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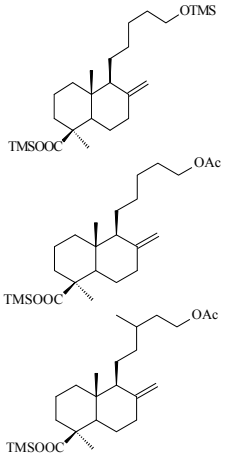
Graphical abstract



Title page

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Labdane-type diterpenoids from *Juniperus communis* needles

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Abstract

Herein we report the extraction of labdane-type diterpenoids from the needles of the common juniper (*Juniperus communis ssp. communis var. communis L.*), and identification of them as the major class of compounds in this species. Furthermore, and to the best of our knowledge we provide the first-ever report some of these compounds (nor-16-imbricatolic, nor-16-acetylimbricatolic and acetylimbricatolic acids) as natural compounds. Imbricataloic and imbricatolic acids were the most abundant compounds, accounting for 65% of the total extract. We also found long-chain secondary alcohols (*n*-alkan-10-ols) and secondary/secondary alkanediols (4,10-, 5,10-, 6,10-, 7,10-), which we report here for the first time ever in the genus *Juniperus*. In total, we identified 127 compounds: of these, 48 (nonacosan-10-ols, γ-tocopherols and lignans) are described here for the first time in *Juniperus* and 54 (including alkanols, phytol, sterols and flavonoids), for the first time in common juniper.

Highlights

- 127 phytochemical constituents of the needles of *Juniperus communis* were investigated with imbricatolic and imbricatalic acid as the most abundants
- 3 Labdane-type diterpenoids are new natural products
- 17 compounds have no precedent in *Juniperus* genus and 32 are described for the first time from *Juniperus communis*

Keywords: *Juniperus communis*; Common juniper; Diterpenoids; Labdane-type; Alkanediols; Imbricatolic acid; Gas chromatography-Mass spectrometry (GC/MS); Trimethylsilyl (TMS) ether derivatives.

1. Introduction

The genus *Juniperus* (Cupressaceae) comprises 68 species and 36 varieties growing principally in the northern hemisphere (Gonny *et al*., 2006). The chemical composition of the genus *Juniperus* has been extensively reviewed by Seca & Silva (2006). Table 1 summarizes the phytochemical characteristics of the principal compounds from the genus *Juniperus*, excluding terpenes, secondary alcohols and secondary diols, which we discuss separately in a latter section.

The species *Juniperus communis L*. comprises four subspecies, including the common juniper (*J. communis* ssp. *communis*), a coniferous evergreen shrub that has a broad geographic range throughout the holarctic region. In fact, it exhibits the largest range of any woody plant, spanning the cool, temperate, part of the Northern Hemisphere: from mountains in the southern arctic, to roughly 30ºN latitude in North America, Europe and Asia. It tolerates a variety of climatic and edaphic conditions.

The surface layer of plant leaves typically contains many classes of compounds, including alkanes, primary alcohols, acids, aldehydes, terpenes and phenols. However, there are very few reports on the overall composition of common-juniper needles. Thus, in the study reported here, we sought to analyze the soluble fraction of common-juniper needles.

We analyzed the total extract of common-juniper needles and identified each wax-class of compounds, discovering many acyclic and cyclic compounds from three major groups: *terpenoids* (*e.g.* sesquiterpenes and diterpenes); *phenols* (including phenolic acids, flavonoids and lignans); and *aliphatic alcohols* (secondary alcohols and secondary/secondary alkanediols). We describe here three newly discovered labdane-type diterpenes, which we have named *nor-16-imbricatolic acid*, *nor-16-acetylimbricatolic acid* and *acetylimbricatolic acid*.

2. Material and methods

2.1. Sampling site

Needles from the branches of the upper third of a common juniper were collected from a chose field site located in the southern limit of the village of Calders (Bages, Catalonia). The coordinates were 46º14'55''N by 4º6'46''E, and the elevation was 330 m.

2.2. Plant material

Branches of *J. communis* were collected in May 2004 from the sampling site indicated above (Section 4.1) and were identified by Dr. Joan Simon (joansimon@ub.edu) of the University of Barcelona. A voucher specimen (BCN 106843) was deposited at the Herbarium of the Faculty of Pharmacy at the University of Barcelona, in Barcelona, Catalonia.

2.3. Analytical procedures

2.3.1. Extraction

Fresh green needles from the twigs of the common juniper were collected, mixed with sand (1:1, w/w) and finally, crushed manually with a mortar and pestle. A sample (7 g) of needles was introduced into a cellulose cartridge, and then extracted in pentane/dichloromethane (7:3, v/v) in a Soxhlet apparatus. Given the non-polarity of these solvents, the compounds in the extract were expected to be more hydrophobic than hydrophilic. To facilitate MS analysis of polar compounds (alcohols and carboxylic acids), their corresponding TMS derivatives were studied. These were

prepared by treating the total extract with *N,O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA [Merck]; 250 μL; 1 h; 70 ºC).

2.3.2. Gas-chromatography/Mass-spectrometry (GC/MS)

For GC-MS analysis of the derivatized total extract of needles of the common juniper, a sample of the extract was dissolved in 1 μL dichloromethane, and then injected in splitless mode into a Fisons Instruments GC 8000/MD 800 integrator (injector temperature: 275 ºC.), using helium as carrier gas. The column used was a fused-silica capillary column (DB-5MS; 5% phenylmethylpolysyloxane; 30 m x 0.25 mm i.d.; film thickness: 0.25 μm [J&W Scientific, Folsom, CA]). The oven temperature to heat the column was programmed as follows: hold at 40 ºC for 1 minute; ramp up to 230 ºC at a rate of 20 ºC/min; ramp up to 300 ºC at a rate of 2 ºC/min; and finally, hold at 300 ºC for 20 min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, the mass range was from *m/z* = 50 Daltons to *m/z* = 650 Daltons, and the scan time was 1.0 s.

2.4. Identification of the compounds

The structures were elucidated by mass spectrometry and subsequent comparison of the resulting spectra with literature data. Silylated derivatives were identified based on mass-fragmentation patterns, by matching (on computer) the mass spectra data with those from the NIST library or using relevant literature data whenever possible, and according to retention times, by comparison with published results.

2.5. Quantification

The compounds were quantified based on the integration of the individual peak voltage areas of the chromatographic profile of the characteristic ions. Compound concentrations (expressed as mg/kg dry weight [dw]) were calculated using an internal standard (friedeline); this method afforded semi-quantitative results based on total ion-current peak areas. The mass fragmentation peaks were integrated by assuming similar fragmentation for each compound in a given homologous series. The quantification ions were: *m/z* = 85 for *n*-alkanes; *m/z* = 103 for *n*-alkanols; *m/z* = 132 for *n*-alkanoic acids; *m/z* = 129 for *n*-alkenoic acids; *m/z* = 204 for *n*-ω-hydroxy acids; *m/z* = $[M-265]^+$ for tocopherols; *m/z* = $[M-129]^+$ for sterols; *m/z* = 121 for diterpenoids; *m/z* = 229 for secondary alcohols; *m/z* = 317 for secondary/secondary alkanediols; *m/z* = 136 for monoterpenoids; and *m/z* = 179 for phenols and phenolic acids.

3. Results and discussion

3.1. General description

In this study, we have established the chemical composition of needles from the common juniper. The compounds represent several classes of acyclic (*n*-alkanes, *n*-alkanols, *n*-alkanoic and *n*-alkenoic acids, *n*-ω-hydroxy acids and monoglycerides) and cyclic compounds (tocopherols, sesquipenoids, diterpenoids, phenolic acids, lignans and flavonoids). To the best of our knowledge, we provide here the first-ever report of several compound classes in the genus *Juniperus* and/or in the species *Juniperus communis*. We identified a total of 127 compounds, of which three were detected for the first time as natural compounds; 48, for the first time in *Juniperus*; and 54, for the first

time in *J. communis*. Figure 1 shows the total ion current (TIC) corresponding to the total extract of the needles of the common juniper; the acyclic compounds are listed in Table 2, and the cyclic ones, in Table 3. Detailed mass-spectrometry (MS) characteristics of the identified secondary alcohols, alkanediols and diterpenoids, and histograms showing the relative amounts of several of the compound classes compounds discussed in the text, are presented in the Supplementary Information.

The chromatographic profile of the needles from *Juniperus communis* exhibits two major peaks, each corresponding to a labdane-type diterpene: *imbricataloic acid* [15-oxo-8(17)-labden-19-oic acid] and *imbricatolic acid* [15-hydroxy-8(17)-labden-19-oic], which have previously been identified only in the species *Pinus* (Spalding *et al*., 1971; Zinkel and Critchfield, 1974; Zinkle and Clark, 1985; Zinkel and Magee, 1991) and in the berries of *J. communis* (De Marino *et al*., 2011). Other compounds found in the extract included long-chain secondary alcohols and secondary/secondary alkanediols, the most abundant of which were nonacosan-10-ol, 5,10-nonacosanediol and 4,10-nonacosanediol. The distribution of these alcohols was very similar to that reported for the needles of *Picea abies* and of *Picea sitchensis* (Prügel & Lognay, 1996).

