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1 **Ecophysiological and phytochemical responses of *Salvia sinaloensis* Fern. to drought stress**

2

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20 **Abstract**

21 *Salvia sinaloensis* Fern. (sage) is a medicinal plant containing plant secondary metabolites (PSMs) with
22 antioxidant properties. The current study investigated the effects of drought stress on *Salvia sinaloensis*
23 morphological and ecophysiological traits, and active constituent production. Sage plants were
24 cultivated in controlled conditions for 34 days and exposed to full irrigation as control, half irrigation,
25 and no irrigation. Changes in growth index (G.I.), dry biomass, leaf water potential (LWP), physiological
26 parameters, active compounds, volatilome (BVOCs) and essential oils (EOs) were determined. Not
27 irrigated plants showed a decrease in total chlorophyll content (~-14.7%) and growth (G.I., ~-59.4%)
28 from day 18, and dry biomass at day 21 (-56%), when the complete leaf withering occurred (LWP, -1.10
29 MPa). Moderate drought stressed plants showed similar trends for chlorophyll content and growth but
30 kept a constant LWP (-0.35 MPa) and dry biomass throughout the experiment, as control plants.
31 Carotenoids were not affected by water regimes. The photosynthetic apparatus tolerated mild to severe
32 water deficits, without a complete stomatal closure. Plants under both stress conditions increased the
33 percentage of phenols and flavonoids and showed altered BVOC and EO chemical profiles. Interestingly
34 Camphor, the main EO oxygenated monoterpene, increased in moderate stressed plants while the
35 sesquiterpene hydrocarbon Germacrene D decreased. The same trend was seen in the headspace under
36 stress severity. The data evidenced a possible role of the active molecules in the response of *S.*
37 *sinaloensis* plants to drought stress. Taking together, these findings point at *S. sinaloensis* as a potential
38 drought adaptive species, which could be used in breeding strategies to obtain sages with high quality
39 PSMs, saving irrigation water.

40
41 **Keywords:** Antioxidant activity, BVOCs, Drought stress, EOs, Monoterpenes, Sage

42
43 **Abbreviations**

44	BVOCs	Biogenic volatile organic compounds
45	EOs	Essential oils
46	PSMs	Plant secondary metabolites
47	MAPs	Medicinal and aromatic plants
48	CC	Container capacity
49	LWP	Leaf water potential
50	C_i	Internal CO ₂ concentration
51	E	Transpiration rate

52	g_s	Stomatal conductance
53	A	Net photosynthetic rate
54	G.I.	Growth index
55	FRAP	Ferric reducing antioxidant power
56	Fe ³⁺ -TPTZ	Ferric tripyridyl triazine
57	SPME	Solid phase micro extraction
58	GC-EIMS	Gas chromatography–electron impact mass spectrometry
59	WUE	Water use efficiency
60	FW	Fresh weight
61	DW	Dry weight

62 **Introduction**

63 Biogenic volatile organic compounds (BVOCs) and essential oils (EOs) are plant secondary metabolites
64 (PSMs), with important ecological functions within defence, protection, and signalling mechanisms
65 (Loreto and Schnitzler 2010; Raut and Karuppayil 2014). Plants modulate their concentration to
66 withstand stress-related conditions (Fleta-Soriano and Munné-Bosch 2016), such as those provoked by
67 drought (Selmar and Kleinwachter 2013; Kleinwachter et al. 2015). Water deficit affects many aspects
68 of plant physiology and biochemistry, activating root to shoot signalling hydraulic and hormones-
69 mediated processes (Gulen and Eris 2004; Jaleel et al. 2008; Schachtman and Goodger 2008; Pirbalouti
70 et al. 2014; Fleta-Soriano and Munné-Bosch 2016; Ali et al. 2017; Caser et al. 2017). Plants respond by
71 closing their stomata and accumulating compatible solutes to maintain a low water potential and avoid
72 dehydration (Skirycz and Inze 2010). The increased diffusion barrier impairs influx of carbon dioxide
73 (CO₂) into leaves. In consequence, lower amounts of CO₂ are fixed via the Calvin Cycle, and then less
74 reduction equivalents (NADP⁺) are available as electron acceptors (Kleinwachter et al. 2015). In this
75 state, all reactions must be promoted to consume NADPH + H⁺ such as the biosynthesis of highly
76 reduced secondary compounds, i.e. phenols, terpenoids and alkaloids.

77 BVOCs and EOs are also sources for pharmaceutical, food additives, flavours, and fine chemicals
78 (Zhao et al. 2005). In the last years the global EO production was around 50 to 100 tonnes per annum
79 (Lubbe and Verpoorte 2011; Rauter et al. 2012; Ben Farhat et al. 2013). In medicinal and aromatic plants
80 (MAPs), drought stress enhanced total phenolics and flavonoids production in *Labisia pumila* Benth. &
81 Hook. (Jaafar et al. 2012) and monoterpene hydrocarbons and oxygenated sesquiterpenes in
82 *Helichrysum petiolare* Hilliard & B.L. (Caser et al. 2016).

83 *Salvia* species is well known for possessing numerous secondary metabolites with significant
84 benefits to human nutrition and health, used in folk medicine for a long time and therefore many species
85 were listed in the official Pharmacopoeias. Antiinflammatory and antioxidant properties were observed
86 in leaf extracts of *S. officinalis* L. (Munné-Bosch et al. 2001), anticonvulsant and sedative properties in
87 leaves and seed extracts of *S. leriifolia* Benth (Hosseinzadeh and Arabsanavi 2001), and analgesic,
88 neuroprotective and antiparkinson properties in roots of *S. miltiorrhiza* Bunge (Du and Zhang 2004).
89 However, other *Salvia* species, such as in *S. sinaloensis* Fern, had beneficial properties (Abreu et al.
90 2008) but little information is available.

91 Despite the enormous richness of MAP species, breeding activities are advanced only for those
92 plants with high demand and cultivation area (e.g. *Origanum vulgare* L. and *Matricaria recutita* L.).
93 Wild collection and simple domestication are therefore still the main production strategies (Novak 2017).

94 The need of support and speed up MAPs breeding also for minor crops impelled us to deepen the
95 knowledge about *S. sinaolensis* characteristics in relation to cultivation practices.

96 In sage, imposed drought stress already proved to increase EOs, total polyphenols content and
97 monoterpenes in *S. officinalis* (Abreu and Munne-Bosch 2008; Bettaieb et al. 2009; Bettaieb et al. 2011;
98 Radwan et al. 2017) and active constituents in *S. miltiorrhiza* (Liu et al. 2011).

