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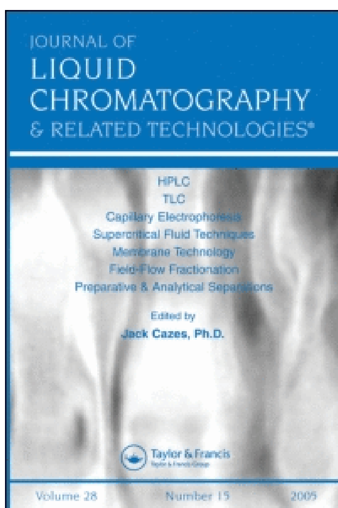

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LOW TEMPERATURE PLANAR CHROMATOGRAPHY-DENSITOMETRY AND GAS CHROMATOGRAPHY OF ESSENTIAL OILS FROM DIFFERENT SAGE (*SALVIA*) SPECIES

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□ *Essential oils of plant origin are the multicomponent mixtures of mono-, di-, tri-, and sesquiterpenes. Due to their recognized curative, cosmetic, and nutritional properties on the one hand and an outstanding modern analytical potential on the other, qualitative and quantitative composition of essential oils currently is in the focus of interest for phytochemistry and pharmacognosy. Due to the recognized volatility of the essential oil components, in their case the analytical method of choice is gas chromatography with mass spectrometric detection (GC-MS). However, great versatility of planar chromatography has resulted in a number of successful applications of this relatively simple and inexpensive separation technique to the investigations on composition of the volatile plant constituents as well. Generally, the low temperature preparative layer chromatography (PLC) is used for preliminary fractionation of the essential oils, and the separated fractions are further analyzed by means of GC-MS. In this study, we scrutinized a possibility of using the low temperature analytical thin-layer chromatography (TLC) to fingerprinting of the essential oils originating from the five different sage (*Salvia*) species, i.e.,* S. lavandulifolia, S. staminea, S. hians, S. triloba, *and* S. nemorosa. *We also used the low temperature PLC for the preliminary fractionation of these essential oils prior to the GC-MS analysis. It was shown that the low temperature TLC can successfully be applied to fingerprinting the different sage (*Salvia*) species. Fractionation of the essential oils from the sage species by means of the low temperature PLC prior to the GC-MS analysis is also possible, although individual stages of the approach still need an additional optimization.*

Keywords essential oils, GC-MS, low temperature planar chromatography, PLC-densitometry, *Salvia* espe., terpenes, TLC-densitometry

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INTRODUCTION

Essential oils have been recognized for ages now as substances of plant origin that possess highly appreciated curative, cosmetic, and nutritional properties. In purely chemical terms, essential oils are the multicomponent mixtures of mono-, di-, tri-, and sesquiterpenes. Structurally, these compounds can belong to the groups of hydrocarbons, alcohols, aldehydes, ketones, esters, and ethers. At ambient temperature, many of them appear as liquids and display an oily consistency. Their positive impact on somatic and mental health has been recognized in a remote antiquity. Ancient Egyptians highly valued the olfactory properties of the sage species. Rich men and women have used aromatic essential oils to moisturize their bodies and protect their skin against the influence of an arid climate. Ancient Greeks adapted the Egyptian medical knowledge and utilized aromatic essential oils for curative massages.

In present time, essential oils are widely used in aromatherapy (for different inhalations, aromatic baths, and massages). However, essential oils usually are complicated mixtures and their composition is, in most cases, not fully established. Due to that, they cannot be prepared in laboratories, neither can they be considered as strictly curative formulations by doctors and pharmacists, which is a good reason to intensify studies on essential oils. Their pharmacological action is diverse. Owing to disinfection properties, essential oils hamper the growth of various different pathogenic microorganisms, e.g., they prove helpful in combating skin parasites and fungi. Individual constituents often appear in high concentrations (e.g., in order to obtain one drop of rose oil, ca. 30 fresh flowers are needed) and for this reason, essential oils cannot be applied to human skin in a pure form.

