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# *Larrea divaricata* volatilome and antimicrobial activity against *Monilinia fructicola*

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ABSTRACT

*Monilinia fructicola* is the most destructive pre- and postharvest pathogen in stone fruit worldwide. A great attention has been paid to plant extracts, a rich source of bioactive chemicals, for the sustainable pest control. Volatile organic compounds from plant matrices have proved important antimicrobial activity. In the present work, an analytical procedure for the characterization of the volatile profile in *L. divaricata* extract by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography/mass spectrometry (GC–MS) was proposed. Moreover, the biological activity of *L. divaricata* extract was evaluated for their contact-phase and vapor-phase effects on *M. fructicola* mycelial growth. In order to improve the absorption of volatile compounds, the following experimental parameters were optimized: fiber coatings, time and temperature extraction. As a result of the optimization, CAR/PDMS fibers, 30 °C and 30 min were selected for the extraction volatile compounds from *L. divaricata* extract. A total of 79 VOCs were identified in the extract. Among them, aromadendrene was found at highest concentration. Other bioactive volatile compounds were detected such as eugenol, carvacrol, limonene and thymoquinone. The antimicrobial activity of *L. divaricata* extract on *M. fructicola* mycelial growth was evaluated under contact- and vapor-phase conditions. *L. divaricata* extract showed significant antimicrobial activity on *M. fructicola* mycelial growth in both conditions. The results reveal that *L. divaricata* is a promising antifungal agent which could be used as bio-fungicide in the protection of fruits and vegetables against postharvest infections.

## 1. Introduction

*Monilinia fructicola* (Winter) is the most destructive pre- and postharvest pathogen in stone fruit worldwide [1, 2]. This pathogen causes brown rot leading to important economic losses in food industry [3]. Current management of this disease still relies heavily on the use of synthetic chemical fungicides [4]. However, public concern over the potential impact of fungicides on the environment and human health, as well as the development of resistance strains, have promoted the search of novel methods for the control of *M. fructicola* [5]. Among the alternative treatments to chemical products, plant extracts have received growing attention due to their potential antimicrobial properties [6]. Several studies have demonstrated that plant extracts constitute a rich source of bioactive chemicals such as phytosterines, saponines, phenolic compounds, alkaloids, volatile compounds, glucosinolates, quinons, tannins, sterols [7, 8].

*Larrea divaricata* Cav. (Zygophyllacea) is a perennial woody shrub with widely distribution in Argentina. In folk medicine, different *L. divaricata* extracts have been used as anti-inflammatory, antirheumatic, dysphoretic, amenagogic and antimicrobial agents [9, 10]. Although phytochemical studies had reported the presence of secondary metabolites such as lignans, essential oils, phenolic compounds and glycosides [11, 12], the volatile profile of *L. divaricata* extract has not yet been reported.

The totality of volatile organic compounds (VOCs) synthesized by plants has been termed the volatilome (or volatome) [13], being terpenes, fatty acid derivatives, benzenoids, phenylpropanoids and amino acid derived metabolites, the most relevant groups. These compounds play an important role in plants; acting as signals in plant-plant communication, in the attraction of pollinators as well as in the defense against organisms pathogens [14, 15]. Thus, the volatile organic compounds in plant extracts could play an effective role in the control of postharvest diseases.

Analytical procedures for the analysis of volatile constituents from

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plants require sample preparation methods for the extraction and preconcentration of target compounds [16, 17]. Headspace-solid-phase microextraction (HS-SPME) is one of the most commonly used methods combining sampling, extraction, concentration and cleanup in a single step [15, 18]. This technique presents remarkable advantages for volatilome analysis, including high selectivity and sensitivity, possible automation of extraction, solvent-free process and small sample volume [14]. Extraction efficiency is affected by experimental factors [19, 20]. The fiber coating is a key factor in SPME, different coatings interact with analytes through different adsorption/absorption mechanisms [16, 21, 22].

In the present work, an analytical procedure for the characterization of the volatile profile in *L. divaricata* extract by headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography/mass spectrometry (GC–MS) was proposed. Moreover, the biological activity of *L. divaricata* extract was evaluated for their contact-phase and vapor-phase effects on *M. fructicola* mycelial growth.

## 2. Materials and methods

### 2.1. Plant extract preparation

*Larrea divaricata* (10 plants) was cultivated at a greenhouse under natural radiation were identified by means of morphological, anatomical, and histochemical analyses. Leaves were harvested during flowering, immediately frozen in liquid nitrogen and then lyophilized in darkness. The extract was prepared from *L. divaricata* leaves (20 g) using the methodology proposed by Widmer and Laurent with modifications [23]. Then, the leaves were placed into Erlenmeyer flasks containing 200 mL of distilled water and autoclaved for 45 min at 121 °C at 1 atm. The liquid was filtered and the volume was reduced by boiling in laminar-flow hood to an approximate volume of 20 mL. Finally, the extract was centrifuged and the supernatant was autoclaved for 20 min at 121 °C at 1 atm. The extracts were stored at 4 °C until analysis.