3.2. Aliphatic hydrocarbons

3.2.1. n-Alkanes

We detected and quantified nine long-chain *n*-alkanes in the wax extracted from the needles of the common juniper (0.3%; 101 mg/kg dry weight). The compounds range in formula from *n*-C_{27} to *n*-C_{35} and were dominated by the odd-C-numbered homologs, the most abundant of which (in the unimodal distribution) was tritriacontane (61%). The respective concentrations are shown in Table 2. In previous work on *Juniperus*

communis, Dodd and Poveda (2003) found that *n*-C_{33} was the most abundant alkane in the cuticular waxes that they identified (range: *n*-C_{21} to *n*-C_{35}), and De Pascual-Teresa (1977) reported a series of *n*-alkanes ranging in formula from *n*-C_{23} to *n*-C_{31}. Furthermore, in a similar study to ours, but in *Juniperus scopulorum*, Tulloch and Bergter (1981) identified tritriacontane as the major hydrocarbon (68%).

3.3. Fatty alcohols

3.3.1. n-Alkan-1-ols

Another minor class of wax epicuticular components that we identified in the common juniper was primary alcohols (0.2%; 53.9 mg/kg dry weight). The homologous series (Table 2) ranges in formula from *n*-C_{16} to *n*-C_{30}, shows even-C-numbered predominance, with the most abundant compound being *n*-docosanol (27.5 mg/kg dw; 53%). These results parallel those reported by Tulloch and Bergter (1981) for *Juniperus scopulorum*, in which the *n*-C_{22} homolog accounted for 30% of the hydrolyzed wax. In a study on the bark of *J. brevifolia*, Seca & Silva (2008) found the homologs *n*-C_{20} and *n*-C_{22}, and in a later study (Seca & Silva, 2010), they identified docosanol in the leaves of the plant. Herein we report thirteen *n*-alkan-1-ols that, to the best of our knowledge, have never previously been described in the common juniper.

3.3.2. Phytol

We detected one isoprenoid alcohol in the needles of the common juniper: phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol). Its concentration (9.9 mg/kg dw) was lower than that normally reported in the literature for angiosperms. Interestingly, and to the best of our knowledge, this compound has never previously been described in the common juniper. However, this compound has previously been reported in the leaves of

three other species: *J. chinensis* (Fang *et al*., 1993), *J. phoenicea* (Barrero *et al*., 2004) and *J. thurifera* var. *Africana* (Barrero *et al*., 2004).

3.3.3. Asymmetric n-secondary alcohols

In plants, asymmetric *n*-secondary alcohols usually appear as a single major homolog in which the hydroxyl group is located at C-10, although it has been described in other positions (C6 to C17). As expected from the literature precedent on these alcohols in plants, in the extract of the common juniper we identified a homologous series of 5 isomers, all of which contain a hydroxyl group at C-10. To the best of our knowledge, four of these secondary alcohols have not previously been reported in the genus *Juniperus*, and none of them has ever been reported in the common juniper. An overview of the literature on secondary alcohols isolated from plants is provided in Table S2 *(Supplementary Material)*.

We detected 10-*n*-nonacosanol (also known as *gimnol*) in minor amounts (see Table 2), together with 10-*n*-heptacosanol (10-*n*-C_{27}), 10-*n*-octacosanol (10-*n*-C_{28}), 10-*n*-triacontanol (10-*n*-C_{30}) and 10-*n*-hentriacontanol (10-*n*-C_{31}). Interestingly, 10-*n*-nonacosanol is the most widely reported secondary alcohol from the genus *Juniperus*: it has been described in the needles of species such as *J. pinchotti* (Kircher, 1982) and *J. scopulorum* (Tulloch and Bergter, 1981) as well as in the berries of the common juniper (De Pascual Teresa *et al*., 1977). This aliphatic alcohol has also been identified in the waxes from other conifers, in which it constitutes waxy, tubular, crystalloid aggregates (known as *nonacosanol tubules*) that can be observed throughout the surface of the needles of various plants (Holloway *et al*., 1976; Jetter and Riederer, 1994; Barthlott *et al*., 1998; Matas *et al*., 2003; Stabentheiner *et al*., 2004; Koch *et al*., 2009). In a

previous study on the secondary alcohols from several plants, Holloway *et al*. (1976) reported that the mass spectrum of 10-*n*-nonacosanol is dominated by two prominent ions in the middle, which result from α-cleavage of the TMS group. As they explain, the two fragments enable localization of the OH group in a parent alcohol. For example, the mass spectrum of the TMS-ether derivative of the homolog *n*-C_{29} shows two intense peaks: one at *m/z* = 229 (base peak), which corresponds to the fragment $C_{10}H_{20}OSi(CH_3)_3^{\cdot+}$, generated by α-cleavage at the shorter-chain end; and one at *m/z* = 369, which corresponds to $C_{20}H_{40}OSi(CH_3)_3^{\cdot+}$, generated by α-cleavage at the longer-chain end. Table 4 shows distinctive spectrometric characteristics of the TMS-ether derivatives of these secondary alcohols from the common juniper.

3.3.4. Secondary/secondary alkanediols (7,10-; 6,10-; 5,10-; 4,10-)

We identified another class of long-chain aliphatic hydroxyl compounds in the common juniper: *secondary/secondary alkanediols*. The literature on such alkanediols from plants is summarized in Table 5. We identified a mixture of eight homologs: 5,10-pentacosanediol; 5,10-heptacosanediol; 5,10-octacosanediol; 7,10-nonacosanediol; 6,10-nonacosanediol (newly discovered in *Juniperus*); 5,10-nonacosanediol; and 4,10-nonacosanediol. The respective concentrations of these compounds in the needle-extract are listed in Table 2. We also detected 5,10-hexacosanediol, albeit only in trace amounts; thus, we did not quantify it. The secondary/secondary alkanediols found in this study are predominantly odd-C-numbered, as reported for similar compounds in the literature (Holloway and Brown, 1977; Franich *et al*., 1979). The total concentration of these alkane diols was 393 mg/kg (dw), 93% of which corresponded to just two homologs: 5,10-*n*-C_{29} and *n*-4,10-C_{29}. Diols of 29 carbon atoms have previously been reported in *Juniperus pinchotii* (*n*-4,10-C_{29}, *n*-5,10-C_{29} and *n*-7,10-C_{29}), in a GC study

by Kircher (1982); in *Juniperus scopulorum* (*n*-4,10-C_{29}, *n*-5,10-C_{29}, *n*-7,10-C_{29} and *n*-13,14-C_{29}), by Tulloch and Bergter (1981); in the wax from *J. oxycedrus*, (nonacosane-5,10-diol), by De Pascual Teresa and Sáez (1973); and in the wax from *J. communis* (also nonacosane-5,10-diol), by De Pascual Teresa *et al*. (1977). Furthermore, the distribution pattern that we observed is similar to that described for the needles of *Pinus radiate* by Franich *et al*. (1979). In addition to containing high concentrations of asymmetric secondary alcohols (predominantly, nonacosan-10-ol), the aforementioned nonacosanol tubules also contain asymmetric alkanediols (Koch *et al*., 2009 and references therein). For instance, 5,10 and 4,10-nonacosanediols have been reported to be constituents of the epicuticular wax crystals that comprise the nanotubules that protrude from the surface of the needles of *Taxus baccata* (Wen *et al*., 2006).

3.4. Fatty acids: n-alkanoic and n-alkenoic acids

We identified a homologous series of fatty acids in the total extract of the common-juniper needles (Table 2). We confirmed eight saturated fatty acid homologs (as their corresponding TMS derivatives), of formulas ranging from *n*-$C_{12:0}$ to *n*-$C_{24:0}$ and dominated by even-C-numbered chains. The most prominent homolog of the unimodal distribution was palmitic acid (*n*-$C_{16:0}$). Only three unsaturated fatty acids were detected and quantified: *n*-$C_{18:2}$ $\Delta^{9,12}$ (linoleic acid), *n*-$C_{18:1}$ Δ^{9} (oleic acid) and *n*-$C_{18:3}$ $\Delta^{9,12,15}$ (α-linolenic acid). Our results differ from those obtained by Mongrand *et al*. (2001), who studied the fatty acid composition of 137 gymnosperm species from 14 families, including *Juniperus communis*: whereas we found the most abundant compounds in the common juniper to be *n*-$C_{16:0}$, *n*-$C_{18:2}$ $\Delta^{9,12}$, *n*-$C_{18:1}$ Δ^{9}, *n*-$C_{14:0}$ and *n*-$C_{12:0}$, they found the most abundant ones to be *n*-$C_{18:3}$ $\Delta^{9,12,15}$, *n*-$C_{20:2}$ $\Delta^{11,14}$, *n*-$C_{16:0}$, *n*-$C_{18:2}$ $\Delta^{9,12}$ and *n*-$C_{20:0}$. Interestingly, in a study on the bark of *J. brevifolia*, Seca & Silva (2008) found the most

abundant compounds to be the saturated and unsaturated homologs *n*-C_{16}, *n*-C_{18}, *n*-$C_{18:2}$ $\Delta^{9,12}$, *n*-$C_{18:1}$ Δ^{9}, *n*-C_{18}, *n*-C_{22} and *n*-C_{24}, and in a subsequent study on its leaves, found the most abundant compound to be palmitic acid *n*-$C_{16:0}$ (Seca & Silva, 2010).

3.5. n-ω-Hydroxyacids

We found four homologs of *n-ω-hydroxyacids* in the total extract of the common-juniper needles. The most abundant of these compounds was 16-hydroxypalmitic or juniperic acid (11.1 mg/kg dw). We also identified together with juniperic (*n*-C_{16}) and sabinic (*n*-C_{12}) acids, which have previously been reported in the common juniper by Seca & Silva (2005), the homolog 14-hydroxy *n*-alkanoic acid, which, to the best of our knowledge, has not been previously reported in this species. Lastly, we found 22-hydroxydocosanoic fatty acid (*n*-C_{22}), which represented only small proportions of the total extracted amount of *ω-hydroxyacids* (17.2 mg/kg dw) and, to the best of our knowledge, has not been previously been reported in the genus *Juniperus*.