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There are several sage oils used for aromatherapy purposes which are often confused (e.g., Clary Sage, Common Sage, and Spanish Sage).[1–3] They have differing aromatic uses. Some of them (e.g., *S. sclarea*) have a high chemical component of esters and are generally considered as non-sensitizing and non-toxic. These essential oils are considered antiseptic, digestive, sedative, deodorant, and aphrodisiac. Apart from being well known for their euphoric and ‘feel good’ actions, they are also used for menopausal symptoms, nervous fatigue, scanty periods, varicose veins, and in the anti-aging and the post-natal depression treatments.

Some of essential oils derived from sage, with a high percentage of ketones in their chemical composition (e.g., *S. officinalis*) are considered as more toxic. These essential oils are analgesic, anti-viral, bactericidal, antiseptic, diuretic, decongestant, and abortive in their actions. They can be used for the treatment of angina, menopausal symptoms, irregular periods, conceptual difficulties, anxiety, rheumatism, thrush, herpes, and nervous debility. They are also a source of natural anti-oxidants.

Certain essential oils derived from sage (e.g., *S. lavandulifolia*) are composed mainly of alcohols, but also contain a certain amount of ketones. Actions of this group of essential oils is recognized as anti-depressant, anti-inflammatory, analgesic, balancing, immune boosting, and hormonally balancing. They can be used to treat skin infections, stress, gum infections, hair loss, fluid retention, amenorrhea, and dysmenorrhoea, headaches, muscular aches and pains, asthma, and arthritis. They also have the properties of being astringent, digestive, nerve tonic, and deodorant. It should be emphasized that the safety of using certain sage derived essential oils is questioned by some therapists because of a high level of thujone, which has been found as a neurotoxic compound.

Appreciating high versatility of planar chromatography, we tested its applicability in the analytical (TLC) and preparative (PLC) mode to fingerprinting and fractionating essential oils obtained from the five different sage (*Salvia*) species, i.e., from *S. lavandulifolia, S. staminea, S. hians, S. triloba*, and *S. nemorosa.*

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EXPERIMENTAL

Materials and Reagents

Samples of the five different sage (*Salvia*) species (*S. lavandulifolia, S. staminea, S. hians, S. triloba*, and *S. nemorosa*) investigated in this study were collected in the Pharmacognosy Garden of Medical University, Lublin, Poland, on August 20th, 2007. Plant material was dried for 40 h in an oven with a forced air flow at 35 to 40°C. Finally, 50 g of each plant species was weighed and powdered in a porcelain mortar. Three replicates of each sample were processed in an identical way.

In our investigations, solutions of the pine, mint, eucalyptus, and juniper essential oils with a strictly defined chemical composition were employed (manufactured by Apotheca Pacis, Rybnik, Poland), which helped to attribute the retention times of the volatile sage components to the terpenes contained in these standards.

Thin-layer chromatographic separations were performed on commercial glass plates (20 cm × 20 cm) precoated with 0.25 mm layers of silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany; cat. no. 1.05715) and preparative-layer chromatographic separations were performed on commercial glass plates (20 cm × 20 cm) precoated with 2 mm layers of silica gel 60 F_{254} (Merck, cat. no. 1.05745).

For planar chromatography, methanol, hexane, toluene, and ethyl acetate were used (all solvents were of analytical purity grade and manufactured by POCh, Gliwice, Poland). Planar chromatograms were developed in sandwich DS chambers (Chromdes, Lublin, Poland).

Vapor Distillation of Essential Oils from *Salvia* Species

The dried plant material (50 g) was placed in the round bottomed flask and 400 mL water was added. Vapor distillation was carried out for 3 h with use of the Deryng apparatus. The procedure is described in Polish Pharmacopoeia VI[4] and earlier it has proved more effective than traditional solvent extraction and instrumental accelerated solvent extraction (ASE) for this particular purpose, as documented in our comparative study.[5]

Volumes of the distilled essential oils were the following ones: with *S. lavandulifolia* and *S. triloba*, 0.5 mL per 50 g of the dried plant material (ca. 1%, *v/w*); and with *S. hians, S. staminea*, and *S. nemorosa*, 0.05 mL per 50 g of the dried plant material (ca. 0.1% v/w). Thus, the first two sage species (*S. lavandulifolia* and *S. triloba*) can be regarded as rich in essential oils, while the remaining three species (*S. hians, S. staminea*, and *S. nemorosa*) yielded roughly ten times less essential oils and consequently, they cannot be regarded as particularly oily species.