### 2.2. HS-SPME/GC–MS

#### 2.2.1. Reagents and materials

The SPME tested fibers were Stable Flex™ polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm), polydimethylsiloxane (PDMS, 100 μm), Stable Flex™ carboxen/polydimethylsiloxane (Car/PDMS, 85 μm), and Stable Flex™ divinylbenzene/carboxen/polydimethylsiloxane (Car/PDMS/DVB, 50/30 μm); SPME support and holder manual for SPME were purchased from Supelco (Bellefonte, PA, USA). Magnetic stirrer bar and 10 mL glass vial with PTFE-faced silicone septa were supplied by Varian (Lake Forest, CA, USA). Magnetic stirrer Ret Control Visc IKAMAG Safety Control (IKA, Wilmington, USA) was used. Fibers were conditioned following the instructions from manufacturers, and cleaned at 250 °C for 5 min. Ultrapure water was obtained from a RiO/Elix3-Sinergy185 purification system (Millipore, Sao Pablo, Brazil). The internal standard (IS) anisole was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Methanol chromatographic grade was purchased from Merck (Darmstadt, Germany).

#### 2.2.2. HS-SPME conditions

In order to improve the absorption of volatile compounds, the following experimental parameters were optimized: four coating fibers (PDMS, CAR/PDMS, DVB/CAR/PDMS and PDMS/DVB); extraction temperature (25–40 °C); extraction time (10–60 min). As a result of the optimization, *L. divaricata* extract (2 mL) was spiked with an internal standard solution to obtain a final concentration of 1 μg $mL^{-1}$. Then, the extract was placed into a 10 mL glass screw-top vial with polytetrafluoroethylene/silicone septa. The sealed vial was placed in a thermoblock at 30 °C and stirrer at 1000 rpm. After thermal equilibration for 30 min, the SPME needle (CAR/PDMS fiber) was inserted through the septum and the fiber exposed in the HS (2 cm) for 30 min. After extraction, the SPME fiber was removed from the vial and inserted into the injection port of GC–MS. All the analyses were performed in triplicate.

#### 2.2.3. GC–MS analyses

GC–MS analyses were performed on a Varian CP-3800 gas chromatograph with a Saturn 2200 Ion Trap Mass Spectrometric detector (Varian, Walnut Creek, CA, USA). The system was operated by Saturn GC–MS Workstation software Version 6.41. The column was a Factor Four TM capillary column VF-5MS (50 m × 0.25 mm I.D., with 0.25 μm film thickness; Varian, Lake Forest, CA, USA).

The oven temperature program was: 40 °C (2 min), 4 °C $min^{-1}$ to 220 °C (5 min), 30 °C $min^{-1}$ to 280 °C (10 min). The 1079 injector was equipped with a glass insert for SPME and the temperature was programmed isothermally. Desorption temperature was set at 220 °C based on previous studies carried out in our laboratory [24]. The injection was in split/splitless mode for 2 min, and then the split ratio was 1/50. The carrier gas flow rate (Helium 6.0, Linde, Buenos Aires, Argentina) was constant at 1 mL $min^{-1}$. The electron impact energy was 70 eV. Transfer line and ion trap temperature were 200 °C and manifold temperature was 40 °C. Mass spectrometry acquisition was carried out using the continuous scanning mode (5 μscan $s^{-1}$) from *m/z* 30 to *m/z* 300. Qualitative analysis was performed by comparison of mass spectra and retention times with standard compounds and/or by comparison of the mass spectrum with those of reference compounds in the NIST Mass Spectral Search Program (NIST Version 2.0). The relative amounts of volatile compounds were expressed with respect to IS (R2 = 0,998) as μg $g^{-1}$ of plant extract. The volatile compounds were expressed as μg $mL^{-1}$. All data were reported as the mean ± SD for three replications and analyzed by Statgraphics Plus Version 5.0 program (Manugistic Inc., Rockville, MD, USA) [32].

### 2.3. Antimicrobial activity of Larrea divaricata extract on Monilinia fructicola mycelial growth

*Monilinia fructicola* was obtained from the microorganism's collection of the Cátedra de Fitopatología de la Facultad de Ciencias Agrarias de la Universidad Nacional de Cuyo (Mendoza, Argentina). The fungus was maintained on Potato Dextrose Agar (PDA). The effect of *Larrea divaricata* extract on *M. fructicola* mycelial growth was evaluated under contact- and vapor-phase conditions. The vapor phase was tested by using the technique of Ma et al. [25], in Petri plates (90 mm × 20 mm; 130 mL air space) containing Potato Dextrose Agar (PDA) [25]. Subsequently a 4 mm disk of *M. fructicola* obtained from the edge of 10 days old culture was placed in the center of Petri dishes. A filter paper disk (4 cm in diameter), impregnated with the extract at different concentrations (50 to 750 mg $mL^{-1}$), was placed on the inner surface of the Petri plate lid, in order to prevent direct contact of *M. fructicola* with the extract. Control was performed by replacing extract with sterile distilled water. Inoculated Petri plates were sealed immediately and were incubated at 21 ± 2 °C for 5 days. Each treatment contained three replicates and the assay was repeated three times. After incubation period, the *M. fructicola* colony area was measured using Axio Vision 4.8 software. The results were expressed as inhibition percentage of mycelial growth using the formula (1).

$$\text{Inhibition } (\%) = [(C - T)/C] * 100, \quad (1)$$

where C and T correspond to the fungus colony area ($cm^2$) of control and treatments ($cm^2$), respectively.

