

## Chiral characterization of monoterpenes present in the volatile fraction of *Myrtus communis* L. growing in Algeria

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**Abstract.** The chiral and achiral monoterpenes present in the volatile fraction of *Myrtus communis* have been determined by headspace-Solide Phase MicroExtraction (HS-SPME) combined with enantioselective gas chromatography/mass spectrometry (GC/MS). HS-SPME has shown good potential to reproduce the same results as the commonly used hydrodistillation extraction technique. With circa 29 %, limonene was the major compound in the volatile fraction followed by cis- $\beta$ -terpineol,  $\alpha$ -pinene and eucalyptol with 23 %, 16% and 13%, respectively. The enantiomeric distribution of chiral monoterpenes revealed that for  $\alpha$ -pinene, the (+)-enantiomeric form was present with more than 95 %. Limonene was exclusively present as (+)-enantiomere while  $\beta$ -pinene,  $\beta$ -phellandrene and neomenthol showed rather a racemate.

**Keywords:** HS-SPME, monoterpenes, chiral analysis, *Myrtus communis* L., GC/MS.

### 1. Introduction

Plants produce a large number of volatile organic substances, which includes isoprene and terpenoid compounds, alkanes, alkenes, carbonyl compounds, alcohols, and esters. Terpenoid compounds are the principal components usually found in the volatile fraction [1]. These volatile compounds are responsible for multiple interactions between plants and other organisms, such as pollinating animals and predators of herbivore creatures [2] in addition to their well established therapeutic and pharmacological activities.

The genus *Myrtus* belongs to the family Myrtaceae and comprises about 50 species native to the Mediterranean basin. Among them, *Myrtus communis* is a familiar species. It is an evergreen shrub (1-3 m tall) with white flowers which grows spontaneously in many countries. It is traditionally used as antiseptic, disinfectant drug and hypoglycemic agent [3].

Nowadays, several extraction methods are available for extracting essential oils from plant materials. Hydrodistillation (HD), distillation-solvent extraction (SDE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) are among the most used extraction techniques. The extraction technique of Solid-Phase MicroExtraction (SPME) has considerable potential for use in studies of plant volatiles. Invented in 1989 by Pawliszyn and co-workers [4,5], SPME has been applied successfully in many fields of research, including pharmaceuticals, food research, and environmental organic pollutants in air (i.e., [6,7]) and water.

The consideration of stereochemical aspects in food analysis has been widely demonstrated. For instance, chiral analysis has been extensively applied to the quality assurance of natural flavors and essential oils and the differences in sensory properties and biological activities between pairs of enantiomers are well documented.

In the present work, SPME has been combined with chiral GC/MS to study monoterpene composition of myrtus. Here we report the enantiomeric and nonenantiomeric composition of monoterpenes present in the

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headspace of *Myrtus communis* growing in Algeria using HS-SPME and enantioselective gas chromatography/mass spectrometry.

## 2. Experimental

### 2.1. Headspace SPME

A manually operated SPME holder was used throughout these experiments. The three beds 50/30  $\mu\text{m}$  divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibre from Supelco (Taufkirchen, Germany) has been used in this study and selected based on its high performance towards monoterpenes (see [10]). Each new fibre was thermally conditioned before use, according to the manufacturer recommendations.

Headspace-SPME sampling was carried out by weighing 200 mg of detached leaves of myrtus into a 20 mL vial fitted with a Teflon/silicone septum (Supelco) and the SPME holder needle was inserted through the septum and (DVB-CAR-PDMS) was exposed to the headspace for a 5 min. Each extraction was repeated 3 times. In order to reach the equilibrium between the sample and the headspace, the samples were kept 30 min inside the vial before the start of the extraction.

### 2.2. Standards

GC grade standards of enantiomeric and non-enantiomeric monoterpenes were obtained from SIGMA-ALDRICH (Taufkirchen, Germany). These pure standards were used to: optimise the separation conditions; to determine the elution order of enantiomer pairs; and to provide positive identification of the monoterpenes present in the two plant species.

### 2.3. Chromatographic analysis

Immediately after SPME sampling, the SPME needle was introduced into the split/splitless injector of the gas chromatograph. A glass inlet liner with a narrow internal diameter (0.75 mm I.D., Supelco) was used in order to improve the GC resolution and the peak shape. Desorption was achieved in splitless mode at 250 °C for 2 min. These settings were found to be sufficient for a quantitative desorption of all the analytes studied. This was established by subjecting the analysed fibre to a second desorption and observing no carry-over peaks.