99 Here we investigated *S. sinaloensis* dynamics in response to water limitation through
100 morphological, ecophysiological and phytochemical analyses, contributing to our understanding of the
101 mechanisms underlying plant adaptation and PSMs production.

102

103 **Materials and methods**

104 **Plant material and treatments**

105 Three year old plants from the CREA-FSO collection (Code database RGV FAO Salv095, Sanremo,
106 Imperia, Italy - 43°81'60.28''N Lat, 7°76'67.38''E Long) were cloned by cuttings in the glasshouse of
107 Dept. of Agricultural, Forest and Food Sciences of the University of Torino (Italy, 45°06'23.21''N Lat,
108 7°57'82.83''E Long), and cultivated in vases (9 cm in diameter – one plant per vase) containing peat
109 (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite® (70:30), and fertilized with 4 g L⁻¹,
110 Osmocote (15:11:13; Scotts Europe, The Netherland). When plants reached 20 cm in height, one
111 hundred and twenty pots were transferred in a growth chamber at 20 °C, 60% relative humidity and 16
112 h photoperiod (constant average PAR of 300 μmol·m⁻²·s⁻¹).

113 The experimental design was a split-plot design with three treatments and four replications per
114 treatment. Plants were randomly divided in three groups and subjected to irrigation at 100% of container
115 capacity (CC, control), half of the irrigation volume provided to CC controls (50% CC - moderate
116 drought stress), or no irrigation (0% CC). The value of 50% CC was set to determine moderate drought
117 stress in *S. sinaloensis* plants in a previous study (Caser et al., 2012). All the water contents were kept
118 constant throughout the experiment. Gravimetric determinations of water contents were made by
119 weighing soil samples before and after oven-drying to constant weight at 80 °C for one week. These
120 values were used to calibrate all measurements of the moisture content of the substrate in the container.
121 Container capacity was determined 48 h after irrigation and was calculated according to the equation of
122 Paquin and Mehuys (1980). The soil moisture levels were maintained by manual irrigation and checked
123 by weighting individual container every two days. The experiment lasted for a total of 34 days.

124

125 **Ecophysiological measurements**

126 Leaf water potential (LWP, Ψ_w), internal CO₂ concentration (C_i), transpiration rate (E), stomatal
127 conductance (g_s) and net photosynthetic rate (A) were measured twice a week (10-12 a.m.). In each
128 treatment, LWP was counted in three leaves of five plants with a Scholander chamber (Soil Moisture
129 Equipment, Santa Barbara, CA, USA) (Scholander et al. 1965).

130 C_i , E , g_s and A were measured through a gas analyzer ADC-LCPro+ (The Analytical Development
131 Company Ltd, Hoddesdon, UK). For each treatment three apical leaves of five plants were clamped in
132 the leaf chamber, and routines of measurements were performed according to Caser et al. (2016). The
133 CO₂ concentration (450-470 ppm) and vapour pressure deficit (19.46 ± 1.67 Pa/KPa) were kept constant
134 during the experiment. The ratio between A and E was used to calculate the instantaneous water use
135 efficiency (WUE).

136

137 **Determination of pigment content**

138 Fifty mg from four fresh fully formed leaves per treatment were used to determine chlorophyll and
139 carotenoids twice a week. After an over-night extraction in 5 ml of methanol at 4 °C in the dark, pigments
140 were spectrophotometrically determined at 665, 652, and 470 nm using a Ultrospec 2100 pro (Amersham
141 Biosciences, UK) as described by Lichtenthaler (1987).

142 The Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc., Osaka, Japan) was used to
143 measure the relative quantity of chlorophyll present in five randomly selected leaves per treatment.

144

145 **Determination of growth index and biomass**

146 Height and diameter of each plant per treatment were measured to calculate the growth index (G.I.;
147 $\Pi * \{[(W'+W'')/2]/2\}^2 * H$, W' is the broadest diameter, W'' is the perpendicular diameter and H is the
148 height; Hidalgo and Harkess 2002) . At day 34, roots and aerial parts of ten plants per treatment were
149 separated and weighed. After recording their fresh biomass, they were oven-dried at 65 °C for one week
150 and dry biomass was measured.

151

152 **Determination of phenol and flavonoid content, and antioxidant activity**

153 Fresh leaves (100 mg per treatment) were pulverized and homogenized in a mortar with 1 ml of 70%
154 (v/v) methanol to facilitate the extraction. After 30 minutes of incubation on ice, the extracts were
155 centrifuged at 10.000 g for 10 minutes at room temperature to collect the supernatant (methanol extract)
156 to be used for the determination of phenol and flavonoid content, and the antioxidant activity. The
157 content of total phenols was measured by using the Folin-Ciocalteu's phenolic method and determined

158 as reported by Singleton and Rossi (1965). Twenty μl of methanol extracted samples were added and
159 mixed with 0.5 ml of Folin-Ciocalteu's reagent and 0.45 ml of 7.5% (w/v) of saturated sodium
160 carbonate solution. After the incubation at room temperature for 2 h, the absorbance at 765 nm of the
161 samples was detected in UV-VIS spectrophotometer (Cintra 101, GBC Instruments, Australia).

162 Total flavonoid content was determined by applying the colorimetric method of Kim et al.
163 (2003). Twenty-five μl of methanolic extract were added to 225 μl of distilled water and to 75 μl of 5%
164 (w/v) sodium nitrite (NaNO_2). After 5 minutes of incubation were added 75 μl of 10% (w/v) of aluminum
165 trichloride (AlCl_3) and after 5 minutes were added 500 μl of 1M sodium hydroxide (NaOH). The
166 absorbance of the samples was detected at the UV-VIS spectrophotometer (Cintra 101, GBC
167 Instruments, Australia) after 15 minutes at 415 nm. The quantitative determination was made using a
168 calibration curve with, as standard, quercetin 1:1 (w/v) dissolved in absolute methanol.

169 The antioxidant activity was determined by using the ferric reducing antioxidant power (FRAP)
170 method with minor modification (Szöllősi and SzöllősiVarga 2002). The FRAP procedure is based on
171 the reduction of a ferric-tripyridyl triazine complex to its ferrous colored form in the presence of
172 antioxidants. An intense blue color complex was formed when ferric tripyridyl triazine (Fe^{3+} -TPTZ)
173 complex was reduced to the ferrous (Fe^{2+}) form and the absorption at 593 nm was recorded. The
174 calibration curve was plotted with absorbance at 593 nm versus concentration of Fe^{2+} solution which in
175 turn plotted against concentrations of standard antioxidant. A total of 50 μl samples extract were added
176 to 1.5 ml of the FRAP reagent and mixed well. The absorbance is measured at 593 nm using UV-VIS
177 spectrophotometer (Cintra 101, GBC Instruments, Australia). Samples are measured in three replicates.