In the contact phase assay, the biological activity of *L. divaricata* extract against *M. fructicola* was tested using the solid agar bioassay [26]. In this case, a 4 mm disk of *M. fructicola* was placed in the center of Petri dishes containing Potato Dextrose Agar (PDA) amended with different concentrations of extract (50 to 750 mg $mL^{-1}$). Control plates were performed simultaneously, using the growth medium without

extract. A positive control was included using tebuconazole, a commercial fungicide. Stock suspension was prepared by mixing the fungicide in sterile-distilled water at the recommended concentration (tebuconazole 0.3 mL $L^{-1}$). Each treatment contained three replicates and the assay was repeated three times. Petri plates were incubated at 21 ± 2 °C for 5 days. After incubation period, the *M. fructicola* colony area was measured using Axio Vision 4.8 software. The results were expressed as inhibition percentage of mycelial growth using the formula (1).

All data were reported as the mean ± SD for three replicates. Comparison of the means was achieved by analysis of variance (ANOVA) according to Tukey test, $\alpha < 0.05$ using Statgraphics Plus Version 5.0 program (Manugistic Inc., Rockville, MD, USA).

## 3. Results and discussion

### 3.1. SPME optimization

The sensitivity and selectivity of a SPME method can be affected by the type of fiber coating, temperature and time of extraction. These variables were optimized by means of the one-at a time procedure to determine the most suitable conditions for the analysis of volatile compounds of *L. divaricata* extract.

Four different fibers (PDMS, PDMS/DVB, CAR/PDMS and DVB/CAR/PDMS) with a wide range of polarities were evaluated according to their extraction efficiency, expressed as the amount of retained compounds. As can be seen in Fig. 1, the CAR/PDMS fiber showed the highest performance in the extraction of volatile compounds being selected for the following optimization assays. Previous studies have used CAR/PDMS fiber for volatile compounds extraction from plants demonstrating high extraction efficiency when compared with others fiber coatings [27–29].

Then, the effect of extraction temperature was investigated at 25 °C, 30 °C, and 40 °C. The best results concerning extraction efficiency were obtained at 30 °C, consequently it was considered for subsequent optimization (Fig. 2).

Finally, the extraction time of volatile compounds in *Larrea divaricata* extract was studied in the range of 10 to 60 min. The highest extraction efficiency was reached at 30 min (Fig. 3).

As a result of the optimization, CAR/PDMS fibers, 30 °C and 30 min were selected for the extraction volatile compounds from *L. divaricata* extract.

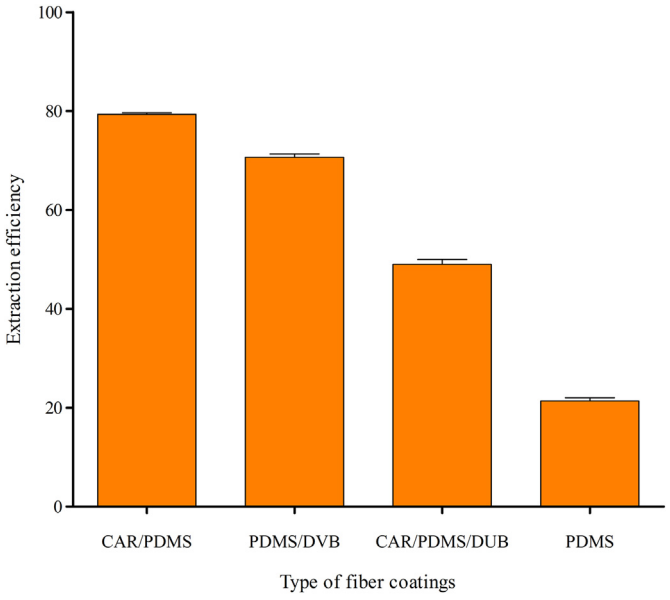


**Fig. 1.** Extraction efficiency of volatile compounds from *Larrea divaricara* extract using different SPME fiber coating (SPME conditions: extraction temperature 40 °C and extraction time 40 min).