For the purpose of the TLC analysis, the aliquots of 0.05 mL essential oil samples with all five sage species were diluted with *n*-hexane to obtain the volume of 1 mL. For the purpose of the PLC analysis, essential oils originating from the two oily species, i.e., from *S. lavandulifolia* and *S. triloba*, were used undiluted.

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Thin Layer Chromatography (TLC)

Thin-layer chromatographic analysis is the pilot procedure, necessary to establish the separation conditions needed at the next step, i.e., for the preparative separation of essential oils. Each essential oil originating from vapor distillation was spotted onto the thin layer in the aliquot of 15 μL *n*-hexane solution using an AS 30 model autosampler (Desaga, Heidelberg, Germany). Development of the chromatograms was carried out at two different temperatures, i.e., at $22 \pm 1°C$ (at the laboratory bench top) and $-10 \pm 0.5°C$ (inside the refrigerator), for a distance of 15 cm in the one dimensional development mode, using the binary mobile phase toluene–ethyl acetate (95:5; *v/v*).[6]

The chromatograms were developed in the sandwich chromatographic chambers saturated with mobile phase for 15 min. Then the developed chromatograms were dried for 3 h at ambient air and eventually evaluated by means of densitometry. Acquisition of the densitograms was carried out with a Desaga CD 60 model densitometer equipped with Windows compatible ProQuant software (Desaga). Concentration profiles of the development lanes for the sage samples were recorded in reflected ultraviolet (UV) light from a deuterium lamp at 340 nm. The dimensions of the rectangular light beam were $2.0\,mm \times 0.1\,mm$. The obtained densitograms were primarily assessed for providing the fingerprint response.

Preparative Layer Chromatography (PLC)

A 40 μL aliquot of an undiluted essential oil extract from both oily sage species, i.e., from *S. lavandulifolia* and *S. triloba*, was applied to a preparative layer by means of the AS 30 model Desaga autosampler. Application was band wise with a band width of 16 cm. These separations were carried out using a procedure analogous to that employed in TLC, i.e., using the binary mixture toluene–ethyl acetate (95:5; *v/v*) as the mobile phase. The chromatographic plates were developed inside the refrigerator at $-10 \pm 2°C$ in the sandwich chambers saturated with mobile phase for 15 min, for a distance of 15 cm. Based on the densitometrically established fingerprints, the preparative chromatograms were divided into two sections. Each section of the silica gel layer was carefully scraped from the plate, mixed with 10 mL methanol, and placed in an ultrasonic bath for 30 min. The ultrasonicated solution was passed through an Anotop syringe filter with aluminium oxide adsorbent in order to separate silica gel particles from the liquid, and the 1 μL aliquots were analyzed by means of GC-MS.

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Gas Chromatography with Mass Spectrometric Detection (GC-MS)

The GC-MS analyses were carried out with use of a TRACE 2000 model capillary gas chromatograph with an MS TRACE model mass detector (ThermoQuest, Waltham, MA, USA), equipped with a CTC Analytics model autosampler (Combi PAL, Basel, Switzerland), working in the head space and non-head space mode. The analyses were run with use of the DB-5 capillary column ($30\,m \times 0.25\,mm$ i.d., film thickness 0.25 mm; manufactured by Agilent Technologies, Palo Alto, CA/formerly J&W Scientific, Folsom, CA, USA). Helium ($p = 100\,kPa$) was used as carrier gas. Gradient analysis was run, using the following temperature program: 40°C (3 min); 40 to 150°C (8°/min); isothermal conditions, 150°C (15 min). The temperature of the injector was kept constant at 150°C. Mass spectrometer was fitted with an EI source operated at 70 eV.