The analysis of SPME samples were conducted using gas chromatograph (Agilent Technologies a GC 6890A) coupled to a mass-selective detector (MSD 5973 *inert*) from the same company. The MS system was operated in electron impact mode with the following conditions: ionization potential 70 eV; source temperature 230°C. The MS system was operated in scan mode (30-350 u). The enantiomeric and non-enantiomeric monoterpenes were separated using a Cyclodex-B capillary column (30 m-long, 0.256 mm I.D., 0.25  $\mu\text{m}$  film thickness) supplied by J & W Scientific (Folsom, CA, USA). With a helium (Messer Griesheim 6.0) gas carrier flow rate of 1 mL min<sup>-1</sup>, the column temperature was maintained at 40°C for 5 minutes, then increased to 180°C at 1.5°C per minute as previously established by Yassaa et al. [9].

Retention time confirmation of individual chiral and non-chiral monoterpenes was performed by analyzing pure standards under the same conditions. The elution order of enantiomers was further confirmed by comparison with our previous work [9,10].

## 3. Results and discussion

Very good separation of enantiomeric pairs of monoterpenes has been obtained using HS-SPME for sampling the volatile fraction and beta-cyclodextrin capillary GC-MS for separation and analysis as demonstrated in Fig. 1 for monoterpene standards. The CAR/DVB/PDMS liquid-solid mixed fibre coating was particularly useful to adsorb wide range of volatilities comprising mono and sesquiterpenes. While DVB phase is mainly mesoporous and ideal for trapping C6-C15 analytes including monoterpenes (C10), CAR is microporous and efficiently traps C2-C6 analytes. Owing to the high sensitivity of this fibre, 5 min sampling time at room temperature was found enough to quantitatively adsorb terpenic compounds without requirement to sample heating. The heating procedure has been avoided in order to prevent possible decomposition of heat-sensitive molecules. The sampling time was selected based on adsorption kinetics which have been established on gas standard mixture of terpenes (see [8]).

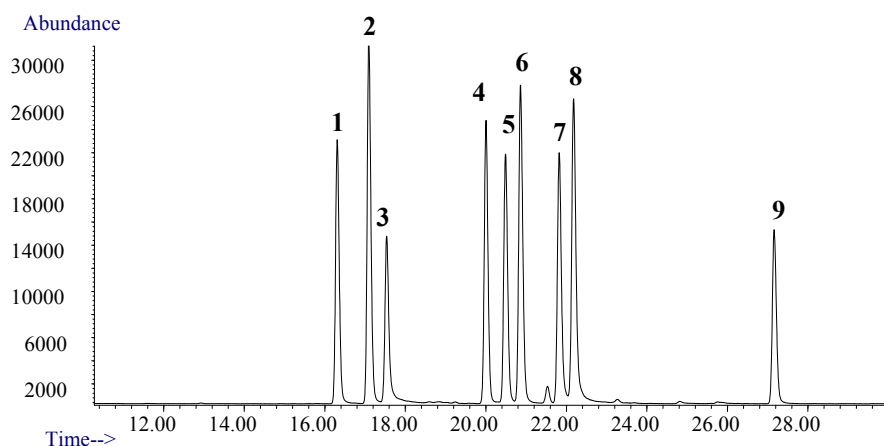


Fig. 1. Reconstructed mass chromatographic profiles (m/z 93) of monoterpenes in standard. (1): (-)- $\alpha$ -pinene, (2): (+)- $\alpha$ -pinene, (3):  $\beta$ -Myrcene, (4): (+)- $\beta$ -carene, (5): (+)- $\beta$ -pinene, (6): (-)- $\beta$ -pinene, (7): (-)-limonene, (8): (+)-limonene and (9): 1,8-Cineol.

Thanks to capillary diffusion system, it was possible to generate monoterpene standards in the gas phase and calibrate the SPME fibre with the same way as with sampling in the headspace of plant material. The enantiomeric pairs of monoterpenes were also identified using pure chiral compounds as well as the elution orders obtained in our previous investigations employing beta-cyclodextrin chiral capillary column.

### 3.1. Non-enantiomeric distribution of monoterpenes

Fig. 1 shows the composition of monoterpenes in the volatile fraction of *M. communis* extracted by HS-SPME. Limonene was the major monoterpene with almost 29 %. Cis- $\beta$ -terpineol,  $\alpha$ -pinene and eucalyptol represented 23 %, 16 % and 13 %, respectively. Para-cymene was also important and accounted for circa 7 %.