178 At the end of the experiment, the total amount of total phenols, flavonoids and antioxidant
179 activity per plant (mg/plantFW) was estimated on the basis of the plant fresh biomass.

180

181 **BVOC analysis**

182 The evaluation of biogenic volatile organic compounds (BVOCs) was conducted at day 14. The
183 headspace of 3 g of twig was measured by applying a Supelco Solid Phase Micro Extraction (SPME)
184 (Supelco, Bellefonte, PA, USA) with polydimethylsiloxane (PDMS, 100 μm). Each sample was
185 introduced into a 100 ml glass conical flask and equilibrated for 30 min. After the equilibration time, the
186 fiber was exposed to the headspace for 15 min at room temperature; once sampling was finished the
187 fiber was withdrawn into the needle and transferred to the injection port of the Gas Chromatography–
188 Electron Impact Mass Spectrometry (GC-EIMS) system where the fiber was desorbed. GC-EIMS
189 analysis was performed with a Varian CP 3800 gas chromatograph (Varian, Inc., Palo Alto, CA)

190 equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian
191 Saturn 2000 ion trap mass detector chromatograph (Varian, Inc., Palo Alto, CA). Analytical conditions
192 were as follows: injector and transfer line temperature were 250 °C and 240 °C, respectively; oven
193 temperature was programmed from 60 °C to 240 °C at 3 °C min⁻¹; helium as carrier gas was set at 1 mL
194 min⁻¹; and the injection was in splitless mode. Identification of the constituents was based on comparison
195 of their retention times with those of authentic samples, and on computer matching against commercial
196 (NIST 98 and ADAMS) and home-made library mass spectra built from pure substances and MS
197 literature data (Swigar & Silvestein, 1981; Davies, 1990; Adams, 1995).

198 The relative proportions of the volatile constituents were percentages obtained by peak-area
199 normalisation, and all relative response factors were taken as one.

200

201 **EO analysis**

202 Twenty g of dried leaves were used for the distillation of essential oils (EOs) by using a Clevenger-type
203 apparatus (2 h) (Farmacopea Ufficiale della Repubblica Italiana, vol I. IXth edn. Zecca dello Stato:
204 Rome, 1991). The yields of distillation were not determined due to the low amount of the starting plant
205 material. The EOs obtained were solubilized in *n*-hexane, filtered over anhydrous sodium sulphate and
206 stored in a vial at 4 °C in the dark until use. GC-EIMS was used to analyse all EOs (injection of 0.2 μL,
207 10% hexane solution) as reported above.

208

209 **Statistical analysis**

210 Data were previously tested for the variance homogeneity. Ryan-Einot-Gabriel-Welsch-F post-hoc test
211 (REGW-F) ($P < 0.05$) was applied by means of the SPSS statistical package (version 19.0; SPSS Inc.,
212 Chicago, Illinois).

213

214 **Results and discussion**

215 **Changes in ecophysiological parameters**

216 In this study, moderate drought stress did not affect LWP compared to control (Fig. 1). In both cases it
217 was constant throughout the experiment, with a mean value of -0.35 MPa. On the opposite, no irrigation
218 significantly reduced LWP to -0.64 MPa at day 7. Then, a decreasing trend was observed till day 21 (-
219 1.10 MPa) when the complete leaf withering occurred. Within the genus, similar results were found by
220 Eakes et al. (1991) in *S. splendens* Sellow ‘Bonfire’ whose leaves reached a LWP ranging between -1.10
221 and -1.40 MPa when exposed to episodic drought (moisture stress conditioned). In other xerophytic sage

222 species, drought induced much lower LWP. *S. officinalis* plants subjected to 50% CC and 25% CC
223 reached LWP equal to -3.00 and -4.80 MPa, respectively (Bettaieb et al. 2011), and *S. mellifera* Greene
224 under severe drought condition reached LWP equal to -8.0 MPa, even if severely affected by embolism
225 (Hargrave et al. 1994).

226 Stomatal closure is the primary mechanism by which plants regulate water loss. It is widely
227 observed that plants reduce stomatal conductance of their leaves in response to declining water potential
228 (Oren et al. 1999). Here, drought did not affect internal CO₂ (C_i) concentration with the exception for
229 the 0% CC treatments that led to increase C_i at day 21 (Fig. 2A). More differences were found for E , g_s
230 and A , showing similar trends (Fig. 2B-C-D). Not irrigated plants showed lower values from day 14,
231 shifted to day 21 (as regarding g_s and A) and from day 25 (E). At the end of the experiment no differences
232 were observed between control and half irrigation for all measured traits. Thus, drought stress led to
233 partial stomata closure (Fig. 2B). As result, the influx of CO₂ is diminished, visible by the strongly
234 decreased rate of photosynthesis in the stressed plants (Fig. 2C) and the lower growth rate (Fig. 3).
235 However, as the stomata closure is not very distinct, the actual CO₂ concentration in the stressed plants
236 is not significantly lower than in the controls. Obviously, the steady state concentration, at least in part,
237 is determined by the photosynthetic action (i.e. the affinity of RuBisCO). This is confirmed by the fact
238 that in the severely stressed plants, the internal CO₂ concentration increased, when CO₂ consumption is
239 stopped due to the lacking photosynthetic activity after 21 days. Results suggested that the
240 photosynthetic apparatus of *S. sinaloensis* could tolerate mild to severe water deficits and decreasing in
241 photosynthesis couple with reduced stomatal function (Eakes et al. 1991; Galmes et al. 2007).

242 Instantaneous water use efficiency (WUE, A/E) is used as an indicator of the water amount
243 applied for growing by plants. Different authors indicated that stressed plants showing higher A/E are
244 more able to utilize energy obtained by photosynthesis per unit of water transpired (Liu and Stützel
245 2004; Monclus et al. 2006). In this study, drought did not affect WUE except for the 0% CC treatment
246 at day 21 (Fig. 2E). This finding indicates that *S. sinaloensis* could efficiently use water resources under
247 moderate and severe drought stress condition at least under control conditions. In many species WUE
248 was improved under water limitation and low g_s (Liu et al. 2005). Among these, *Trifolium alexandrinum*
249 L. (Lazaridou and Koutroubas 2004), *Spartina alterniflora* Loisel (Hessini et al. 2009), *Callistemon*
250 (Alvarez et al. 2011), *Hybanthus floribundus* Lindl. (Kachenko et al. 2011), *Rosa hybrida* L. (Cai et al.
251 2012), *Vitis vinifera* L. ‘Grenache’ and ‘Tempranillo’ (Lovisololo et al. 2010; Medrano et al. 2015), and
252 *Helichrysum petiolare* (Caser et al. 2016). In sage, Lambrecht et al. (2011) observed that leaf
253 physiological activity of *S. mellifera* was not limited by imposed water deficits as well as indicated in

254 our study. The photosynthetic apparatus was not completely affected by drought stress also in *S.*
255 *splendens* ‘Bonfire’ (Eakes et al. 1991) and in *S. pitcheri* L. (Hamerlynck et al. 1997), highlighting the
256 positive water use efficiency attitude of different species within the genus *Salvia*.