Identification of the compounds originating from the respective essential oils vapor distilled from sage and preliminarily fractionated by means of the low temperature PLC was based on an agreement between retention times of the known components of the ether oil standards (i.e., of the pine, mint, eucalyptus, and juniper essential oil) with those obtained from the sage species, and on a comparison of the mass spectra of the sage components with those from the NIST Mass Spectral Library with the Search Program in the GC/MS system software.

RESULTS AND DISCUSSION

Thin Layer Chromatography (TLC)

In the pilot TLC study, we elaborated the temperature conditions for further fractionation of the essential oils by means of PLC. Within the framework of this study, we compared two different temperatures of running the chromatographic development (i.e., ambient temperature of 22°C and the temperature of −10°C obtained inside the refrigerator). The development carried out at −10°C provided considerably better results both in qualitative and quantitative terms than that carried out at 22°C (which can easily be deduced from a comparison of the respective densitograms shown in Figs. 1a,b). These densitograms (i) witness to a possibility of separating essential oils by means of planar chromatography, and at the same time (ii) they clearly demonstrate superiority of developing the chromatograms at −10°C than at an ambient temperature, which is rather understandable with volatile compounds.

For our purpose, we tested the mixed mobile phase earlier reported in publication[6] as an eluent suitable for the low temperature preparative layer separation of the essential oils contained in *Carum carvi* L., *Mentha piperita* L., *Iuniperus communis* L., and *Chamomilla recutica* L.). This suitability was considered in terms of a sufficient elution power in combination with reasonable viscosity, and we managed to show that the same eluent is well suited for the low temperature separation of essential oils originating from the sage species also.

Finally, it can be stated that the low temperature TLC densitometry has proved very useful in fingerprinting essential oils contained in the different sage species as a well performing analytical technique in its own right. This conclusion can be drawn from the distinct and differentiated densitogram patterns presented in Fig. 1b.

Preparative Layer Chromatography

In Figs. 2a,b, we showed the densitograms derived from the preparative layer chromatograms valid for *S. lavandulifolia* and *S. triloba*. Due to greater thickness of preparative layers compared with analytical ones, and also to incomparably higher sample aliquots spotted onto the preparative layers (because of an acceptable layer overload in the PLC mode), the separation performance of PLC was worse than that of TLC, and the concentration profiles derived from PLC were less diversified than those derived from TLC. Consequently, the preparative layer chromatograms obtained for *S. lavandulifolia* and *S. triloba* could only be divided into the two fractions (as marked in Figs. 2a,b).

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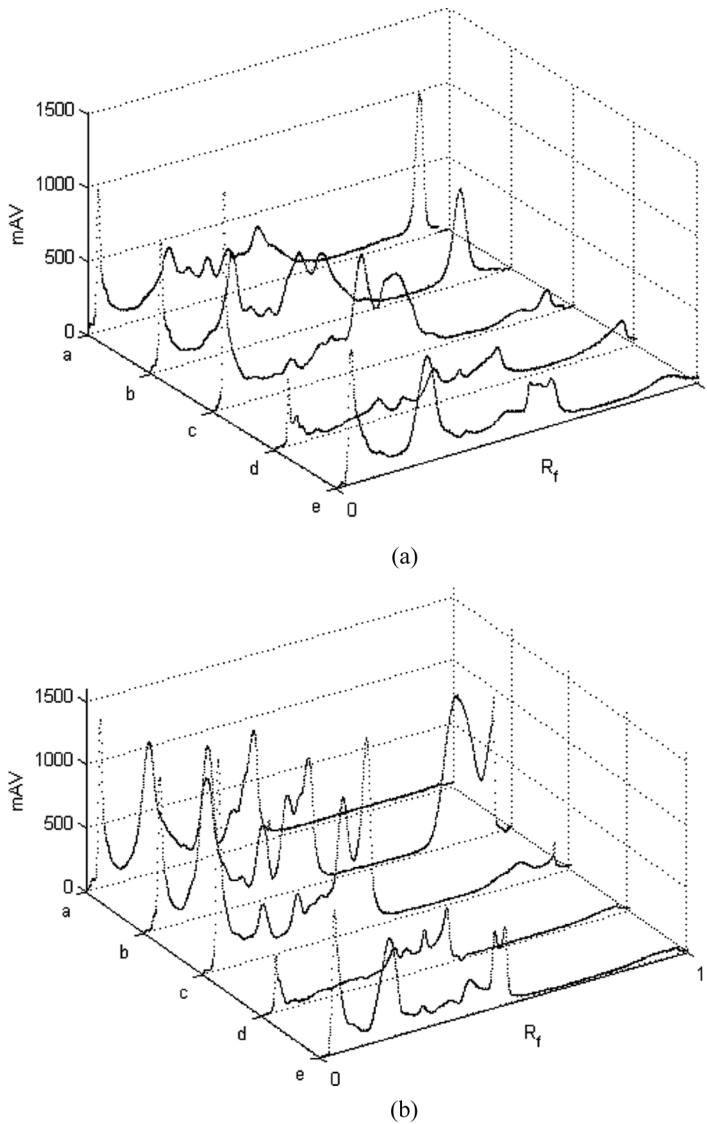