3.6. Diterpenoids

3.6.1. General considerations

We identified twenty-two diterpenic compounds of the labdane (10), abietane (8) and pimarane (4) classes in the pentane-dichloromethane extract of the needles of the common juniper (Fig. 2 and 3; Table 6). These include three newly discovered products that, to the best of our knowledge, have never previously been reported as natural products: nor-16-imbricatolic acid, nor-16-acetylimbricatolic acid and acetylimbricatolic acid. The preliminary GC analyses of their corresponding TMS derivatives revealed two labdane-type diterpenoids, which we identified as imbricataloic acid ($[M]^{\cdot+}$ = 392) (8460 mg/kg dw; 27.2%) (Fig. 4) and imbricatolic acid ($[M]^{\cdot+}$ = 466)

(11800 mg/kg dw; 7.8%) (Fig. 5), which were the two most abundant components of the total extract of the juniper. Furthermore, 94% of all of the diterpenes detected corresponded to only seven compounds: pimaric, sandaracopimaric, *trans*-communic, isopimaric, imbricataloic, imbricatolic and isocupressic acids. The diterpenic profile was similar to that previously reported for *Pinus ponderosa* needle oleoresin by Zinkel & Magee (1991, and references therein), who also found labdane diterpenes, imbricataloic acid and imbricatolic acid, as well as isocupressic, acetylimbricatolic acid, dihydroagathic acid (also known as *junicedric acid*) and acetylisocupressic acid. The diterpenoid region in the GC/MS chromatogram presented a pattern of peaks different from that reported by Gardner *et al*. (1999) in *J. communis*: our pattern indicated a minor amount of imbricatolic acid, which their pattern did not. The acid fractions obtained from hexane extract of leaves of *J. communis* in a previous work (San Feliciano *et al*., 1991) showed isocupressic and isopimaric acids as the main compounds.

3.6.2. Tricyclic abietane-type diterpenoids

In our study on common-juniper needles, labdane-type diterpenes were the most abundant compounds and abietane-type diterpenes were the least abundant compounds. However, Lee *et al*. (1995), in their study of needles and bark of *J. chinensis*, reported the opposite trend. For example, they mention dehydroabietic acid, which is the main diterpenoid in several gymnosperms yet was nearly absent in our extract of common-juniper needles. Also, Seca & Silva (2008) found abietanoids to be prevalent in the bark of *J. brevifolia*; however, these compounds are generally associated with *Pinaceae*.

We also identified four phenolic terpenoids (ferruginol, dehydroabietol, totarol and sugiol [7-ketoferruginol]), albeit in minor amounts. However, they have been reported as biomarkers of the Cupressaceae (cypress) family according to Cox *et al*. (2007). Totarol has previously been reported in the needles of the common juniper (Gordien *et al*., 2009). The most abundant abietanes in our study were palustric acid and dehydroabietol (Table 6). We also found, through MS characterization, minor amounts of pisiferol and hinokiol, which are scarcely reported in the literature (Otto and Simoneit, 2001).

3.6.3. Tricyclic pimarane-type diterpenoids

Based on the work of Cox *et al*. (2007), we identified four pimaranes in the common-juniper needles: pimaric acid, sandaracopimaric acid, isopimaric acid and levopimaric acid, all these which have a molecular ion at *m/z* 374. Isopimaric acid and sandaracopimaric acid have previously been reported in the needles of this plant (De Pascual *et al.*, 1980).

3.6.4. Bicyclic labdane-type diterpenoids

As we mentioned above, in addition to finding large amounts of imbricatolic acid and imbricataloic acid, we also identified others labdanoids in the common juniper (see Table 6). These include *trans*-communic acid ($[M]^{\cdot+}$ = 374), which in our study exhibited a similar MS pattern to that indicated by Cox *et al*. (2007). This compound was also found in the needles of the common juniper by De Pascual *et al*. (1980) and by Gordien *et al*. (2009), and isocupressic acid was previously reported in this plant by De Pascual *et al*. (1980).

Imbricataloic acid (also known as *dihydroisocupressic acid*) was first reported as a natural compound in plants by Spalding *et al*. (1971), who found it in the needles and the cortex oleoresin of *Pinus elliottii*. Since then, this compound has been reported several times in plants, chiefly, in the genus *Pinus*. However, it has previously been reported in the common juniper (De Pascual *et al*., 1980; and San Feliciano *et al.*, 1991), inlcuding in the berries of this plant (De Marino *et al*., 2011).

Figure 3 shows the mass spectrum of the TMS-ether derivative of imbricataloic acid, together with its fragmentation pattern. Several peaks can be observed: the molecular ion at *m/z* = 392; a peak at *m/z* = 377, which corresponds to loss of a methyl group (most likely, at C-10 [Conner *et al*., 1980]); a very prominent peak at *m/z* = 274, corresponding to the fragment $[M\text{-}HCOOTMS]^{\cdot+}$, generated upon loss of the silylated carboxylic group at C-4; the base peak at *m/z* = 73; the very abundant, characteristic diterpenoid ion at *m/z* = 121 (C_9H_{14}); and finally, characteristic labdane fragment ions at *m/z* 143 ($[TMSO\text{-}CH_2\text{=}CH\text{-}C(CH_3)]^{\cdot+}$), *m/z* = 161 (M-231), *m/z* = 175 ($C_{13}H_{19}$) and *m/z* = 189 ($C_{14}H_{21}$), which have previously been described for similar compounds (Scalarone *et al*., 2003).

We also identified three imbricatolic acid analogs that are oxygenated at C-19 and C-15 in the total extract of the needles from *Juniperus communis* (see Fig. 4): nor-16-imbricatolic acid ($[M]^{\cdot+}$, *m/z* = 452), acetylimbricatolic acid ($[M]^{\cdot+}$, *m/z* = 436) and nor-16-acetylimbricatolic acid ($[M]^{\cdot+}$, *m/z* = 422). To the best of our knowledge, these compounds are reported here for the first-time ever.

The mass spectrum of the TMS-ether derivative of each bicyclic labdane-type diterpenoids described above showed an M-15 peak, as is expected for silylated compounds. The mass spectrum of imbricatolic acid (Fig. 4(a)) is characterized by a prominent peak at *m/z* = 348, corresponding to loss of the silylated carboxylic group ($[M\text{-}HCOOTMS]^{\cdot+}$) (M-118); in the center of the spectrum, the doublet *m/z* = 258/259, corresponding to the fragment generated when the former ion loses its derivatized hydroxyl group ($[M\text{-}HCOOTMS\text{-}TMSOH]^{\cdot+}$) ([M-118]-90); a prominent peak at *m/z* = 238, $[M\text{-}OTMS\text{-}]^{\cdot+}$ (M-118), corresponding to simultaneous cleavage of the 9,10- and 5,6-bonds; in the upper part of the spectrum, a less abundant peak at *m/z* = 376 (M-90), which corresponds to the loss of the silylated hydroxyl group at C(15) ($[M\text{-}HOTMS]^{\cdot+}$); the minor ion *m/z* = 308 ($[TMSO\text{-}(CH_2)_2\text{-}CH(CH_3)\text{-}CH_3]^{\cdot+}$) (M-158), representing the fragment generated upon cleavage of the side chain; in the center of the MS, a peak at *m/z* = 293, which represents loss of a methyl group; in the lower part of the MS, very prominent peaks at low *m/z* values that are characteristic of all labdanoids and diterpenoids under electron-impact ionization, including the base peak at *m/z* =121, corresponding to the metastable ion C_9H_{14}, a peak at *m/z* = 189 ($C_{14}H_{21}$), at *m/z* 175 ($C_{13}H_{19}$); and a peak at *m/z* = 73, corresponding to loss of the TMS group.

Figure 4 (b) shows the mass spectrum of a compound that contains most of the same diagnostic peaks as those found for imbricatolic acid (Fig. 4 (a)), minus a methyl group and identified as nor-16-imbricatolic acid. The molecular ions ($[M]^{\cdot+}$, *m/z* = 452), $[M\text{-}HCOOTMS]^{\cdot+}$ (*m/z* = 348) and $[M\text{-}HCOOTMS\text{-}TMSOH]^{\cdot+}$ *m/z* = 258) are fourteen units (m/z = 14) lower than for imbricatolic acid, due to loss of a methyl group. However, the spectra for both compounds share the peaks at *m/z* = 73, *m/z* = 121, *m/z* = 175 and *m/z* = 189, suggesting that the methyl group implicated in the fragmentation pathway had probably been located at C-16 in the parent compound.

The mass spectrum of the labdane-derivative acetylimbricatolic acid, which was characterized by an acetyl group, shows a mass peak at $[M]^{\cdot+}$, *m/z* = 436 (Fig. 4 (c)); a characteristic fragment ion (Popova *et al*., 2010) formed by the loss of [HCOOTMS] (*m/z* = 118) (Cox *et al*., 2007), which gives a peak at *m/z* = 318; and a peak at *m/z* = 258, corresponding to elimination of an AcOH unit (*m/z* = 60) (Dijkstra *et al*., 2010).

Finally, we identified nor-16-acetylimbricatolic acid in the mass spectra, (Fig. 4 (d)) based on loss a methyl group (fourteen units) from the molecular ions $[M^{+}]$, $[M\text{-}15^{+}]$, $[M\text{-HCOOTMS}]^{\cdot+}$ and $[M\text{-HCOOTMS-}CH_3COOH]^{+}$. However, as we previously observed for other compounds in this series, the spectrum for this compound, and that for imbricatolic acid, share the peaks at *m/z* = 73, *m/z* = 121, *m/z* = 175 and *m/z* = 189, indicating that, as we mention above, the methyl group implicated in the fragmentation pathway in each ase had probably been located at C-16 in the corresponding parent compound.