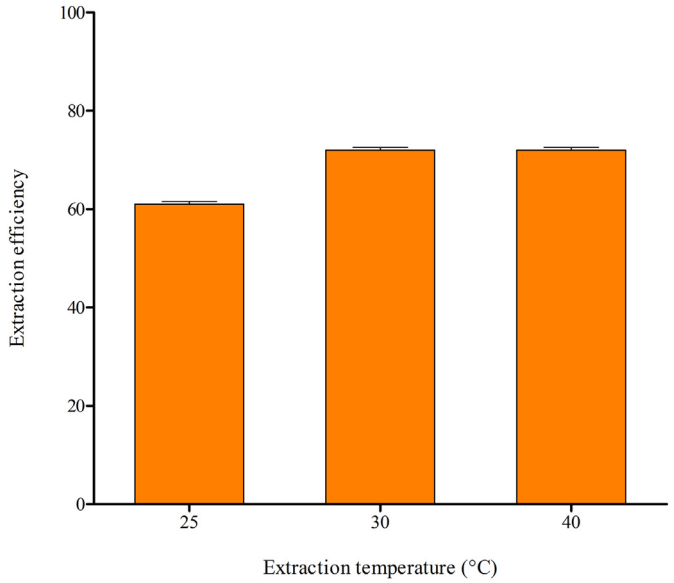


**Fig. 2.** Extraction efficiency of volatile compounds from *Larrea divaricara* extract using different extraction temperature (SPME conditions: fiber CAR/PDMS and extraction time 40 min).

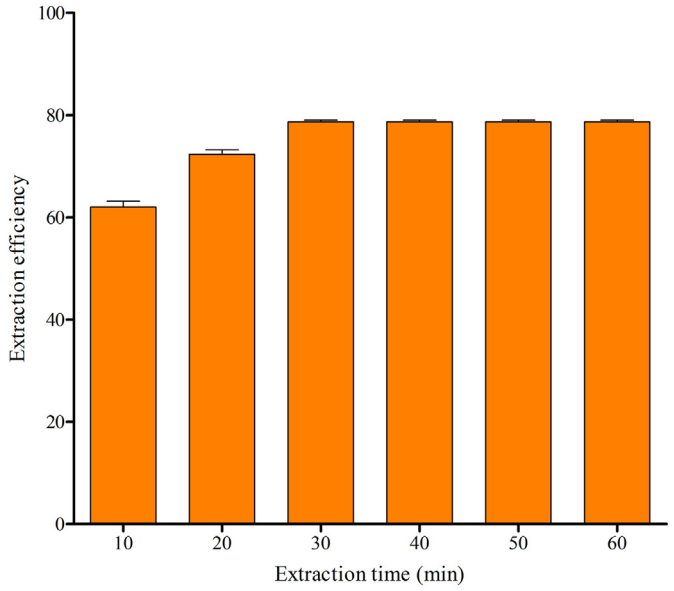


**Fig. 3.** Extraction efficiency of volatile compounds from *Larrea divaricara* extract using different extraction time (SPME conditions: fiber, CAR/PDMS and extraction temperature 30 °C).

### 3.2. Performance of the analytical method

Analytical quality parameters were evaluated to assess the performance of the HS-SPME/GC–MS procedure with the selected conditions. The repeatability (within day precision) of the method was determined by a series of six replicates carried out on the same day. In all cases, the percentage of relative standard deviation (RSD %) was lower or equal to 12%. The reproducibility of the method was tested in different days with six replicates analyses each day, the values were lower or equal to 11%.

