratio, 1:50.

Gas Chromatography-Mass Spectrometry

GC/MS analysis was performed using a Hewlett-Packard 5970 with HP-1 column, (50 m × 0.22 mm, film thickness 0.25 μm). The operating conditions were as follows: injector and detector temperatures were 300ºC. The column temperature was kept at 80ºC for 2 min, heated to 150ºC with 7ºC/min rate and then heated to 300ºC with 12ºC/min rate and kept constant at 300ºC for 20 min. Split ratio, 1:50. Scan time: 45 minutes. Acquisition mass range: 32–420 daltons. Helium was used as carrier gas with a flow rate of 1 mL/min. MS were taken at 70 eV. The identification of the isolated constituents of the oil components was established from their GC retention indices, relative to C_6–C_{24} n-alkanes, by comparison of their MS spectra with those reported in the literature,[8] and by computer matching with the NBS-Reve mass spectral library.

Thin Layer Chromatography

For TLC, we used glass plates (20 × 20 cm) covered with silica gel G60 and GF_{254} Merck® (1:10) with 0.5 mm thickness of stationary phase. The plates have been activated in a kiln at 140ºC for 4 h (adsorption chromatography). The eluents used for essential oil separation were acetone/hexane in proportions (v/v) 1:30 and hexane/dichloromethane (v/v) 1:1.3 with dielectric constants (ε) equal to 2.65 and 5.88, respectively.

RESULTS AND DISCUSSION

The analytical study of the oil from the leaves of *A. gratissima* had as its result the isolation and identification of secondary metabolites of the terpene class. The fractionation of the initial sample (oil + chloroform) in preparative scale was done by thin-layer chromatography (TLC)

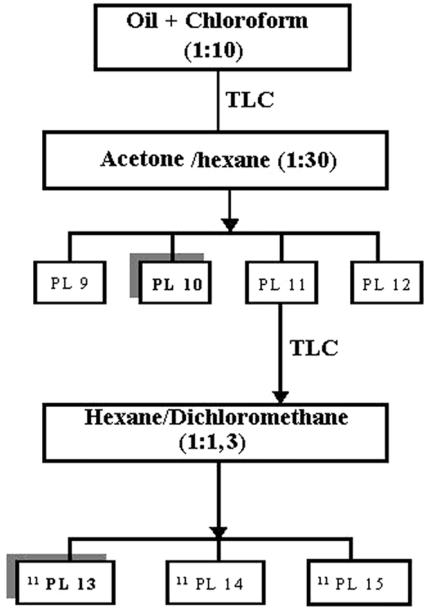

Figure 1. Fractionation of essential oil from leaves of *Aloysia gratissima* by TLC.

and the best mixtures of tested solvents were acetone/hexane in proportions (v/v) 1:30 and hexane/dichloromethane (v/v) 1:1,3, as shown in Figure 1.

For the mixture of solvents acetone/hexane (1:30), the TLC plates showed four shades when radiated with UV light, which has been codified as PL9, PL10, PL11 and PL12. It was possible to obtain informations only from PL10 and PL11 fractions and they were subjected in this study.

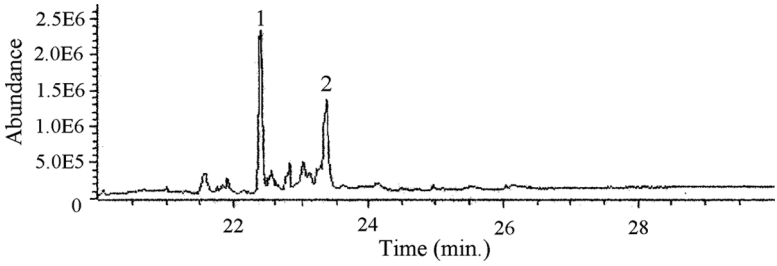

Figure 2. Chromatographic analysis of the PL10 fraction.

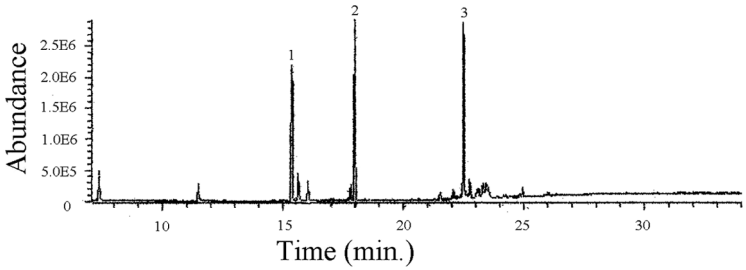

Figure 3. Chromatographic analysis of the PL11 fraction.