To the best of our knowledge, the three aforementioned bicyclic labdane-type diterpenoids (nor-16-imbricatolic acid, acetylimbricatolic acid and nor-16-acetylimbricatolic acid) are described here for the first time ever.

3.7. Sterols

We found only two sterols in the extract of the common-juniper needles (see Table 3): campesterol (C_{28}; 47 mg/kg dw) and β-sitosterol (C_{29}; 364 mg/kg dw), which were present in relatively small amounts relative to the diterpenes. Both compounds have previously been reported in this plant (Seca & Silva, 2005).

3.8. Tocopherols

In the needles of the common juniper, we detected three of the four tocopherols (α-, β-, γ- and δ-) typically found in plants (see Table 3). Of the total tocopherol content, 98% corresponded to α-tocopherol (141 mg/kg dw), as was expected, since this compound is predominant in the leaves of nearly all plants; the remainder comprised two isomers of γ-tocopherol (combined concentration: 1.2 mg/kg dw). The α-tocopherol content we observed is very similar to that (145 mg/kg) previously reported for the same plant (Szymanska & Kruk, 2008). To the best of our knowledge, the two γ-isomers have not previously been described in the genus *Juniperus*.

3.9. Monoglycerides

We identified five homologs of a series of monoglycerides in the common juniper, according to Coello *et al*. (2007), the two most abundant of which were the *n*-C_{16} and *n*-C_{22} homologs. To the best of our knowledge, these compounds have not previously been reported in the genus *Juniperus*.

3.10. Monoterpenes and sesquiterpenes

We isolated five monoterpenes from the wax cuticles of the common-juniper needles (see Table 7): 2-carene (65%), tricyclene (18%), terpinen-4-ol (12%), α-pinene (22%), and isobornyl acetate (7.5%). As was expected from their high volatility, the monoterpenoids in the Söxhlet extract were present in only low levels (total amount: 137 μg/kg dw). Our findings for these compounds differed to those reported in the literature for similar plants: for example, Gonny *et al*. (2006) reported that the most abundant monoterpenes in the common juniper (*J. communis* subsp. *alpina*) were limonene (30.9%), α-pinene (24.4%) and β-phellandrene (12.6%); and Orav *et al*. (2010) found that the prevailing monoterpene in the oil from the needles of *J. communis*

was α-pinene (33.7% to 36.4%). To the best of our knowledge, we provide here the first reports of tricyclene in the genus *Juniperus* and of isobornyl acetate in the common juniper.

After diterpenes, sesquiterpenes were the most abundant structural class that we identified in the common juniper, representing 5% of the total compounds (Table 7). We identified eight sesquiterpenoids, in agreement with previous studies (Shahmir *et al*., 2003; Seca & Silva, 2005; Cabral *et al*., 2012): β-elemene, β-caryophyllene, (*Z*)-β-farnesene, α-humulene, α-curcumene, germacrene D, bicyclogermacrene and germacrene D-4-ol. Of these eight compounds, the most abundant were β-elemene (28%), germacrene D (25%), β-caryophyllene (17%) and germacrene D-4-ol (15%). Having reviewed the relevant literature we can affirm that, to the best of our knowledge, we provide here the first-ever report of β-elemene, α-curcumene and bicyclogermacrene in the genus *Juniperus*, and of (*Z*)-β-farnesene in the common juniper. In an earlier study on common-juniper needles, Martz *et al*. (2009) reported germacrene D-4-ol and germacrene D as the terpenoids and that together they represented 40% of the total sesquiterpenoid content. Gonny *et al*. (2006), in their study of the common juniper (*J. communis* subsp. *alpina*), reported that the dominant sesquiterpenes were δ-cadinene, γ-cadinene and τ-cadinol.

3.11. Phenolic compounds

Phenolic compounds are a large, diverse class of compounds that among the major families of secondary metabolites in plants. However, there is scarce literature on the phenolic composition of common juniper-needles (Martz *et al*., 2009).

3.11.1. Phenols and phenolic acids

We identified 20 phenols and phenolic acids in the needles of the common juniper (see Table 3). These compounds, all of which are monocyclic, include benzoic acid and several related derivatives (salicylic acid, vanillin, 4-hydroxybenzoic acid, vanillic acid and syringic acid); cinnamic acid and related derivatives (*cis*-ferulic acid, *trans*-*p*-coumaric acid, *trans*-sinapyl alcohol and *trans*-ferulic acid); tyrosol; and hydroxytyrosol. The total amount of these compounds in the extract from the common-juniper needles was 244 mg/kg. The most abundant compounds were dihydro-*p*-coumaryl alcohol (4-hydroxybenzene propanol) (35%), 4-hydroxybenzaldehyde (26%) and *trans*-ferulic acid (14%). In an earlier study on *Juniperus drupacea* berries, Miceli *et al*. (2011) reported a total phenolic content of 48 mg/kg, in which tyrosol was the predominant phenol. In a study on the needles of *J. foetidissima*, Lesjak *et al*. (2013) found fourteen phenolic acids, including *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid, although in higher concentrations than those we found for the common juniper. In a very recent study on *Juniperus macrocarpa*, Lesjak *et al*. (2014) reported eleven phenolic acids (Table 1), the most abundant of which were protocatechuic acid and gallic acid (neither of which we detected in the common juniper), followed by *p*-hydroxybenzoic, *p*-coumaric, ferulic acid and vanillic acid (all of which we identified in our study). To the best of our knowledge, we report here for the first time fourteen phenols and phenolic acids in the common juniper.

3.11.2. Lignans

Given that the order Pinales, which includes the genus Cupressaceae, is the greatest plant source of lignans (Yamamoto *et al*., 2004 and 2010; Willför *et al*., 2006), which are phenols that contain two phenylpropane units, we expected to find these compounds

in the extract of the common-juniper needles. We indeed identified eight lignans in the extract, albeit in minor amounts (total concentration: 99.1 mg/kg dry weight). These compounds comprised (from most to least abundant): two lignanolides from the matairesinol group (3',4'-methylenedioxy-5-methoxymatairesinol-4-methyl ether ($[M]^{\cdot+}$ = 400) and the parent compound matairesinol ($[M]^{\cdot+}$ = 502)); the furofuran pinoresinol ($[M]^{\cdot+}$ = 502); two isomeric tetrahydrofuran derivatives (secoisolariciresinol ($[M]^{\cdot+}$ = 650) and lariciresinol ($[M]^{\cdot+}$ = 576)); two neolignans with a dihydrobenzofuran skeleton (dihydrodehydrodiconiferyl alcohol ($[M]^{\cdot+}$ = 576)) and a related isomer with an hydroxyl group at C-7; and the aryltetralin deoxypodophyllotoxin ($[M]^{\cdot+}$ = 398).

Matairesinol and secoisolariciresinol have previously been reported in the leaves and seed cones of *Juniperus macrocarpa* (Lesjak *et al.*, 2014) and in the berries of the common juniper (De Marino *et al*., 2011). Interestingly, the cyclic lignan deoxypodophyllotoxin was isolated by Carpenter *et al*. (2012) from the aerial parts of the common juniper and subsequently studied for its potent antitumor, antiviral and anti-inflammatory activities. To the best of our knowledge, deoxypodophyllotoxin identified by Carpenter *et al.* (2012) together with dihydrodehydrodiconiferyl alcohol (Castro *et al*., 1996) are the only ones of the eight lignans identified that has previously been reported in the needles of the common-juniper.

3.11.3. Flavonoids

We identified two flavonoids in the needles of the common juniper (Figure 1 and Table 3): 2-(3,4-methylenedioxyphenyl)propane-1,3-diol (165 mg/kg dw) and, in lesser amounts, 7-hydroxycoumarin (known as *umbelliferone*; 116 mg/kg dw).

2-(3,4-methylenedioxyphenyl)propane-1,3-diol, which has been reported in *Juniperus chinensis* (Fang *et al*., 1992), is considered unusual due to its atypical (for flavonoids)

branched skeleton. Figure 5 shows the mass spectrum of the TMS derivative of 2-(3,4-methylenedioxyphenyl)propane-1,3-diol. The spectrum is characterized by peaks at *m/z* = 340; *m/z* = 73 (the base peak) $[SiMe_3]^{+\cdot}$; *m/z* = 135 and *m/z* = 148 (both previously described by Fang *et al*. [1992]); and *m/z* = 325 $[M\text{-}CH_3]^+$; *m/z* = 250 $[M\text{-}90]^+$; and *m/z* = 103 $[CH_2OSiMe_3]^{+\cdot}$.

Umbelliferone is an anti-microbial (phytoallexinic and fungicidal) benzopyrone that was first studied by Jurd *et al*. (1971). Umbelliferone has previously been reported as the most abundant flavonoid in the needles of *Juniperus chinensis* (Fang *et al*., 1992), in the needles of *J. foetidissima* (Lesjak *et al*., 2013), and in the leaves and seed cones of *J. macrocarpa* (Lesjak *et al*., 2013). Interestingly, its derivatives are coumarins that help plants resist diverse bacteria, yeasts and molds. To the best of our knowledge, we provide here the first-ever report of these two flavonoids in the common juniper.

3.13. Miscellaneous compounds

In the needles of the common juniper, we also identified some compounds that do not fall into any of the classes described above. These comprised squalene and the metabolites phosphoric acid, glycerol, succinic acid and fumaric acid.