257

258 **Changes in chlorophyll, carotenoids and plant growth**

259 The decrease of chlorophyll and carotenoids in drought stressed plants is reported by several authors
260 (Kaminska-Rozek and Pukacki 2004; Pastenes et al. 2005; Guerfel et al. 2009). Here, moderate and
261 severe drought stress reduced the total chlorophyll content starting from day 18 up to the end of the
262 experiment (Supplementary Table 1), while carotenoids and Chl:Car ratios were not affected by the
263 imposed irrigation regimes. Plants of *S. officinalis* treated with 50% CC and 25% CC presented similar
264 values and trends (Bettaieb et al. 2011). Similarly, a chlorophyll decrease was observed also in plants of
265 *Catharanthus roseus* L. (Jaleel et al. 2008) and *Helichrysum petiolare* (Caser et al. 2016). Jaleel et al.
266 (2009) indicated that carotenoids play an important role in protecting different processes from reactive
267 oxygen species damages. Here, the presence of constant amount of carotenoid during the experiment
268 suggests a possible role to cope with oxidative damages. Similar results were observed by Caser et al.
269 (2016) in *H. petiolare* plants subjected to 50% CC water regime.

270 The evaluation of SPAD units by the use of the chlorophyll meter is commonly used to associate
271 leaf damages and abiotic stresses (Caser et al., 2013) and leaf photosynthesis (Castelli et al. 1996). Here,
272 no significant results were highlighted (data not shown). Similarly, Caser et al. (2012) showed no
273 differences of SPAD values in *S. dolomitica* Codd and *S. sinaloensis* plants under drought conditions
274 with the exception for those irrigated with 20% CC in which was observed an increase.

275 The decrease of chlorophyll content might cause a reduction in growth parameters of plants
276 under water stress conditions (Viera et al. 1991). In this study, all the treated plants grew similarly till
277 day 11 (Fig. 3). Then, drought stressed plants significantly reduced their growth. Not irrigated plants
278 reached the lowest G.I. at day 21 (2654.00 cm³), while plants under 50% CC kept constant G.I. from
279 day 18 onward, ranging between 6908.73 cm³ and 5686.40 cm³. These results are in agreement with a
280 preliminary study on *S. sinaloensis* plants treated with different irrigation regimes where irrigation with
281 20% CC and 40% CC significantly reduced the plant growth compared to 80% CC and controls (Caser
282 et al. 2012). However, as shown in Table 1, here only severe drought stress reduced drastically the total
283 dry mass of plants (-56%), affecting particularly the roots (-67%). Thus, the R:A ratio (-55%), favoring
284 the shoot as showed in other species under severe drought stress conditions (Comas et al. 2013; Caser et
285 al. 2016). A reduction of shoot dry weight, leaf area, stem length and root length was similarly seen in

286 *S. splendens* (Eakes et al. 1991; Burnett et al. 2005). On the opposite, half irrigated plants showed an
287 increase in root dry biomass (+14%) and consequently in R:A ratio (+37%) compared to control. Since
288 roots are the only source to acquire water from soil, the root growth, its density, proliferation and size
289 are key responses of plants to drought stress (Kavar et al. 2007). The drought tolerance of tea, onion and
290 cotton was increased by improved root growth and root functioning (Farooq et al. 2009).

291

292 **Changes in phenols and flavonoids and antioxidant activity**

293 Polyphenols and flavonoids are among the most adaptable PSMs, helping plants to cope with different
294 stress conditions (Di Ferdinando et al. 2014). In this study the total content of phenols in full-irrigated
295 plants decreased during the whole experiment, reaching the lowest value between days 21 and 25 (0.98
296 mg/g FW) (Fig. 4A). Conversely, at the same data point moderate stressed plants exhibited the highest
297 amounts (14.26 mg/g FW and 13.16 mg/g FW at the days 21 and 25, respectively). A different behavior
298 was also observed in severe stressed plants, that showed higher content of phenols from day 11 to day
299 18 with the highest value at day 11 (15.74 mg/g FW). A similar dynamic was observed for the total
300 flavonoids accumulation (Fig. 4B). In control plants, flavonoids increased during the first 7 days of
301 cultivation. Subsequently, they dramatically decreased reaching minimum values between days 21 (1.19
302 mg/g FW) and 25 (1.16 mg/g FW). By contrast, in moderate stress condition flavonoid content kept
303 constant until day 21, later increased with higher values from day 18 till the end of the experiment,
304 reaching the maximum content at day 25 (2.89 mg/g FW). In the not irrigated plants, the total content of
305 flavonoids peaked at day 11 (3.73 mg/g FW). In literature, different works showed that in drought
306 stressed plants was observed an increase in secondary metabolites content. Plants of *Prunus persica* L.,
307 *Echinacea purpurea* L., *Hypericum brasiliense* Choisy, *Trachyspermum ammi* L. and *Labisia pumila*
308 Benth. & Hook. subjected to drought stress showed significant increase in total phenols (Kubota et al.
309 1988; Gray et al. 2003; de Abreu and Mazzafera 2005; Azhar et al. 2011; Jaafar et al. 2012) and *Pisum*
310 *sativum* in flavonoids (Nogués et al. 1998). In *Salvia officinalis*, an increase in phenols was observed in
311 half irrigated plants (Bettaieb et al. 2011). Here, a mid to late increase in total phenols and flavonoids
312 occurred in plants subjected to 50% CC. According with Paulsen and Selmar (2016), imposed drought
313 stress generally results in decrease of aerial biomass gain and the corresponding dry or fresh weight are
314 strongly influencing the content of natural products in MAPs. Here, as indicated in Table 2, moderate
315 drought conditions resulted in a significant increase of the total content of phenols in comparison to
316 severe drought stressed plants and controls (16.44, 22.85 and 10.43 mg/plant in 100% CC, 50%CC and
317 0% CC, respectively). While, no differences were noted in flavonoid content between moderate drought

318 stress and control conditions (6.42, 6.47 and 2.73 mg/plantFW of flavonoids in 100% CC, 50%CC and
319 0% CC, respectively). These findings should be taken in consideration for future industrial purposes.