The chromatogram of the PL10 fraction (Figure 2) showed two distinct peaks, with retention times of 22.40 min. (peak 1) and 23.78 min. (peak 2). The major peak, which was identified as *guaiol*, represents 12.7% of the raw essential oil.[3] Furthermore, the other smaller peak observed was identified as *elemol*. The fraction PL11 represented by Figure 3, indicated three major peaks in their retention times of 15.20 min. (peak 1); 18.02 min. (peak 2) and 22.42 min. (peak 3). These compounds were identified as pinocanphone (*trans*-3-pinanone), *cis*-pinocarvyl acetate, and acorenone, referring to peaks 1, 2, and 3, respectively.

In order to achieve further separation of these three compounds previously identified in the PL11 fraction, it was rechromatographed using preparative thin-layer chromatography with the mixture of solvents hexane/dichloromethane (1:1,3). Then, three shades have been also observed and they were codified as ^{11}PL13, ^{11}PL14 and ^{11}PL15, but only in the ^{11}PL13 was it possible to obtain information and it was subjected to this study. Thus, the rechromatographed fraction ^{11}PL13 shows one peak which was identified as *acorenone*, as shown in Figure 4.

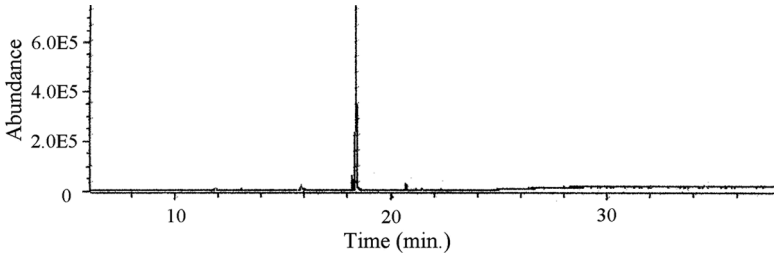

Figure 4. Chromatographic analysis of the ^{11}PL13 fraction.

CONCLUSION

This work is a continuation of the study of the plant *Aloysia gratissima*, showing a way to prove the existence of the some compounds through isolation by thin-layer chromatography, previously identified, and suggesting a system of eluents for its separation. The isolation of five compounds from *Aloysia gratissim*a essential oil was demonstrated: the compounds guaiol and elemol were isolated from the PL10 fraction using the mixture of solvents ethyl acetone/hexane in proportion 1:30; acorenone, pinocanphone (*trans*-3-pinanone), and *cis*-pinocarvyl were isolated from the PL11 fraction, also using the system of eluents acetone/hexane in proportion 1:30. Acorenone was isolated from the PL10 fraction (^{11}PL13) using the system of eluents hexane/dichloromethane (1:1.3). Thus, some systems of eluents were suggested for some compounds of the essential oil of the *Aloysia gratissima* could be isolated, giving continuation to the work previously done with this species.

REFERENCES

1. Anitescu, G.; Doneanu, C.; Radulescu, V. Isolation of *coriander* oil: Comparison between steam distillation and supercritical CO_2 extraction. Flavour Fragr. J. **1997**, *12*, 173–176.
2. Bohlmann, J.; Keeling, C.I. Terpenoid biomaterials. Plant J. **2008**, *54* (4), 656–669.
3. Trovati, G.; Sanches, E.A.; Chierice, G.O. Essential oil composition of *Aloysia gratissima* from brazil. J. Ess. Oil Res. **2008**, DOI: RN-2570 (in press).
4. Alice, C.B.; Siqueira, N.C.S.; Mentez, L.A.; Brazil e Silva, G.A.A.; José, K.F.D. *Plantas medicinais de uso popular: Atlas farmacognóstico*; Editora Ulbras. Press: São Paulo, Brazil, 1995; 205pp.
5. Troncoso, N.S. Los gêneros de Verbenaceae de Sudamérica extratropical. Darviniana. Revista Inst. Bot. Darwin **1974**, *18*, 295–412.
6. Bailac, P.N.; Dellacasa, A.D.; Duschatzky, C.B.; Firpo, N.; Ponzi, M.I. Composición del aceite esencial y actividad antimicrobiana de *Aloysia gratissima* de San Luis, Argentina. Ann. Asoc. Quim. Arg. **1999**, *87*, 149–153.
7. Botta, S.M. Las espécies argentinas del gênero *Aloysia* (Verbenaceae). Darwiniana: Revista Inst. Bot. Darwin **1979**, *22*, 67–108.
8. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Pub. Corp.: Carol Stream, IL, 1995.

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