4. Conclusions

In conclusion, we have identified 127 compounds from the total resolvable cuticular waxes of the needles of the common juniper (*J. communis*), and semi-quantified nearly all of them. The predominant cuticular lipids of this species were (from most to least abundant): diterpenoids, sesquiterpenoids and secondary alcohols. Other components included a group in which even-C-numbered homologs dominate (encompassing *n*-alkanoic acids, *n*-alkenoic acids, ω-hydroxyacids and primary alcohols), and one in

which odd-C- numbered homologs dominate (including alkanes, secondary alcohols and diols). The only unsaturated fatty acids found were C-18 compounds. Sterols and antioxidants (*e.g.* α-tocopherol) also were detected. Finally, all of the detected alkanediols had secondary/secondary functional groups and odd-C-numbered hydrocarbon backbones. *n*-Alkanes, *n*-alkanols and *n*-alkanoic acids, despite being abundant in the epicuticular structures of the leaves of most higher plants, are present in only small amounts in the common juniper.

Figure captions

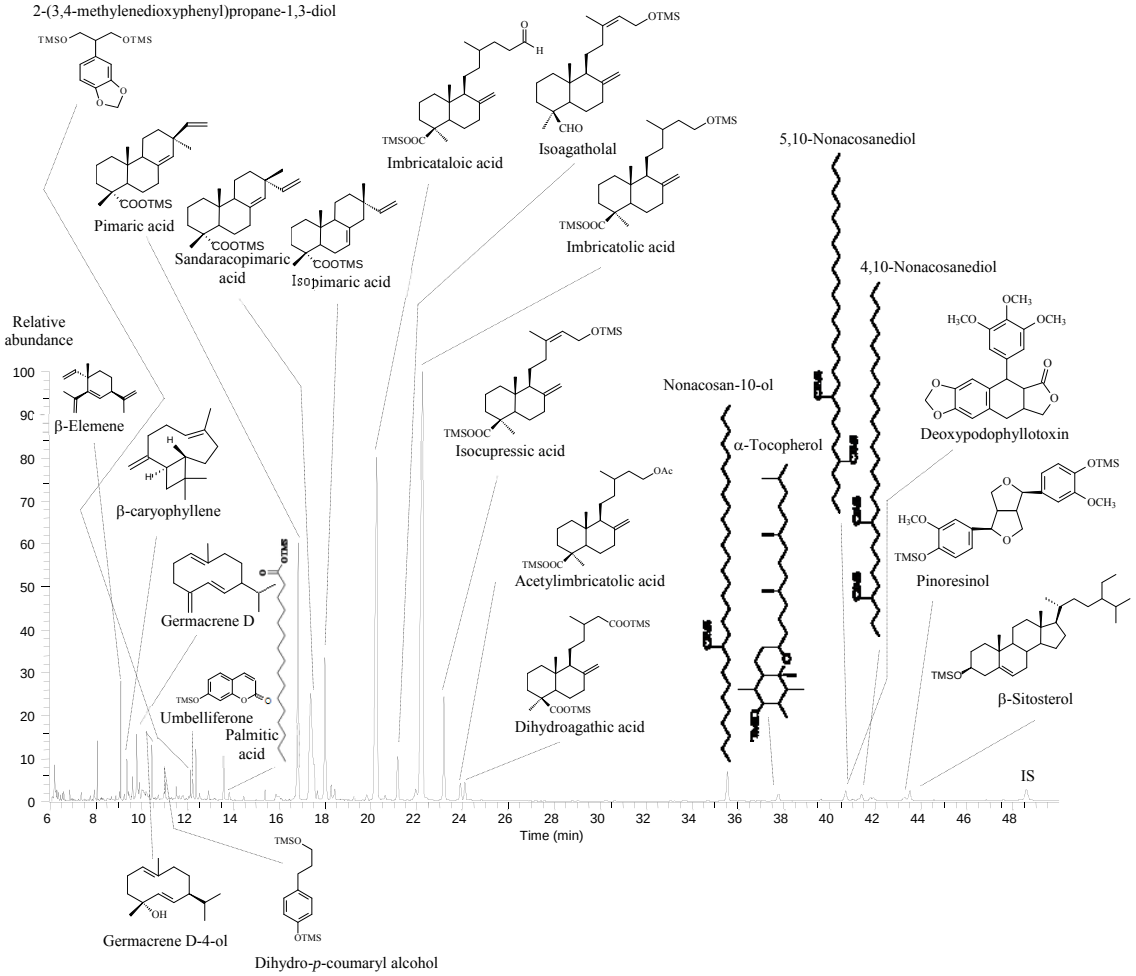
Figure 1. GC-MS Total Ion Current (TIC) chromatogram from the sylilated total extract of the common juniper (*Juniperus communis ssp. communis var. communis*) showing the structure of the major compounds identified. (IS = Internal standard).

Figure 2. Mass fragmentogram *m/z* 121 of silylated total extracts showing the diterpenoid elution region identified in the needles from the common juniper. In bold are marked new labdane-type diterpenoids.

Figure 3. Mass spectra of the labdane diterpenoid imbricataloic acid identified in the sylilated total extract of the needles of *Juniperus communis*.

Figure 4. Mass spectra of the diterpenoids: **(a)** imbricatolic acid, **(b)** nor-16-imbricatolic acid, **(c)** acetylimbricatolic acid and **(d)** nor-16-acetylimbricatolic acid identified in the sylilated total extract of the needles of *Juniperus communis*; **(b)**, **(c)** and **(d)** are new labdane-type diterpenoids.

Figure 5. Electron Impact Mass spectra of the lignin 2-(3,4-methylenedioxyphenyl)propane-1,3-diol identified as its TMSi derivatives in the sylilated total extract of the needles of *Juniperus communis*.



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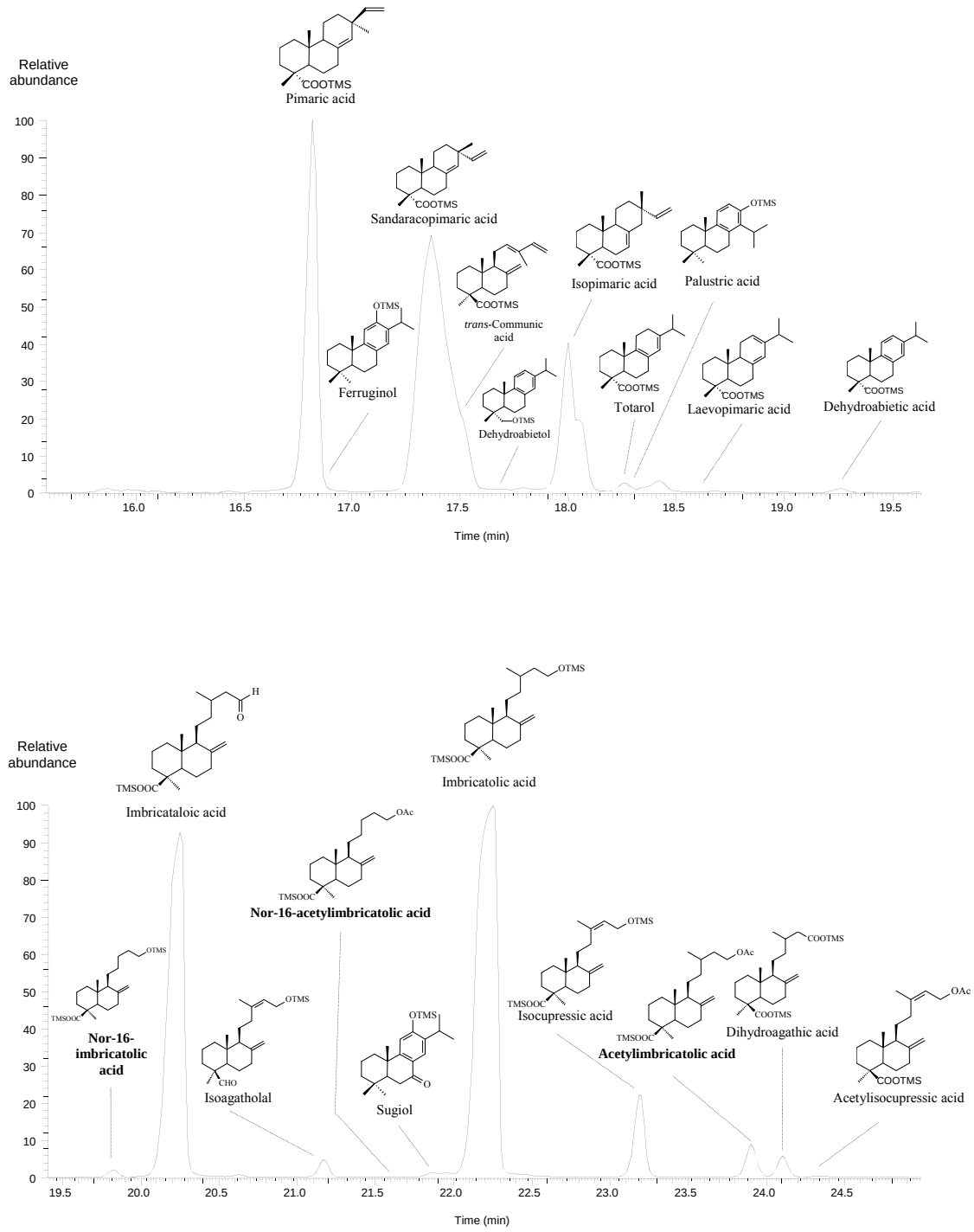