320 Drought induces oxidative stress in plants, in which reactive oxygen species (ROS) are produced
321 (Munné-Bosch and Penuelas 2003). Plant resistance to ROS is associated with an increase in antioxidant
322 activity to prevent stress damage (Bor et al. 2003). In this study, only plants under 50% CC showed a
323 significant increase of antioxidant activity at the end of the experiment (Fig. 4C). Similarly, at the end
324 of the experiment the total amount of antioxidant activity per plant was much superior in moderate
325 drought stressed plants than the others (106.37, 125.98 and 20.86 mg/plantFW in 100% CC, 50% CC
326 and 0% CC, respectively) (Table 2). These findings confirm the ability of this plant to encounter the
327 water limitation (50% CC) activating stress defense signaling and pathways respect to severe drought
328 stress (0% CC) where metabolic and physiological constraints are not able to allow plant survival.

329

330 **Changes in emitted BVOCs**

331 Different authors observed that any stress factor can potentially change the rate of volatile release and
332 alter the bouquet of BVOCs (Dicke and Baldwin 2010; Holopainen and Gershenzon 2010; Niinemets et
333 al. 2013). Their emission can vary drastically depending on the species, organ, developmental stage and
334 environmental conditions (Holopainen and Gershenzon 2010). Abiotic stress can enhances the emission
335 of BVOC rates and patterns by the alteration of the communication with other organisms and
336 photochemical cycles (Loreto and Schnitzler 2010). In the present study, the volatiles emitted and
337 identified from the analyzed twigs are reported in Supplementary Table 2. In total a number of 20, 32
338 and 38 compounds were recognized in plants treated with full irrigation, half irrigation and no irrigation,
339 respectively, accounting for 92.1%, 96.6% and 95.4% of the total compositions, respectively. The
340 volatile fractions were characterized mainly by sesquiterpene hydrocarbons (sh) although a mild
341 reduction by increasing stress conditions was observed (57.5% < 42.8% < 42.7% in full irrigation, half
342 irrigation and no irrigation, respectively). No irrigation affected the production of four of the six reported
343 volatile molecule classes. In particular, an increase in oxygenated monoterpenes (om) (9.7%, 16.2% and
344 23.6% in full irrigation, half irrigation and no irrigation, respectively), was observed. Among the other
345 categories not-terpene derivates (nt), oxygenated sesquiterpene (os) and apocarotenoids (ac) were
346 observed only in few percentage. However, plants under 50% CC exhibited the highest content in
347 monoterpene hydrocarbons (mh) (24.2%, 37.1% and 22.0% in full irrigation, half irrigation and no
348 irrigation, respectively). All investigated headspaces showed different array of the main constituents.
349 The chemical profile in fully irrigated plants was characterized by Germacrene D > β -Caryophyllene >

350 β -Pinene, in moderate stressed plants by β -Pinene > Camphor > β -Caryophyllene and in severe stressed
351 plants by β -Caryophyllene > Camphor > β -Pinene. Among the various constituents, a very sharp
352 decrease (4.4 times) was observed for the Germacrene D (from 22.0% to 5.0% in full irrigation and no
353 irrigation, respectively). On the opposite the oxygenated monoterpene Camphor increased 2.5 times
354 when plants were subjected to 0% CC.

355 The literature is ambiguous concerning BVOC emission in relation to water availability.
356 Several recent reviews addressed the roles of BVOCs in enhancing the tolerance of plants to various
357 general abiotic stressors (Holopainen and Gershenzon 2010; Loreto and Schnitzler 2010; Possell and
358 Loreto 2013; Rinnan et al. 2014). In particular, under drought Mediterranean climatic condition, the
359 emission of oxygenated monoterpene is common (Loreto et al. 2014) and is thought to promote direct
360 and indirect defense by modulating the signaling that biochemically activates defense pathways in
361 response to stressful conditions (Loreto and Schnitzler 2010; Rinnan et al. 2014). Pistelli et al. (2013)
362 reported an increase in oxygenated monoterpenes in *Salvia officinalis* plants under *in vitro* growing
363 conditions. Moreover, there is evidence that the strength of the emission of BVOCs can be quantitatively
364 related to the severity of abiotic stresses (Niinemets et al. 2013). As reported by Niinemets et al. (2004)
365 the emission of oxygenated BVOCs depends on stomatal behavior. The stomatal closure in response to
366 drought stress is therefore expected to drastically affect the emission of these compounds as showed in
367 *Quercus ilex* L. (Bertin and Staudt 1996; Llusia and Peñuelas 1998; Loreto et al. 2001). Here, on the
368 contrary we observed an increase in oxygenated monoterpenes under mild and severe drought stress
369 associated with a not completely stomatal closure. Progressing soil water deficiency enhanced
370 monoterpene emissions also in *Pinus halepensis* Mill. and *Cistus albidus* L., whereas did not affect it in
371 *Rosmarinus officinalis* L. and *Quercus coccifera* L. (Ormeño et al. 2007). By contrast, overall emissions
372 of sesquiterpenes were reduced by water deficiency in all the four species (Ormeño et al. 2007).
373 Moreover, Hansen et al. (1997) and Ormeño et al. (2007) noticed that monoterpene emission in *R.*
374 *officinalis* was not dependent by photosynthesis, but may originate from “*de novo*” synthesis in the
375 photosynthetic tissues (Steinbrecher et al. 1999; Ormeño et al. 2009; Nogués et al. 2015). Taking
376 together, all these results on BVOCs could represent important findings for studying the plant-
377 environment interactions and can be used in breeding strategies to improve volatile compounds yield
378 and quality.

379

380 **Changes on essential oil**

381 In a wide range of experiments was highlighted that plants under drought stressed showed an increase
382 in secondary metabolites content (Selmar and Kleinwächter 2013). Here, a total of 78 constituents were
383 detected in the investigated EOs of which 59, 41 and 62 in full, half and not irrigated plants, respectively
384 (Supplementary Table 3). Drought stress conditions only slightly increased the total number of
385 constituents identified in the EOs (94.0, 99.6 and 98.8% at full irrigation, half irrigation and no irrigation,
386 respectively). Similar results were observed in *Satureja hortensis* L. (Baher et al. 2002), *Lippia*
387 *berlandieri* Schauer (Dunford and Vazquez 2005), *Petroselinum crispum* (Mill.) (Petropoulos et al.
388 2008), *S. officinalis* (Bettaieb et al. 2009), and *Laurus nobilis* L. (Maatallah et al. 2016) plants treated
389 with similar drought stressed conditions. Regarding the type of constituents, moderate drought stress
390 increased the total amount of monoterpenes (both oxygenated monoterpenes and monoterpene
391 hydrocarbons: 51.5, 77.1 and 54.9% and 1.4, 6.0 and 1.9% in full irrigation, half irrigation and no
392 irrigation, respectively) and deeply reduced the oxygenated sesquiterpenes (29.2, 6.3 and 26.9% in full
393 irrigation, half irrigation and no irrigation, respectively). Similarly, imposed moderate drought stress
394 strongly increased (2 to 4 fold) the content of Camphor, a specific compound in the essential oil, as also
395 reported in *S. officinalis* plants (Bettaieb et al. 2009; Nowak et al. 2010) and in *Picea abies* L. and *Pinus*
396 *silvestris* L. (Turtola et al. 2003). Nowak et al. (2010) reported that moderate stressed condition resulted
397 in a massive increase of concentration of monoterpenes compensating the reduction in biomass. In fact,
398 the stimulation to produce high terpene content under drought stress could be due to the low allocation
399 of carbon to the growth, suggesting a trade-off between growth and defense (Turtola et al. 2003).