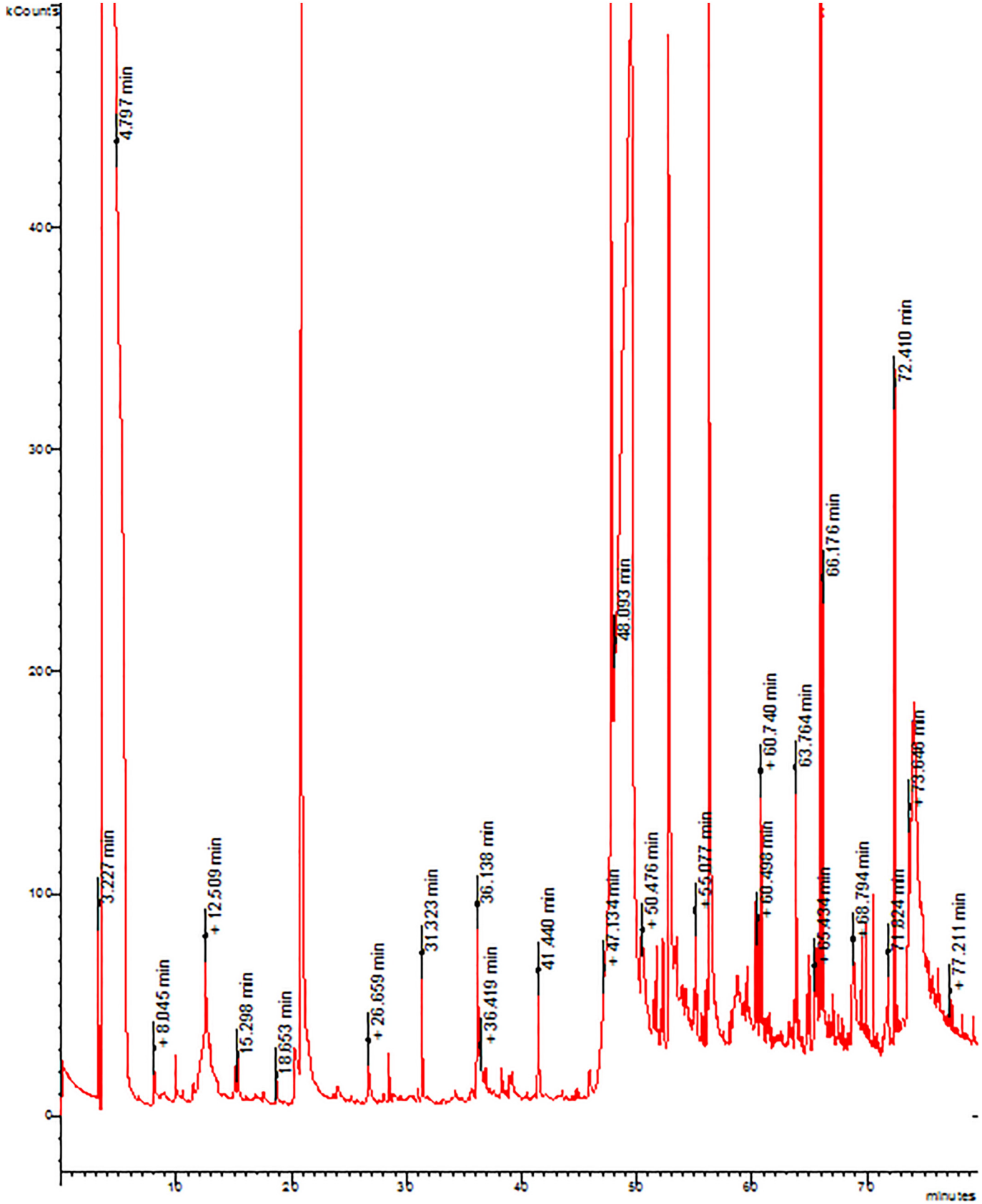


**Fig. 4.** GC/MS chromatogram of *Larrea divaricata* extract volatilome.

**Table 1**
Concentration of volatile organic compounds (μg mL$^{-1}$) determined by HS-SPME/GC–MS analysis in *L. divaricata* extract.