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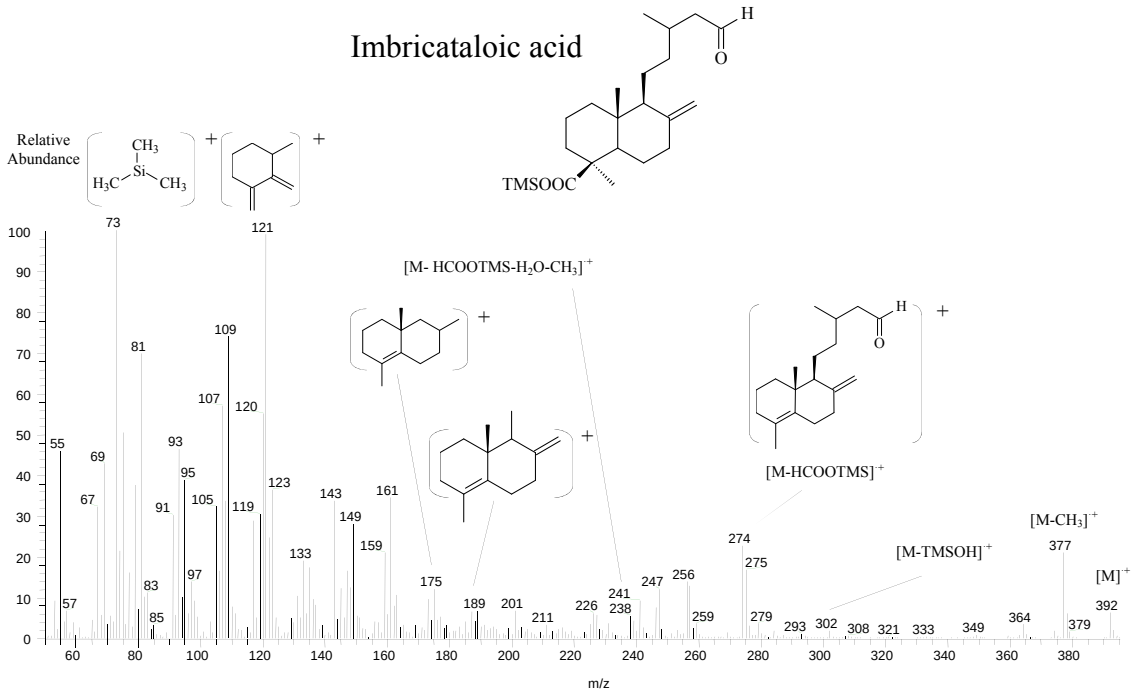

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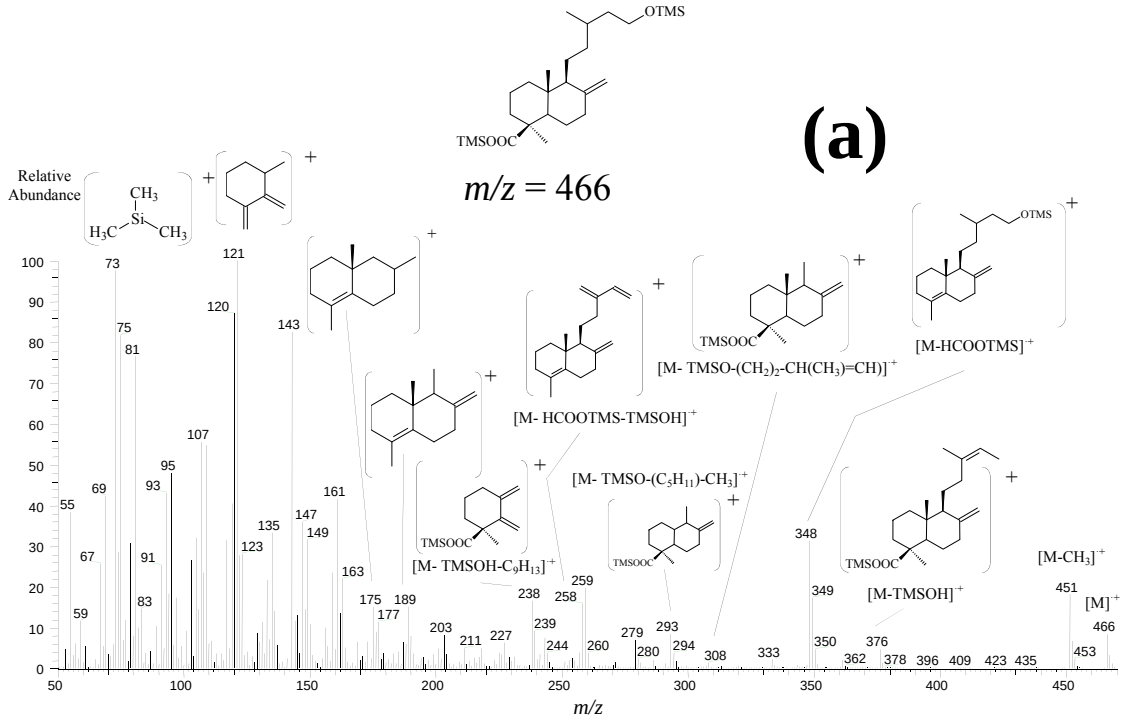
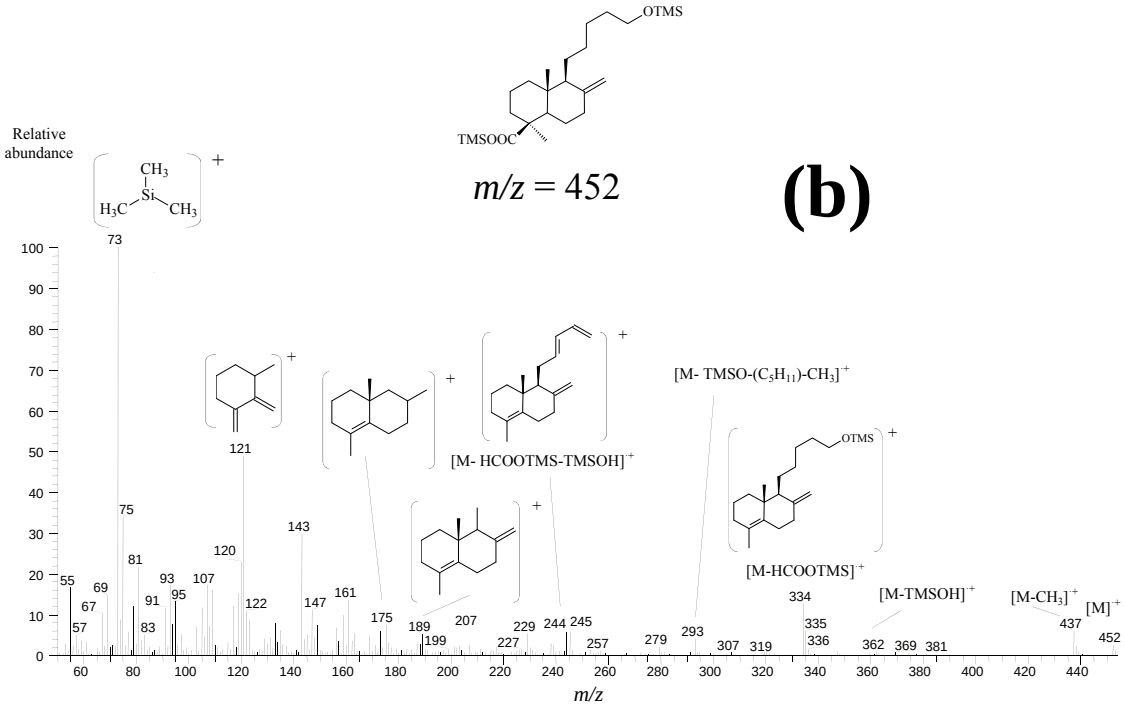
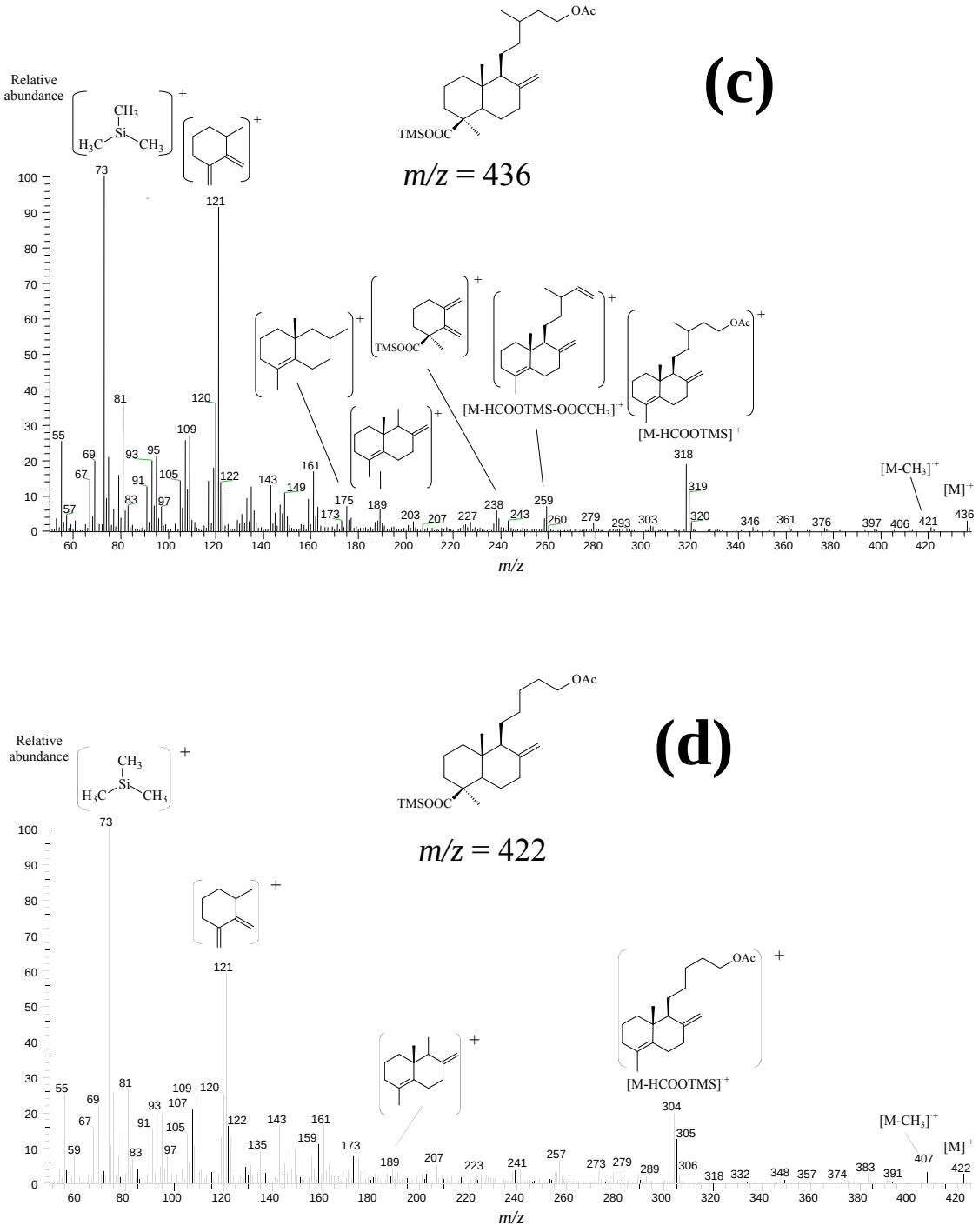