FIGURE 1 A comparison of the densitograms obtained from the analytical thin layer chromatograms developed for essential oils of the five different sage species: a, *S. hians*, b, *S. staminea*, c, *S. nemorosa*, d, *S. triloba.*, and e, *S. lavandulifolia*, depending on the developing temperature; (a) $22 \pm 1°C$ and (b) $-10 \pm 0.5°C$.

Gas Chromatography with Mass Spectrometric Detection (GC-MS)

In Figs. 3a,b, we presented gas chromatograms valid for fraction 1 and 2, respectively, of the essential oil originating from *S. lavandulifolia* and

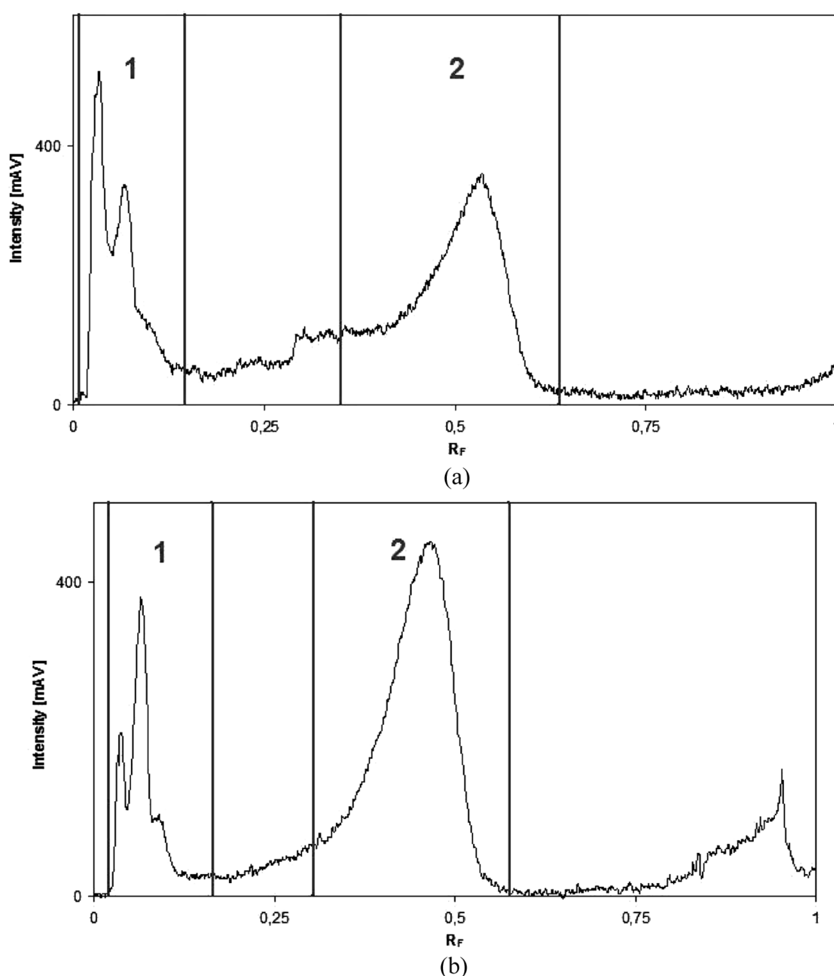

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FIGURE 2 Densitograms of the preparative layer chromatograms for the undiluted essential oils derived from (a) *S. lavandulifolia* and (b) *S. triloba* with use of the Deryng apparatus and spotted on to the plate in the 40 μL aliquot. Development temperature: $-10 \pm 0.5°C$.