| TR[a] | Class[b] | Compounds | Concentration[c] | |
|---|---|---|---|---|
| 23.2 | O | Hydrocinnamoyl bromide | 4.500 | 0.003 |
| 24.5 | HA | 4-Ethyltoluene | 29.910 | 0.001 |
| 27.6 | ES | Hexanoic acid. ethyl ester | 10.720 | 0.001 |
| 29.1 | O | 2-Pentanone. 3.4-epoxy-3.4-dimethyl- | 43.930 | 0.003 |
| 29.1 | AL | p-Menth-1-en-4-ol | 5.240 | 0.001 |
| 29.4 | ES | E-9-methyl-8-tridecen-2-ol.acetate | 3.900 | 0.001 |
| 30.2 | AL | 14-Methyl-8-hexadecyn-1-ol | 2.040 | 0.000 |
| 31.0 | T | Limonene | 10.870 | 0.005 |
| 33.4 | ES | Methyl (9E)-9-octadecen-12-ynoate | 15.520 | 0.000 |
| 34.3 | A | 1.3.4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde | 4.420 | 0.001 |
| 35.7 | O | *N*-Methoxy-4-methylbenzamide | 14.540 | 0.000 |
| 36.7 | ES | Methyl eicosa-8.11.14-trienoate | 1.460 | 0.001 |
| 36.9 | O | Diethyl bis(trimethylsilyl) orthosilicate | 2.160 | 0.001 |
| 37.5 | HA | p-Isopropenyl toluene | 16.120 | 0.002 |
| 38.9 | H | 4-*tert*-Pentyl-1-cyclohexene | 5.370 | 0.001 |
| 39.7 | ES | 2.2-Dimethylpropionic acid. 2-ethylhexyl ester | 2.390 | 0.000 |
| 41.2 | T | Myrcenol | 0.160 | 0.001 |
| 42.5 | O | Methyl 2-methyl-3-oxobutyldithiocarbamate | 0.220 | 0.001 |
| 43.2 | E | 1.3-Dioxolane. 2-benzyl- | 3.620 | 0.000 |
| 43.4 | AL | 5-Octen-2yn-4ol | 12.070 | 0.001 |
| 45.3 | K | 2-(1-Cyclopent-1-enyl-1-methylethyl) cyclopenone | 50.520 | 0.000 |
| 45.7 | T | 4-Terpineol | 12.820 | 0.001 |
| 46.3 | T | 4-Carvomenthenol | 13.270 | 0.001 |
| 46.8 | O | Acetohydrazide.2-hydroxy-2-phenyl-N2-(1-methylcyclohexen-4-yl)- | 3.330 | 0.001 |
| 47.2 | AL | 1-Methyl-4-(à-hydroxyisopropyl)benzene | 7.470 | 0.010 |
| 48.2 | A | Safranal | 11.320 | 0.002 |
| 48.5 | ES | Sabinyl acetate | 4.810 | 0.002 |
| 48.9 | AL | Benzenemethanol. à-(1.1-dimethylethyl)- | 1.400 | 0.001 |
| 49.3 | A | Isoxylaldehyde | 0.230 | 0.001 |
| 49.4 | A | p-Menth-1-en-9-al | 4.750 | 0.001 |
| 50.3 | F | m-Cumenol | 24.960 | 0.001 |
| 50.3 | A | Terephthaldialdehyde monodiethyl acetal | 2.210 | 0.000 |
| 50.4 | H | Bornylene | 2.960 | 0.001 |
| 50.7 | O | 2.4-Dimethoxyacetophenone | 1.020 | 0.000 |
| 50.7 | K | 7a-Methyl-1.2.3.6.7.7a-hexahydro-5H-inden-5-one | 0.410 | 0.000 |
| 52.3 | K | Thymoquinone | 25.640 | 0.001 |
| 52.4 | E | Tricyclo[4.3.1.1(3.8)] undecane.1-methoxy- | 0.300 | 0.002 |
| 54.2 | O | 5-Hydroxy-4a-methyloctahydro-1(2H)-naphthalenone | 17.990 | 0.001 |
| 54.5 | F | p-Cresol. 2-ethyl- | 29.460 | 0.002 |
| 55.2 | AL | Cuminol | 3.150 | 0.001 |
| 55.6 | E | 2-Methyl-5-[1-methyl-1-(5-methyl-2-furyl) ethyl]furan | 7.390 | 0.001 |
| 55.7 | F | Carvacrol | 0.440 | 0.001 |
| 56.9 | HA | Cumol | 2.280 | 0.001 |
| 57.2 | H | 1.5.9.9-Tetramethyl-2-methylene-spiro[3.5] non-5-ene | 29.460 | 0.000 |
| 57.2 | AL | *trans*-Pinocarveol | 12.740 | 0.002 |
| 57.5 | O | Eugenol | 1.080 | 0.002 |
| 58.3 | H | Cyclohexene.1.3-diisopropenyl-6-methyl- | 2.800 | 0.000 |
| 58.6 | A | 4.4-Dimethylpent-2-enal | 13.040 | 0.001 |
| 59.2 | O | 3-Allyl-2-methoxyphenol | 12.330 | 7.120 |
| 59.6 | O | *trans*-m-Propenyl guaiacol | 39.510 | 0.002 |
| 60.5 | K | 4-(7.7-Dimethylbicyclo[4.1.0]hept-3-en-3-yl)-2-butanone | 0.070 | 0.001 |
| 61.4 | HA | 1.4.6-Trimethyl-1.2-dihydronaphthalene | 0.210 | 0.001 |
| 61.5 | H | Oxacyclotetradeca-4.11-diyne | 25.040 | 0.001 |
| 61.7 | O | 2.3 Dehydro-4-oxo-.beta.-ionol | 1.290 | 0.004 |
| 61.9 | HA | 1.2-Dimethylnaphthalene | 2.920 | 0.003 |
| 62.3 | O | Thiocyanic acid.1.1.3-trimethyl-3-phenylbutyl ester | 4.710 | 0.000 |
| 62.9 | AL | Dihydromyrcenol | 0.020 | 0.000 |
| 63.7 | HA | 1.4-Dihydrobiphenyl | 2.330 | 0.001 |
| 63.7 | K | 6-(3-Isopropenylcycloprop-1-enyl)-6-methylhept-3-en-2-one | 0.950 | 0.001 |
| 64.9 | H | Isolongifolene. 4.5.9.10-dehydro- | 22.930 | 0.001 |
| 64.9 | O | 2-(2-Methoxyphenyl)-2-propanol | 6.670 | 0.001 |
| 65.5 | O | 4.6.10.10-Tetramethyl-5-ocxatricyclo [4.4.0.0(1.4)]decen-2-en-7-ol | 0.020 | 0.000 |
| 65.7 | O | 3-Buten-2-one.4-(5.5-dimethyl-1-oxaspiro[2.5] oct-4-yl) | 1.070 | 0.001 |
| 66.0 | F | Phenol. 2.5-di-tert-butyl- | 1.040 | 0.001 |
| 66.2 | E | Aromadendrene oxide-(2) | 311.440 | 0.001 |
| 67.8 | F | 2.4.6-Triisopropylphenol | 1.590 | 0.001 |
| 68.3 | ES | Actinidiolide. dihydro- | 0.130 | 0.001 |
| 68.5 | O | 2-(Phenylsulfanyl)cyclopentanone | 1.390 | 0.010 |
| 68.9 | O | 5-Hydroxy-2.3.3-trimethyl-2-(3-methyl-buta-1.3-dienyl)-ciclohexanone | 7.550 | 0.001 |
| 69.5 | O | Apocynin | 0.350 | 0.001 |
| 71.0 | AL | 2.5.8-Trimethyl-1.2.3.4-tetrahydro-1-naphthol | 2.670 | 0.001 |
| 71.6 | AL | Bicyclo[4.4.0]dec-5-ene.1.5-dimethyl-3-hydroxy-8-(1methylene-2-hydroxyethyl-1)- | 4.910 | 0.001 |
| 71.8 | O | Cumopyran | 0.410 | 0.002 |
| 71.8 | H | Dysoxylonene | 1.200 | 0.001 |
| 72.2 | AL | 5-(7a-Isopropenyl-4.5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol | 0.680 | 0.001 |
| 72.2 | AL | Perhydrocyclopropa[*e*]azulene-4.5.6-triol.1.1.4.6-tetramethyl | 4.770 | 0.002 |
| 72.4 | ES | Methyl (4Z.7Z.10Z.13Z.16Z.19Z)-4.7.10.13.16.19-docosahexaenoate | 2.390 | 0.001 |
| 72.5 | ES | Heptafluorobutyric acid. n-tridecyl ester | 3.950 | 0.001 |
| 72.7 | O | 2.7-Octanedione.4.4-dimethyl-3-[2-(1-hydroxy-1-methylethyl)-3-methyl-3-butenylidene]- | 4.320 | 0.000 |