(a)
OTMS
TMSOOC
m/z = 466
Relative Abundance
[M- HCOOTMS-TMSOH]+
[M- TMSO-(CH2)2-CH(CH3)=CH)]+
[M-HCOOTMS]+
[M- TMSO-(C5H11)-CH3]+
[M- TMSOH-C9H13]+
[M-TMSOH]+
[M-CH3]+
[M]+
m/z


(b)
OTMS
TMSOOC
m/z = 452
Relative abundance
[M- TMSO-(C5H11)-CH3]+
[M- HCOOTMS-TMSOH]+
[M-HCOOTMS]+
[M-TMSOH]+
[M-CH3]+
[M]+
m/z



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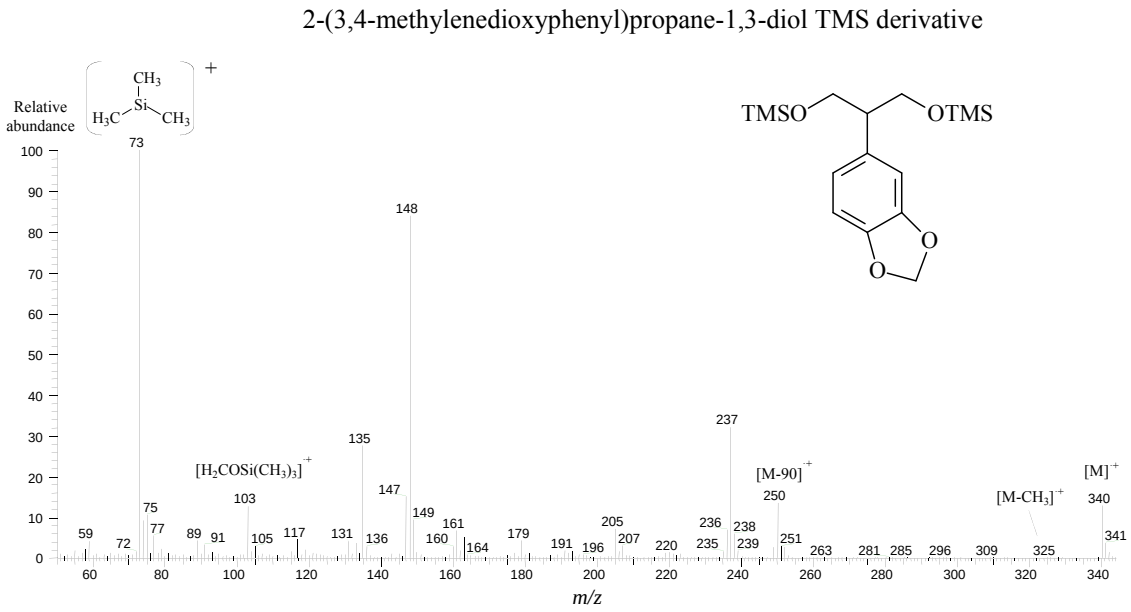

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Table captions

Table 1. Phytochemical characteristics of members of the *Juniperus* genus in the literature excluding terpenes and secondary alcohols and diols.

Table 2. Composition of total cuticular waxes (acyclic compounds) identified and quantified in the needles of *Juniperus communis*.

Table 3. Composition of total cuticular waxes (cyclic compounds) in the leaves of *Juniperus communis*.

Table 4. Spectrometric (EIMS-70 eV) distinctive characteristics (*m/z* (rel. int.)) of the members of the homologue series of secondary alcohols (10-ols) identified in the needles of *J. communis* in this study.

Table 5. Secondary/secondary alkanediols identified in the needles of *J. communis* in other studies.

Table 6. Diterpenes with labdane, pimarane and abietane skeletons identified and quantified as their TMSi derivatives in the total pentane/DCM extract of the needles from the common juniper (*J. communis*).

Table 7. Summary of the chemical composition of cuticular waxes contained i*n* the needles of *Juniperus communis*.

Table 1

Phytochemical characteristics of members of the *Juniperus* genus in the literature excluding terpenes and secondary alcohols and diols.

Species - Part of the plant	Compound	Reference
J. brevifolia - Bark	**Palmitic acid**[†], *trans*-3-hydroxy-4-methoxycinnamic acid, **oleic acid**, **stearic acid**, **eicosanol**, **docosanol**, docosanoic acid, **tetracosanoic acid**, sitosteryl acetate, campesteryl ester, 24-ethylcholest-5,7-dien-3-yl acetate, sitosteryl ester, **campesterol**, stigmasterol, **β-sitosterol**, stigma-4-en-3-one	Seca and Silva, 2008
J. brevifolia - Leaves	**Docosanol** and **palmitic acid**	Seca and Silva, 2010
J. brevifolia - Needles	Stigma-4-en-3-one, **β-sitosterol** and eicosanyl-*trans*-*p*-coumarate	Seca *et al*., 2008
J. chinensis – Bark	*β*-Sitosteryl-1-*O*-*β*-glucopyranoside	Chang *et al*., 2008
J. communis - Needles	**12-hydroxylauric acid**, **16-hydroxypalmitic acid** and two biflavonyls (hinokiflavone and cupressuflavone)	De Pascual *et al*., 1980
J. communis - Needles	***n*-Alkanes (*n*-C_{21}-*n*-C_{35})**	Dodd and Poveda, 2003
J. conferta – Heartwood	**β-Sitosterol**	Doi and Shibuya, 1972
J. drupacea - Berries	Gallic acid, protocatechuic acid, **tyrosol**, 7-hydroxycoumarin, chlorogenic acid, rutin, catechin, hypoalethin-7-pentoside, cupressuflavone, amentoflavone, two biflavones and two methyl-biflavones	Miceli *et al*., 2011
J. formosana - Bark	Oleic acid, nonadecyl ferulate, savinin, eciosyl ferulate, heneicosyl ferulate, **β-sitosterol**, sitostenone, sitosteryl dialdehyde and glycerol.	Kuo and Yu, 1996
J. macropoda - Berries	Hypolaetin 7-glucoside and *β*-sitosterol glucoside	Siddiqui and Sen, 1971
J. macrocarpa – Leaves and seed cones	Leaves: Protocatechuic acid > gallic acid > ***p*-hydroxybenzoic acid** > ***p*-coumaric acid** > **ferulic acid** > **vanillic acid** > 5-O-caffeoylquinic acid > syringic acid > caffeic acid > 2,5-dihydroxybenzoic acid Seed cones: Protocatechuic acid > gallic acid > ***p*-hydroxybenzoic acid** > **vanillic acid** > ***p*-coumaric acid** > **cinnamic acid** > syringic acid > **ferulic acid** > caffeic acid	Lesjak *et al*., 2014
J. phoenicea and *J. thurifera* - Needles	**Phytol**, (*E*,*Z*)-2,4-decadienyl isovalerate, cinnamyl isovalerate, 3',4'-dimethoxycinnamyl isovalerate, 3',4',5'-trimethoxycinnamyl isovalerate, 3',4'-dimethoxycinnamol, yatein, podophyllotoxin, **deoxypodophyllotoxin**, picropodophyllotoxin	Barrero *et al*., 2004
J. pinchotti - Needles	**β-Sitosterol**	Kircher, 1982
J. scopulorum - Needles	**Hydrocarbons (*n*-C_{33})**, esters (*n*-C_{34}- *n*-C_{46}), **free acids** and estolides	Tulloch and Bergter, 1981

[†]In bold are indicated the compounds which were identified in this study.

Table 2

Composition of total cuticular waxes (acyclic compounds) contained in the needles of *Juniperus communis*.