400 The main constituent of EOs in all the studied plants was Camphor (an oxygenated monoterpene).
401 This constituent strongly increased under 50% CC (36.5, 62.8, 38.7% in full irrigation, half irrigation
402 and no irrigation, respectively). Our results showed a chemical profile composed by Camphor >
403 Caryophyllene oxide > α -Cadinol in 100% CC; Camphor > β -Caryophyllene > Borneol in 50% CC; and
404 by Camphor > Caryophyllene oxide > β -Caryophyllene in 0% CC. Abreu et al. (2008) reported
405 interesting antioxidant properties of *S. sinaloensis* due to the presence of phenolic diterpenes in
406 comparison with *S. officinalis* extracts. In the present study, moderate drought stress application induced
407 an increase in oxygenated monoterpenes that are generally the most active components in many EOs
408 (Ruberto and Baratta 2000; Imanshahidi and Hosseinzadeh 2006), such as in *S. cinnabarina* L. with
409 hypotensive effect (Alfieri et al. 2007), *S. dolomitica* with anti-bacterial activity (Kamatou et al. 2007)
410 and in *S. officinalis* with antifungal and anti-inflammatory activity on mammalian cells (Abu-Darwish
411 et al. 2013). These results may contribute to a good utilization of *S. sinaloensis* EOs for plant breeding
412 and pharmaceutical applications.

413

414 **Conclusions**

415 Drought is one of the abiotic stresses that most severely affects plant growth and development.
416 Consequently, plants adjust their structure, metabolism and function to withstand it. Generally, it is
417 characterized by reduction of water content and leaf water potential, turgor loss, closure of stomata and
418 decrease in cell enlargement and growth. However, with respect to MAPs, the water shortage can lead
419 to an enhancement of the content of PSMs, Our results showed that in *S. sinaloensis* drought affects
420 morphological, ecophysiological and chemical traits depending on the stress severity. Plant growth
421 parameters and chlorophyll were generally reduced but the photosynthetic apparatus demonstrated to
422 tolerate moderate to severe drought conditions, without a complete stomatal closure. Interestingly, under
423 moderate stressed conditions, monoterpene hydrocarbons increased in both BVOCs and Eos (e.g.
424 camphene), while volatile oxygenated monoterpenes increased still with increasing stress severity (e.g.
425 camphor). Thus, drought stress did not inhibit per se the biosynthesis of monoterpenes, even better the
426 highest BVOCs and EOs contents were obtained in moderate drought stress condition. Furthermore, this
427 stress condition induced a mid to late increase in phenols, flavonoids and antioxidant activity, suggesting
428 that active molecule accumulation is associated with drought tolerance.

429 In conclusion, here an integrated approach, combining metabolomic and physiological studies,
430 allowed us to get new insights in mechanisms and processes involved in *S. sinaloensis* adaptation to
431 drought stress and PSMs production. Coupling this information, breeders and industries may optimize
432 MAPs breeding and cultivation, in order to produce high quality plant materials adopting sustainable
433 cultivation techniques.

434

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693 **Tables**694 **Table 1**

695 Aerial and root dry mass accumulation and root:aerial (R:A) ratio of *Salvia sinaloensis* plants under full
 696 irrigation (100% container capacity, CC), half irrigation (50% CC), or no irrigation (0% CC). In brackets
 697 are reported the percentage variations referred to controls.

Water regime	Dry mass accumulation (gDW/plant)			R:A ratio
	Total	Aerial part	Root	
100% CC	3.57a (100%)	1.06a (100%)	2.51a (100%)	2.37b (100%)
50% CC	3.75a (105%)	0.88ab (83%)	2.87a (114%)	3.26a (137%)
0% CC	1.59b (44%)	0.77b (73%)	0.82b (33%)	1.06c (45%)
<i>P</i>	**	*	**	*

698 The statistical relevance of 'Between-Subjects Effects' tests (*= $P < 0.05$, **= $P < 0.001$, ns=not significant). Mean values showing the same letter are not
 699 statistically different at $P \leq 0.05$ according to the REGW-F test.

700

701 **Table 2**

702 Total amount (mg/plantFW) of phenols, flavonoids and antioxidant activity at the end of the experiment
 703 in *Salvia sinaloensis* plants under full irrigation (100% container capacity, CC), half irrigation (50%
 704 CC), or no irrigation (0% CC). In brackets are reported the percentage variations referred to controls.

Water regime	Total amount (mg/plantFW)		
	Total phenols	Flavonoids	Antioxidant activity
100% CC	16.44 b (100%)	6.42 a (100%)	106.37 b (100%)
50% CC	22.85 a (139%)	6.47 a (101%)	125.98 a (118%)
0% CC	10.43 c (63%)	2.73 b (43%)	20.86 c (20%)
<i>P</i>	**	*	*

705 The statistical relevance of 'Between-Subjects Effects' tests (*= $P < 0.05$, **= $P < 0.001$, ns=not significant). Mean values showing the same letter are not
 706 statistically different at $P \leq 0.05$ according to the REGW-F test.

707 **Figure captions**

708 **Fig. 1** Dynamics of leaf water potential (LWP) of *Salvia sinaloensis* plants treated with full irrigation
709 (100% container capacity, CC - black line), half irrigation (50% CC - dark grey line), or no irrigation
710 (0% CC - light grey line). Means superscripted by the same letter do not differ significantly, according
711 to REGW-F post-hoc test (NS = non significant).