[a] RT retention time.
[b] H hydrocarbon. HA aromatic hydrocarbons. K ketone. A aldehydes. AL alcohol. E ether. ES esters. T terpene. O others.
[c] Means ± standard deviation of the concentration of volatile compounds in μg mL$^{-1}$ in relation to the concentration of IS.

### 3.3. Volatile profile of Larrea divaricata extract

The HS-SPME optimized method was applied and the volatile compounds from *Larrea divaricata* extract were characterized by GC–MS. Typical chromatogram corresponding to *L. divaricata* extract is shown in Fig. 4.

Seventy nine different volatile compounds were identified and quantified in *L. divaricata* extract belonging *Eucalyptus globulus* to various compound classes: hydrocarbons, aromatic hydrocarbons, ketones, aldehydes, alcohols, phenol, esters, ether, terpene and other (Table 1). Analyzing the results obtained, most of the compounds were found at concentrations between 0.02 and 50 μg mL$^{-1}$. Interestingly, aromadendrene oxide-(2) belonging to esther class, showed the highest concentration (311.44 μg mL$^{-1}$). Several authors have been reported antimicrobial activity of this compound against different microorganism pathogens [30, 31]. Mulyaningsih et al. [32] demonstrated that aromadendrene was the main compound present in the oil. Additionally, these authors reported that the aromadendrene was able inhibit the microbial growth of *Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus pyogenes*, *S. agalactiae* and *Enterococcus faecalis* with minimal inhibitory concentration (0.2 and 2 mg mL$^{-1}$) [32].

Moreover, as can be seen in Table 1, others bioactive compounds in *L. divaricata* extract were determined. Eugenol, carvacrol, limonene and thymoquinone were found. Different authors have reported antioxidant and antimicrobial activities of these volatile compounds [33, 34].

These results suggest that *L. divaricata* extract is a potential source of bioactive compounds that could be used as an alternative to synthetic chemical fungicides in postharvest.

### 3.4. Antimicrobial activity of Larrea divaricata extract on Monilinia fructicola mycelial growth

In order to evaluate the antimicrobial activity of *L. divaricata* extract against *M. fructicola* mycelial growth, under contact- and vapor-phase conditions were tested. For this purpose, the following extract