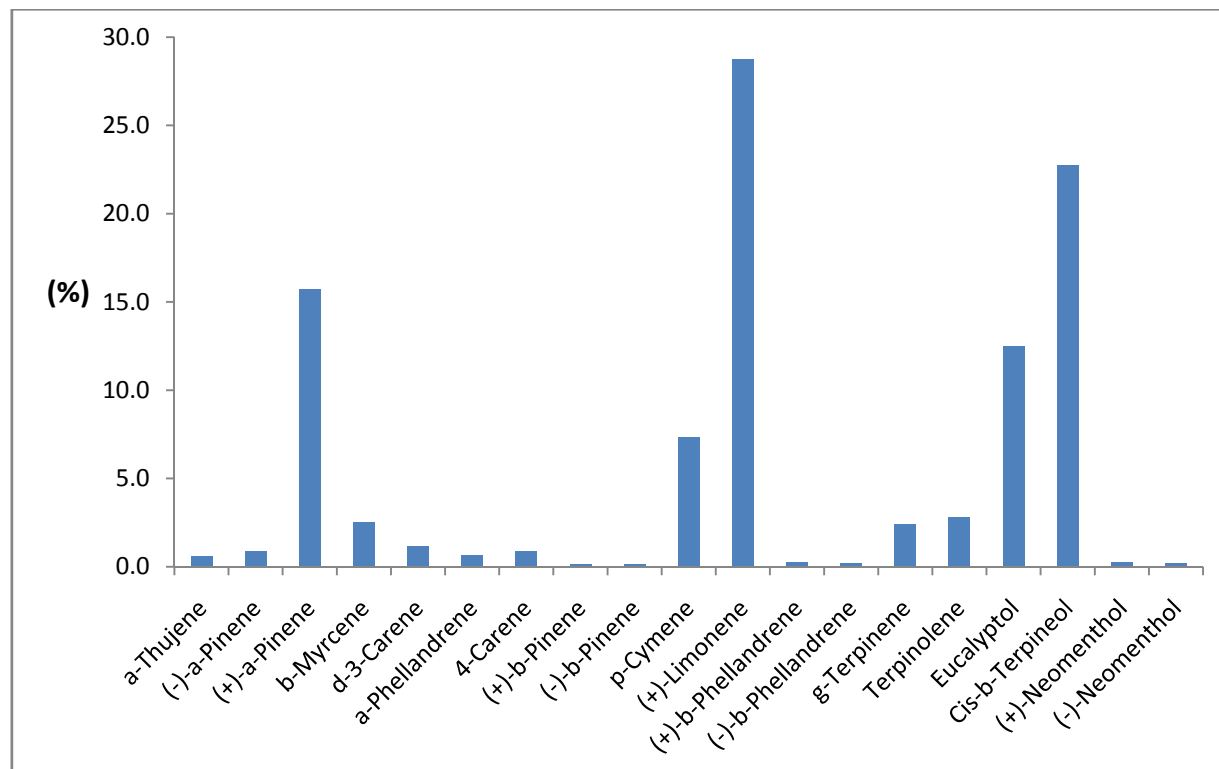


Fig. 2. Composition of monoterpenes in the volatile fraction of *Myrtus communis*

### 3.2. Enantiomeric distribution of monoterpenes

In order to highlight the enantiomeric distribution in a given monoterpene, we preferred the use of relative content (%) of a given enantiomer in the enantiomeric pair rather than enantiomeric excess commonly reported. This enantiomeric percentage is equal to 100% if the given monoterpene is present with only one enantiomeric form and it equals to 50% if the given monoterpene presents a racemate. The enantiomeric distributions of monoterpenes in the headspace of *M. communis* are displayed in Fig. 3. (+)-Antipode of  $\alpha$ -pinene was largely dominated over (-)-antipode with almost 95 %. However, limonene was exclusively present as (+)-enantiomer in all samples.  $\beta$ -Pinene,  $\beta$ -phellandrene and neomenthol showed only a slight preference to (+)-enantiomer.