712

713 **Fig. 2** Gas exchange (internal CO₂ concentration, C_i - A; transpiration rate, E - B; stomatal conductance,
714 g_s - C; net photosynthetic rate, A - D) and dynamic of the instantaneous water use efficiency (WUE, A/E
715 - E) measured on *Salvia sinaloensis* plants treated with full irrigation (100% container capacity, 100%
716 CC - black line), half irrigation (50% CC - dark grey line), or no irrigation (0% CC - light grey line).
717 Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the REGW-F
718 post-hoc test. The statistical relevance of 'Between-Subjects Effects' tests (ns=non significant,
719 *= $P < 0.05$) was evaluated.

720

721 **Fig. 3.** Average values of growth index (G.I., cm³) during the experiment. *S. sinaloensis* plants were
722 treated with three irrigation regimes: full irrigation (100% container capacity, 100% CC, black line), half
723 irrigation (50% CC, dark grey line), or no irrigation (0% CC, light grey line). Means superscripted by
724 the same letter do not differ significantly, according to REGW-F test (NS = non significant; * $P < 0.05$;
725 ** $P < 0.001$).

726

727 **Fig. 4** Leaf total phenols (A) and flavonoids (B), and antioxidant activity (C) of *Salvia sinaloensis* plants
728 under control condition (black line, 100% container capacity, CC), moderate drought stress (dark grey
729 line, 50% CC), and severe drought stress (light grey, 0 % CC). Means superscripted by the same letter
730 do not differ significantly, according to REGW-F test (NS = not significant; * $P < 0.05$; ** $P < 0.001$).

Ecophysiological and phytochemical responses of *Salvia sinaloensis* Fern. to drought stress

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Supplementary files

Supplementary Table 1. Chlorophyll (*a + b*), total carotenoid (Car) and their rate (Chl:Car) measured on *Salvia sinaloensis* plants under full irrigation (100% container capacity, CC), half irrigation (50% CC), and no irrigation (0% CC).

		Days										
		0	4	7	11	14	18	21	25	28	32	34
Chl <i>a + b</i> (mg/g FW)	100% CC	1.40	1.49	1.43	1.38	1.35	1.50a	1.32a	1.36	1.42	1.43	1.45
	50% CC	1.40	1.43	1.25	1.27	1.17	1.15b	1.15b	1.16	1.17	1.24	1.30
	0% CC	1.40	1.39	1.44	1.42	1.38	1.13b	1.07b	-	-	-	-
	<i>P</i>	ns	ns	ns	ns	ns	*	*	*	*	*	*
Car (mg/g FW)	100% CC	0.23	0.29	0.24	0.25	0.25	0.21	0.26	0.24	0.26	0.23	0.26
	50% CC	0.23	0.22	0.21	0.24	0.20	0.16	0.18	0.19	0.24	0.24	0.23
	0% CC	0.23	0.25	0.24	0.23	0.24	0.17	0.19	-	-	-	-
	<i>P</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chl:Car	100% CC	6.20	5.21	5.92	5.51	5.43	7.07	5.17	5.63	5.41	6.12	5.61
	50% CC	6.20	6.54	5.86	5.24	5.97	7.35	6.45	6.25	4.97	5.15	5.76
	0% CC	6.20	5.45	5.96	6.16	5.89	6.48	5.63	-	-	-	-
	<i>P</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The statistical relevance of 'Between-Subjects Effects' tests (*= $P < 0.05$, ns=not significant). Mean values showing the same letter are not statistically different at $P \leq 0.05$ according to the REGW-F test.

Supplementary Table 2. Chemical composition (%) of volatiles emitted from *Salvia sinaloensis* plants after full irrigation (100% CC), half irrigation (50% CC) or no irrigation (0% CC). All constituents are ordered on the basis of their linear retention index (LRI).

Category	Constituents	LRI	100% CC	50% CC	0% CC
mh	α -Pinene	939	4.7	6.5	2.0
mh	Camphene	953	2.6	6.6	2.6
mh	β -Pinene	980	12.0	16.7	7.5
mh	Myrcene	991	0.0	0.5	1.9
mh	Limonene	1031	4.4	3.3	2.3
mh	(<i>Z</i>)- β -Ocimene	1040	0.0	1.9	0.5
mh	(<i>E</i>)- β -Ocimene	1050	0.0	0.9	4.6
mh	Terpinolene	1088	0.5	0.7	0.6
om	<i>Trans</i> -sabinene hydrate	1097	0.0	0.5	0.7
om	Linalool	1098	0.0	0.0	0.6
nt	1-octen-3-yl-acetate	1110	0.0	0.1	4.3
om	α -Campholenal	1125	0.0	0.0	0.4
om	Camphor	1143	8.3	12.9	20.2

om	Borneol	1165	1.4	2.2	0.7
om	α -Terpineol	1189	0.0	0.5	0.7
nt	(<i>N</i>)-decanal	1204	0.0	0.1	0.6
om	Isobornyl acetate	1285	0.0	0.1	0.7
sh	δ -Elemene	1339	1.4	0.6	0.2
sh	α -Copaene	1376	2.0	1.0	0.7
ac-13	(<i>E</i>)- α -damascenone	1380	0.7	0.3	1.0
sh	β -Elemene	1391	1.7	1.7	0.9
sh	Cyperene	1398	0.6	0.5	0.6
sh	β -Caryophyllene	1418	16.0	12.4	24.2
sh	β -Copaene	1429	1.1	1.1	0.3
sh	β -Gurjunene	1432	0.0	0.5	0.4
sh	α -Guaiene	1439	1.7	0.8	0.3
sh	α -Humulene	1454	3.9	3.5	6.5
sh	(<i>E</i>)- β -Farnesene	1458	1.3	2.7	1.0
sh	α -Himachalene	1476	0.0	0.4	0.1
sh	γ -Muurolene	1477	0.0	0.0	0.2
sh	Germacrene-D	1480	22.0	12.1	5.0
sh	α -Selinene	1485	1.5	2.5	0.8
sh	Bicyclogermacrene	1494	4.3	1.4	0.2
sh	Germacrene-A	1503	0.0	0.3	0.2
sh	7-epi- α -selinene	1517	0.0	1.3	0.7
sh	δ -Cadinene	1524	0.0	0.0	0.4
so	Caryophyllene oxide	1581	0.0	0.0	0.5
so	α -Cadinol	1653	0.0	0.0	0.3
Total		92.1	96.6	95.4	
Non Terpene Derivates (nt, %)		0.0	0.2	4.9	
Monoterpene Hydrocarbons (mh, %)		24.2	37.1	22.0	
Oxygenated Monoterpene (om, %)		9.7	16.2	23.6	
Sesquiterpene Hydrocarbons (sh, %)		57.5	42.8	42.7	
Oxygenated Sesquiterpene (os, %)		0.0	0.0	0.8	
Apocarotenoids (ac, %)		0.7	0.3	1.0	

*All the constituents identified belong to non terpene derivates (nt), monoterpene hydrocarbons (mh), oxygenated monoterpene (om), sesquiterpene hydrocarbons (sh), oxygenated sesquiterpene (os), and apocarotenoids (ac).