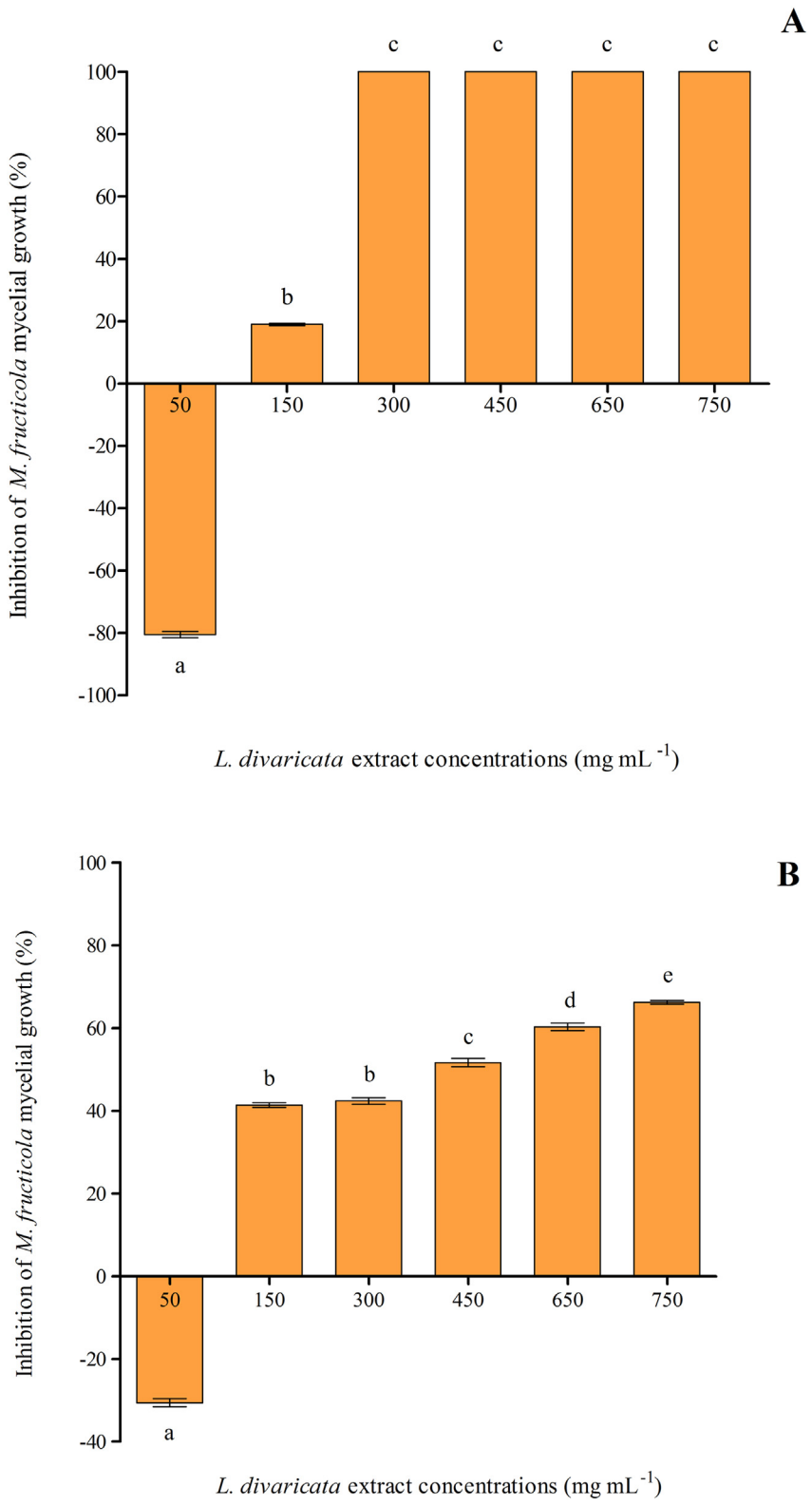


**Fig. 5.** Antimicrobial activity of different dose of *L. divaricata* extract against *M. fructicola* mycelial growth, A) under contact-phase; B) vapor-phase. Diverse letter indicate differ significantly according to Tukey test, $\alpha < 0.05$.

concentrations were selected: 50, 150, 300, 450, 650 and 750 mg $mL^{-1}$.

Analyzing the results obtained (Fig. 5) the inhibition of the mycelial growth was dose-dependent in both conditions (contact and vapor phase). In addition, the lowest concentration tested (50 mg $mL^{-1}$) presented stimulatory effect on *M. fructicola* mycelial growth, whereas highest concentrations showed inhibitory activity. Such dose-dependent behavior has already reported for other plant extracts as well as for pure bioactive compounds. Some reports demonstrated that sub-inhibitory concentrations stimulated fungal growth [35, 36].

As can be seen in Fig. 5a, the extract in the contact-phase at

150 mg mL$^{-1}$ inhibited 20% *M. fructicola* mycelial growth, while at 300 mg mL$^{-1}$ the total inhibition was achieved. Besides, tebuconazole (positive control) completely inhibited *M. fructicola* mycelial growth. In the vapor phase (Fig. 5b), the extract at low concentration (150 mg mL$^{-1}$) revealed a great potential in the inhibition of *M. fructicola* (40%). However, under this condition the highest inhibition obtained was of 67% even at the maximum concentration tested (750 mg mL$^{-1}$). Therefore, *L. divaricata* extract at higher concentrations was more effective in the contact phase than in volatile phase. Nevertheless, it is worth mentioning that at 150 mg mL$^{-1}$ the volatile phase showed a better performance.

Several reports have demonstrated the antimicrobial activity of *Larrea divaricata* extracts against phytopathogens [11, 37]. Hapon et al. [38] demonstrated that this extract at 200 mg mL$^{-1}$ inhibited *Botrytis cinerea* mycelial growth about 94% [38]. Stege et al. reported inhibitory activity of aqueous extract of *L. divaricata* on the growth of *Helicobacter pylori* (40 mg mL$^{-1}$) [39]. Besides, other research studied the effectiveness of organic extracts of *L. divaricata* against *Fusarium graminearum*, *F. solani*, *F. verticillioides* y *Macrophomina phaseolina*. Chloroformic extract showed the highest antifungal activity against all phytopathogen fungus [11].

The results showed that the *L. divaricata* extract is a promising antifungal agent which could be used as bio-fungicide in the protection of fruits and vegetables against postharvest infections.

## 4. Conclusions

The results obtained demonstrated that HS-SPME/GS-MS is a simple and robust method for the volatilome analysis from *L. divaricata* extract. A total of 79 VOCs were identified in the extract. Among them, aromadendrene was found at highest concentration. Other bioactive volatile compounds were detected such as eugenol, carvacrol, limonene and thymoquinone. To the best of our knowledge, the volatile profile of this Argentinean autochthonous plant was assessed for the first time.

This work highlights the capacity of *L. divaricata* extract to inhibit *M. fructicola* mycelial growth. The extract obtained presented an effective activity not only in contact phase but also in vapor phase. This reveals its potential as postharvest protection agents representing an alternative source to chemical fungicides. Further researches are needed to evaluate its effectiveness *in vivo* and adjust the most suitable concentration and formulation for use.

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