fractionated by means of PLC. In Figs. 4a,b, the analogous gas chromatograms are presented, valid for *S. triloba*. *Salvia lavandulifolia* and *Salvia triloba* were specially selected for the PLC fractionation of the respective essential oils as the two species particularly rich in essential oils.

From the results shown in Figs. 3 and 4, it comes out that in both cases, three different terpenes were identified in faction 2 (namely, eucalyptol, thujone, and camphor). In fraction 1, several distinct peaks were observed both for *S. lavandulifolia* and *S. triloba*, yet none of these peaks was identified in this study.

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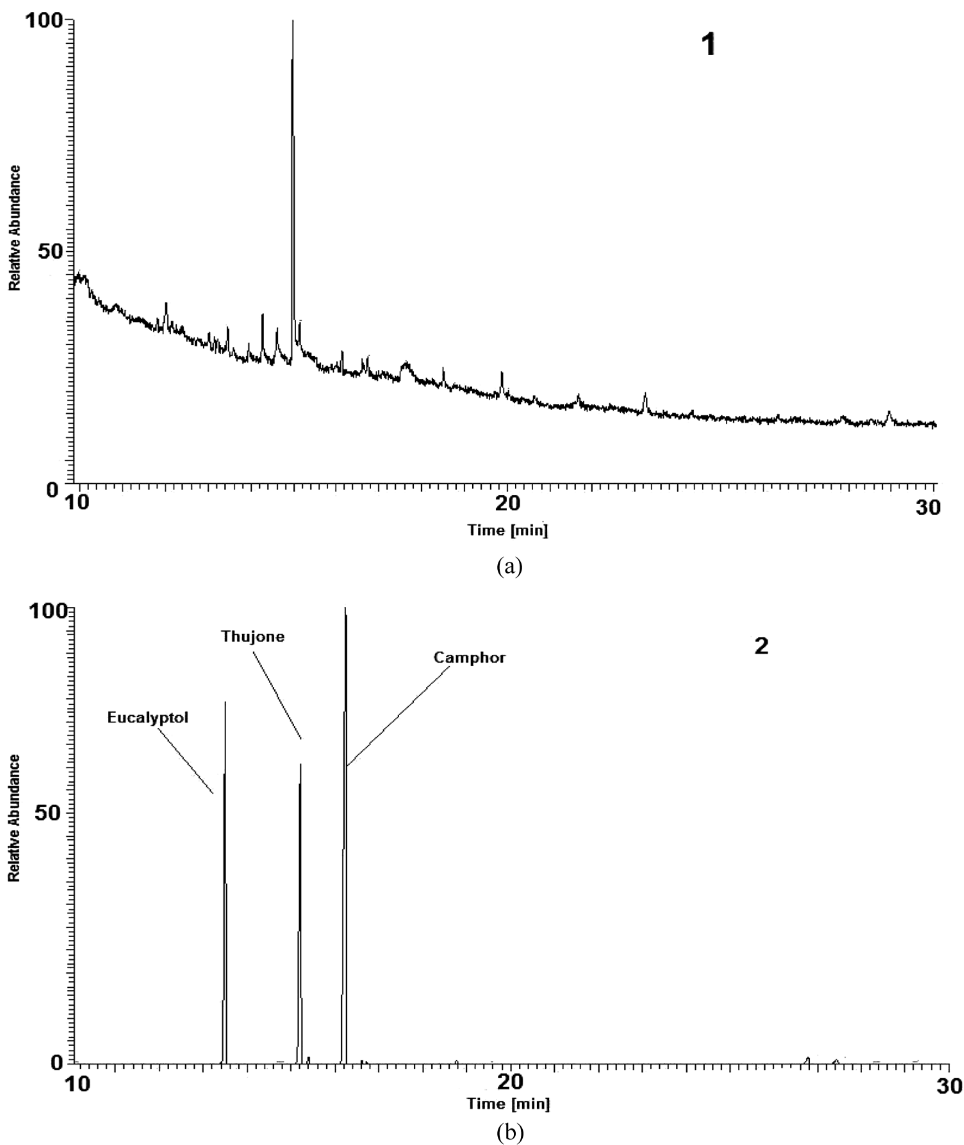