Supplementary Table 3. Effect of full irrigation (100% CC), half irrigation (50% CC) or no irrigation (0% CC) on the essential oil constituents of *Salvia sinaloensis* plants. Constituents are ordered on the basis of their linear retention index (LRI).

Category *	Constituents	LRI	100% CC	50% CC	0% CC
nt	Hexenal	800	0.2	1.0	0.0

nt	(<i>E</i>)-3-hexen-1-ol	851	0.2	0.5	0.0
mh	α -Pinene	939	0.0	0.5	0.1
mh	Camphene	953	0.6	3.6	0.5
mh	Sabinene	976	0.4	1.2	1.0
nt	3-Octanone	988	0.0	0.2	0.0
mh	Myrcene	991	0.0	0.3	0.1
nt	3-Octanol	993	0.0	0.0	0.1
mh	Limonene	1031	0.0	0.1	0.1
om	1,8-Cineole	1033	0.7	0.2	0.1
mh	γ -Terpinene	1062	0.2	0.2	0.0
om	<i>Cis</i> -sabinene hydrate	1068	0.0	0.0	0.4
nt	(<i>N</i>)-octanol	1070	0.0	0.0	0.0
om	<i>Trans</i> -linalol oxide (furanoid)	1074	0.2	0.2	0.2
om-no	Camphenilone	1083	0.2	0.3	0.2
mh	Terpinolene	1088	0.2	0.0	0.0
om	Linalool	1098	0.2	0.2	0.7
nt	1-octen-3-yl acetate	1110	0.5	1.8	1.8
om	α -Campholenal	1125	0.3	0.4	0.9
om	<i>Trans</i> -pinocarveol	1142	0.6	1.4	1.7
om	Camphor	1143	36.5	62.8	38.7
om	Isoborneol	1156	0.0	0.0	0.2
om	Pinocarpone	1162	0.5	0.8	1.1
om	Borneol	1165	7.5	4.7	5.1
om	4-Terpineol	1177	1.3	1.6	1.0
om	Cymen8-ol-para	1187	0.2	0.0	0.2
om	α -Terpineol	1189	0.5	0.7	0.6
om	Myrtenal	1193	1.4	1.5	2.1
om	Verbenone	1217	0.0	0.1	0.2
om	<i>Trans</i> -carveol	1217	0.0	0.0	0.2
om	Isobornyl formate	1233	0.6	0.2	0.3
om	Carvone	1242	0.0	0.0	0.2
om	Perilla aldehyde	1271	0.0	0.0	0.1
om	Isobornyl acetate	1285	1.0	1.0	0.9
nt	2-undecanone	1296	0.1	0.0	0.0
nt	(<i>N</i>)-tridecene	1292	0.1	0.0	0.0
om	α -Terpenyl acetate	1352	0.0	1.1	0.0
nt	Undecanol-N	1374	0.3	0.0	0.1
sh	β -Bourbonene	1384	0.3	0.3	0.0
ac-13	(<i>E</i>)- β -damascenone	1380	0.3	0.0	0.2
sh	Cyperene	1398	0.2	0.0	0.1
sh	α -Gurujene	1409	0.0	0.0	0.9

sh	β -Caryophyllene	1418	1.5	5.7	8.3
sh	β -Gurjunene	1432	0.5	0.0	0.0
sh	α -Guaiene	1439	0.5	0.0	0.0
sh	Aromadendrene	1439	0.3	0.0	0.9
sh	α -Humulene	1454	0.2	0.0	0.1
ac-12	Geranylacetone	1453	0.0	0.0	0.2
sh	(<i>E</i>)- β -farnesene	1458	0.0	0.0	0.2
sh	γ -Himachalene	1476	0.2	0.0	0.0
sh	Germacrene-D	1480	0.0	0.3	0.0
sh	β -Selinene	1485	0.8	0.0	0.6
sh	<i>Cis</i> - β -guaiene	1490	0.2	0.0	0.1
sh	Bicyclogermacrene	1494	0.4	0.0	0.0
sh	Cuparene	1502	1.2	0.3	0.2
sh	7-epi- α -selinene	1517	1.0	0.2	0.5
sh	δ -Cadinene	1524	1.3	0.1	0.3
sh	α -Calacorene	1542	0.3	0.0	0.0
sh	Selina-3,7(11) diene	1542	0.0	0.0	0.2
os	Elemol	1549	0.3	0.2	0.8
os	Longicamphenylone	1559	0.3	0.4	0.2
os	<i>Trans</i> -nerolidol	1564	0.4	0.0	0.2
os	Spathulenol	1576	1.9	0.5	1.6
os	Caryophyllene oxide	1581	9.8	3.0	10.4
os	<i>Cis</i> - β -elemenone	1594	0.4	0.1	0.2
os	Humulene oxide	1606	0.8	0.3	1.3
os	1,10-di-epi-cubenol	1614	1.5	0.3	1.0
os	α -Acorenol/g-Eudesmol	1630	0.6	0.0	0.7
os	β -Acorenol	1634	0.3	0.0	0.1
os	Epi- α -cadinol	1640	0.8	0.0	0.3
os	β -Eudesmol	1649	0.2	0.0	0.2
os	α -Cadinol	1653	9.1	1.4	6.2
os	14-hydroxy-9-epi-E-caryophyllene	1664	1.6	0.0	1.6
os	<i>Cis</i> -14-nor-muurolen 5-en 4-one	1682	0.9	0.1	1.4
os	(<i>E,E</i>)-Farnesol	1722	0.2	0.0	0.2
os	14-oxy-a-muurolene-	1764	0.2	0.0	0.2
os	(<i>Z</i>)- α -santalol acetate	1786	0.0	0.0	0.4
nt	(<i>N</i>)-octadecane	1800	1.5	0.0	0.1
Total			94.0	99.6	98.8
Non Terpene Derivates (nt,%)			2.8	3.5	2.2
Monoterpene Hydrocarbons (mh, %)			1.4	6.0	1.9
Oxygenated Monoterpene (om, %)			51.5	77.1	54.9
Sesquiterpene Hydrocarbons (sh, %)			8.8	6.8	12.4

Oxygenated Sesquiterpene (os, %)	29.2	6.3	26.9
Apocarotenoids (ac,%)	0.3	0.0	0.4

*All the constituents belong to non terpene derivates (nt), monoterpene hydrocarbons (mh), oxygenated monoterpene (om), sesquiterpene hydrocarbons (sh), oxygenated sesquiterpene (os), and apocarotenoids (ac).