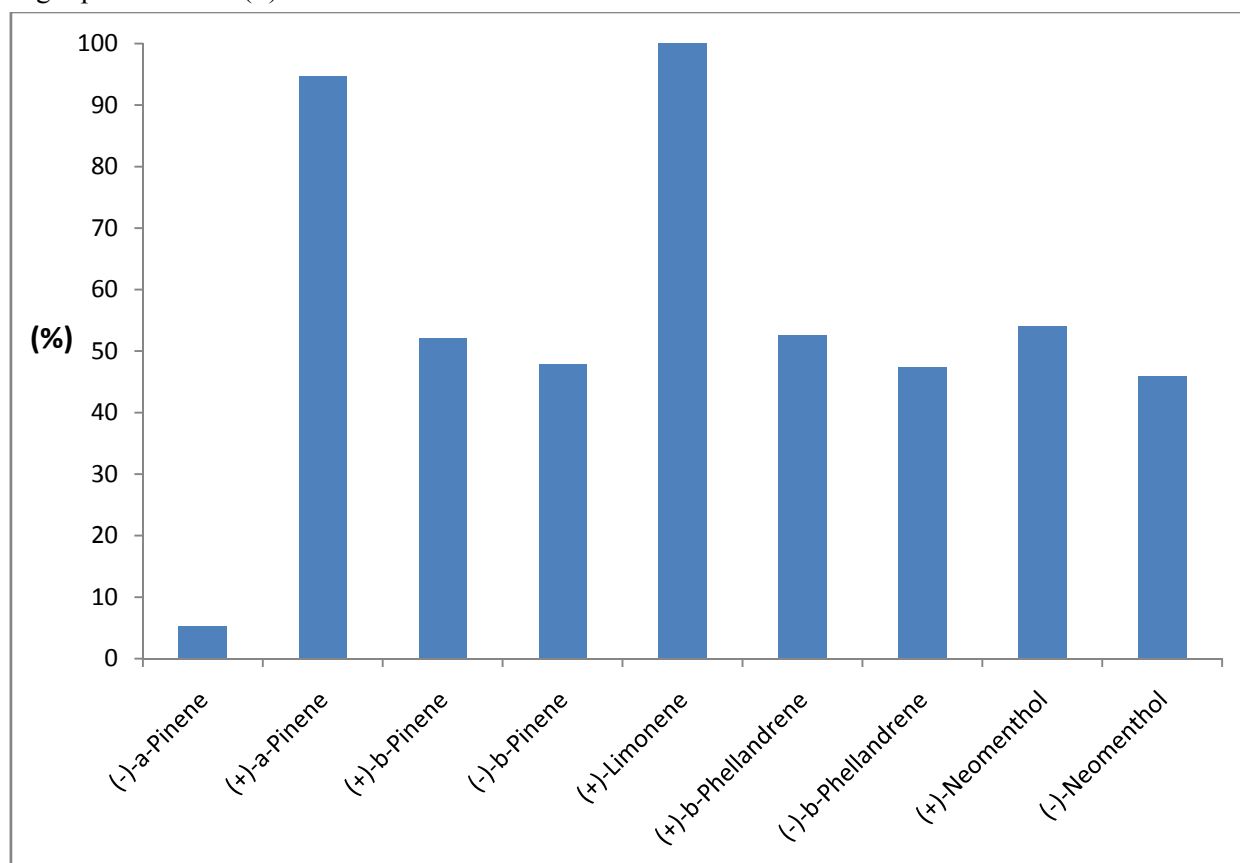


Fig. 3. Enantiomeric distribution of monoterpenes in the volatile fraction of *Myrtus communis*

#### 4. Conclusion

The *monoterpene* fraction of *M. communis* growing in Algeria was characterized by high content of limonene, *cis*- $\beta$ -terpineol, *eucalyptol* and  $\alpha$ -pinene.

The chiral analysis of monoterpenes present in the headspace of aerial part of *Myrtus communis* were performed using HS-SPME and enantioselective GC/MS. Large variability in the enantiomeric distributions of monoterpenes were observed. While limonene was present exclusively as (+)-antipode,  $\alpha$ -pinene showed a strong preference to (+)-enantiomer.  $\beta$ -Pinene,  $\beta$ -phellandrene and neomenthol showed rather a racemate.

Headspace SPME has shown good potential to study chiral and achiral composition of monoterpenes and therefore represent alternative to commonly used extraction techniques. It is cheap, simple, sensitive, fast and completely eliminates the use of organic solvent. This easily automated method when combined with a portable and fast GC, offers good opportunities for *in vivo* and *in-field* study of plant-insect interactions and also for quality control of plant derivative products.

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