FIGURE 3 Gas chromatograms obtained from (a) fraction 1 and (b) fraction 2, respectively, of the essential oil derived from *S. lavandulifolia* by means of PLC (see Fig. 2a). In fraction 2, eucalyptol, thujone, and camphor were identified (based on the NIST library of the spectra and on the pine, mint, eucalyptus, and juniper essential oil standards).

The identification results by means of GC-MS can be regarded as satisfactory, especially when compared with those from our earlier study presented in publication.[7] From the results given in Table 1 of this previous study,[7] it comes out that eucalyptol, thujone, and camphor appear among several most abundant terpenes present in *S. lavandulifolia*. From

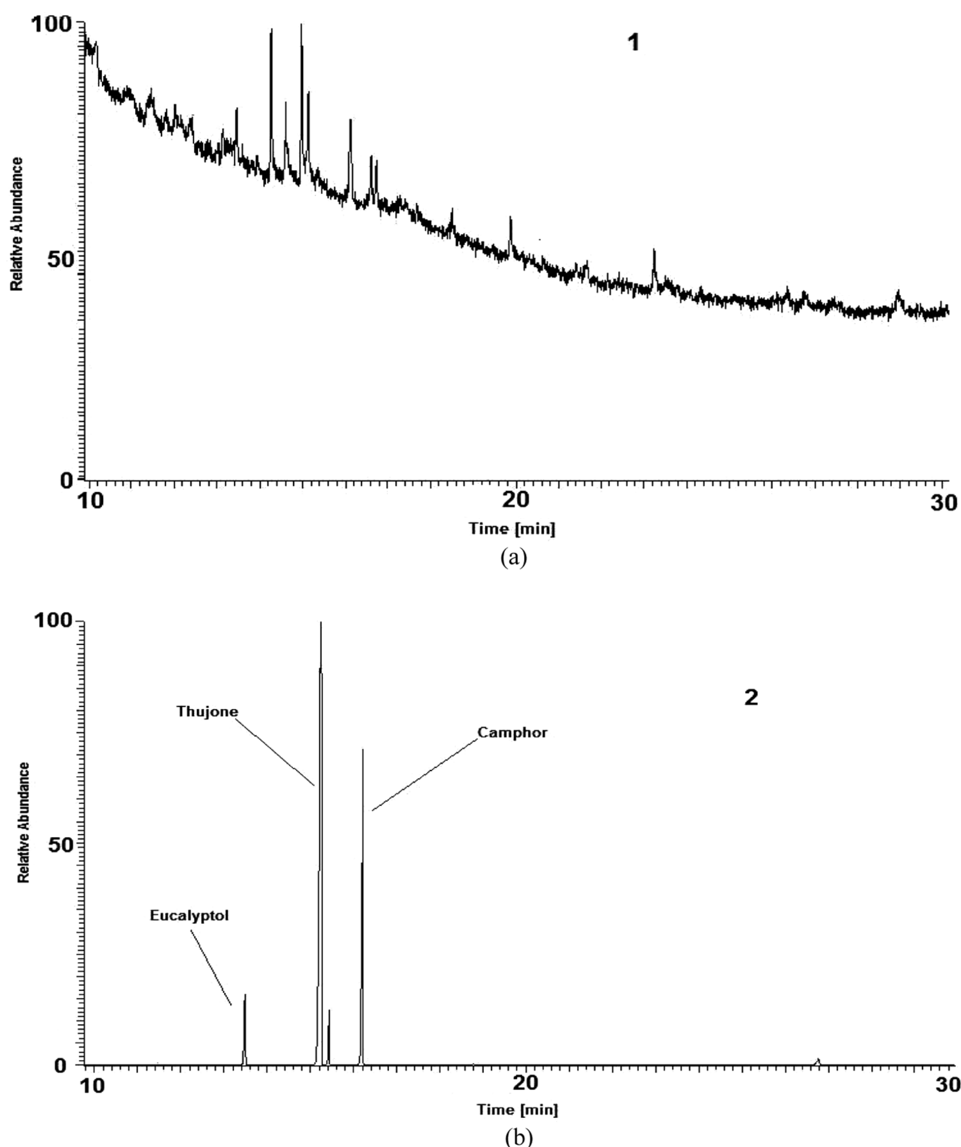

FIGURE 4 Gas chromatograms obtained from (a) fraction 1 and (b) fraction 2, respectively, of the essential oil derived from *S. triloba* by means of PLC (see Fig. 2b). In fraction 2, eucalyptol, thujone, and camphor were identified (based on the NIST library of the spectra and on the pine, mint, eucalyptus, and juniper essential oil standards).

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the same source (Table 1[7]) it also comes out that eucalyptol, thujone, and camphor are three out of four terpenes identified in *S. triloba*, and these three terpenes are the most abundant ones. However, inability to identify several other terpenes present in *S. lavandulifolia* and one terpene (α-pinene) present in *S. triloba* points out to the fact that the aliquots of

essential oils applied to the preparative layer were too low. Thus, a conclusion can be drawn that the essential oil aliquots employed for the preparative layer fractionation have to be carefully optimized (and apparently, in an individual manner, depending on the considered sage species).

The main bottleneck of the PLC fractionation of terpenes is the necessity to use considerable volumes of solvents for washing out fraction components from the scraped silica gel layer and a problem with further removal of these solvents and condensation of the compounds of interest, due to an obvious volatility thereof. Current research carried out in our laboratory is focused on elaborating the possibly most efficient coupling of the low temperature preparative layer fractionation of essential oils with the final step of the GC-MS identification of the constituents of individual fractions.

CONCLUSIONS

Generally, planar chromatography is not dedicated to the analysis of any volatile organic compounds (in this case, the analytical method of choice usually is GC-MS). Hence, planar chromatographic analysis of essential oils is a relatively difficult analytical task.

In certain cases, though, the low temperature planar chromatography can be used for preliminary fractionation of essential oils, in order to facilitate further analysis of the respective fractions by means of GC-MS.

Temperature considerably influences planar chromatographic analysis of essential oils. Lowering of the temperature improves both, separation performance and the yields of the separated fractions.

Silica gel – the most active adsorbent in the planar chromatographic arsenal – is the stationary phase of choice for the low temperature analytical and preparative fractionation of essential oils derived from plant material, as it effectively "fixes" volatile compounds on solid surface through the adsorptive forces.

It was demonstrated that the low temperature TLC densitometry can successfully be used for fingerprinting of essential oils contained in the different sage species as an analytical technique in its own right.

The results presented in this study demonstrate usefulness of PLC for preliminary fractionation of the essential oils derived from the sage species for the purpose of the GC-MS analysis. However, the quantities of essential oils contained in the different sage species can largely differ and hence, separate optimization of the essential oil aliquots applied to the preparative layers is needed for each individual sage species.

This paper is a preliminary report, which confirms usefulness of the low temperature TLC and the low temperature PLC for the analysis of essential oils contained in the sage species.

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