Chain length #	*n*-Alkanes	*n*-Alkanoics	*n*-Alkanols	*n*-10-Secondary alcohols	Monoglycerides	*n*-Alkanediols[†]	*n*-ω-Hydroxyalkanoics
10							
11							
12		12.7 (10.0)					
13							
14		14.7 (11.6)					
15		8.1 (6.4)					
16		**60.8 (48.1)**	0.2 (0.3)		**3.2 (46.6)**		
17		2.5 (1.9)			0.01 (0.2)		
18		11.8 (9.3)	1.3 (2.5)		0.3 (4.2)		
19			1.3 (2.5)				
20		7.3 (5.8)	7,0 (13.4)		0.5 (7.9)		
21			1.1 (2.1)				
22			**28.5 (52.8)**		2.8 (41.1)		**5.9 (100)**
23			0.7 (1.3)				
24		8.6 (6.8)	6.8 (12.7)				
25			0.3 (0.5)			0.6 (0.2)	
26			2.3 (4.4)				
27	5.1 (5.0)		0.3 (0.6)	1.8 (0.4)		14.5 (3.7)	
28	1.2 (1.2)		3.2 (6.0)	1.2 (0.2)		2.7 (0.7)	
29	5.9 (5.8)			**477.4 (98.6)**		**375.6 (95.6)**	
30	1.2 (1.2)		0.5 (0.9)	1.5 (0.3)			
31	10.7 (10.6)			2.3 (0.5)			
32	0.3 (0.3)						
33	**61.7 (61.0)**[‡]						
34	3.0 (3.0)						
35	11.9 (11.9)						
Total	**101.1 (100)**	**126.5 (100)**	**53.9 (100)**	**484.2 (100)**	**6.8 (100)**	**392.8 (100)**	**5.9(100)**

[†]Compounds of each class are ordered by increasing retention time, in elution order in GC on an non-polar column (DB-5).

[‡]In bold is indicated the predominant homologue.

[§]The homologues *n*-C_{25}, *n*-C_{27} and *n*-C_{28}, are the isomer 5,10-diol while in the case of the *n*-C_{29} member the amount indicated corresponds to the sum of the contributions from 7,10-, 6,10-, 5,10- and 4,10-diols.

Table 3

Composition of total cuticular waxes (cyclic compounds) in the leaves of *Juniperus communis*.

Compound[†]		Concentration (mg/kg dry weight) and (%)
Tocopherols		
γ_1-Tocopherol		1.2 (0.8)
γ_2-Tocopherol		1.3 (0.9)
α-Tocopherol		**145.8 (98.3)**
	$Total_1$	**148.4 (100)**
Sterols		
Campesterol		48.6 (11.4)
β-Sitosterol		**377.3 (88.6)**
	$Total_2$	**426.0 (100)**
Monoterpenes		
Tricyclene		7.2 (17.2)
α-Pinene		1.2 (2.8)
2-Carene		**26.7 (63.7)**
Isobornyl acetate		6.8 (16.2)
	$Total_3$	**41.8 (100)**
Sesquiterpenes		
β-Elemene		**503.4 (28.0)**
β-Caryophyllene		302.8 (16.8)
(Z)-β-Farnesene		40.1 (2.2)
α-Humulene		123.5 (6.8)
α-Curcumene		12.3 (0.7)
Germacrene D		451.4 (25.0)
Bicyclogermacrene		90.7 (5.0)
Germacrene D-4-ol		277.9 (15.4)
	$Total_4$	**1802.2 (100)**
Flavonoids		
2-(3,4-Methylenedioxyphenyl)propane-1,3-diol		**165.3 (58.8)**
Umbelliferone		115.9 (41.2)
	$Total_5$	**281.2 (100)**
Lignans		
Secoisolariciresinol		12.8 (12.9)
Dehydrodehydrodiconiferyl alcohol isomer		1.4 (1.5)
Dehydrodehydrodiconiferyl alcohol		0.9 (0.9)
Matairesinol		18.0 (18.1)
3'4'-Methylenedioxy-5-methoxymatairesinol-4-methylether		17.3 (17.5)
Lariciresinol		2.9 (2.9)
Deoxypodophyllotoxin		**30.8 (31.1)**
Pinoresinol		14.9 (15.1)
	$Total_6$	**99.1 (100)**

Table 3 (*Continued*)

Compound[†]	Concentration (mg/kg dry weight) and (%)
Phenolic acids and phenols	
Benzyl alcohol	14.9 (6.1)
Benzoic acid	20.5 (8.4)
Catechol	0.2 (0.1)
4-Hydroxybenzaldehyde	**60.2 (24.7)**[‡]
Resorcinol	1.5 (0.6)
4-Hydroxybenzyl alcohol	2.6 (1.1)
Salicylic acid	0.1 (0.03)
Vanillin	1.0 (0.4)
trans-Cinnamic acid	3.3 (1.3)
Tyrosol	0.6 (0.2)
4-Hydroxybenzoic acid	0.6 (0.2)
Vanillyl alcohol	2.3 (0.9)
Dihydro-*p*-coumaryl alcohol	80.2 (32.9)
Vanillic acid	6.9 (2.8)
Hydroxytyrosol	0.2 (0.1)
Dehydroconiferyl alcohol	13.6 (5.6)
cis-Ferulic acid	0.6 (0.2)
trans-*p*-Coumaric acid	2.3 (0.9)
trans-Sinapyl alcohol	0.4 (0.1)
trans-Ferulic acid	32.0 (13.1)
$Total_7$	**244.1 (100)**

[†]Compounds of each class are ordered by increasing retention time, in elution order in GC on an non-polar column (DB-5)

[‡]In bold is indicated the most abundant compound

Table 4

Spectrometric (EIMS-70 eV) distinctive characteristics (*m/z* (rel. int.)) of the members of the homologue series of secondary alcohols (10-ols) identified in the needles of *J. communis* in this study.

Homologue	Retention time (min)[†]	$[M]^+$ (%)	Key fragment ions (GC/MS *m/z* (Relative intensity)[‡]
n-C_{27}-10-ol-TMS = O-TMSi-10-*n*-heptacosanol	30.086	468 (-)	453 (-), **341** (30), **229** (59), 129 (16), 103 (20), 83 (28), 75 (59), 73 (100)
n-C_{28}-10-ol-TMS = O-TMSi-10-*n*-octacosanol	32.786	482 (-)	467 (-), **355** (25), **229** (35), 129 (19), 103 (14), 83 (23), 75 (44), 73 (85)
n-C_{29}-10-ol-TMS = O-TMSi-10-*n*-nonacosanol[ǂ]	35.553	496 (-)	481 (2), **369** (48), **229** (100), 129 (10), 103 (13), 83 (21), 75 (35), 73 (50)
n-C_{30}-10-ol-TMS = O-TMSi-10-*n*-triacontanol	38.320	510 (-)	495 (-), **383** (48), **229** (100), 129 (11), 103 (9), 83 (14), 75 (28), 73 (59)
n-C_{31}-10-ol-TMS = O-TMSi-10-*n*-hentriacontanol	41.103	524 (-)	509 (-), **397** (12), **229** (22), 129 (27), 103 (11), 83 (22), 75 (42), 73 (80)

[†]Order of elution are given on the DB-5 non-polar column

[‡]Compounds after silylation with BSTFA (TMSi ether derivatives)

[ǂ]MS of this compound was very similar to that described by Tulloch and Bergter (1981): $[M]^+$ not detected, 481 $[M-15]^+$ (1), 369(43), 229(100), 73(82)

Table 5

Secondary/secondary alkanediols previously identified in other studies.

Species – plant structure	Homologues detected	Reference
Abies balsamea - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol	Tulloch, 1987
Encephalartos spp. - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol	Osborne and Stevens, 1996
Juniperus pinchotti - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol; 10,20-*n*-C_{29}-diol	Kircher, 1982
Juniperus scopulorum - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol; 10,13-*n*-C_{29}-diol	Tulloch and Bergter, 1981
Myricaria germanica - leaves	8,10-*n*-C_{25}-diol; 6,8-*n*-C_{27}-diol; 8,10-*n*-C_{27}-diol; 10,12-*n*-C_{27}-diol; 3,10-*n*-C_{29}-diol; 8,10-*n*-C_{29}-diol; 10,12-*n*-C_{29}-diol; 2,12-*n*-C_{31}-diol; 3,12-*n*-C_{31}-diol; 4,12-*n*-C_{31}-diol; 5,12-*n*-C_{31}-diol; 6,12-*n*-C_{31}-diol; 7,12-*n*-C_{31}-diol; 8,12-*n*-C_{31}-diol; 10,12-*n*-C_{31}-diol; 12,13-*n*-C_{31}-diol; 12,14-*n*-C_{31}-diol; 12,15-*n*-C_{31}-diol; 12,16-*n*-C_{31}-diol; 12,17-*n*-C_{31}-diol; 12,18-*n*-C_{31}-diol; 1,9-*n*-C_{32}-diol; 1,11-*n*-C_{32}-diol; 1,13-*n*-C_{32}-diol; 8,10-*n*-C_{33}-diol; 8,14-*n*-C_{33}-diol; 6,12-*n*-C_{33}-diol; 8,14-*n*-C_{33}-diol; 10,12-*n*-C_{33}-diol; 10,14-*n*-C_{33}-diol; 12,16-*n*-C_{33}-diol; 14,18-*n*-C_{33}-diol; 1,11-*n*-C_{34}-diol; 8,10-*n*-C_{35}-diol; 10,12-*n*-C_{35}-diol; 8,10-*n*-C_{36}-diol; 9,11-*n*-C_{36}-diol; 8,10-*n*-C_{37}-diol; 10,12-*n*-C_{37}-diol; 8,10-*n*-C_{38}-diol; 9,11-*n*-C_{38}-diol; 10,12-*n*-C_{38}-diol; 8,10-*n*-C_{39}-diol; 8,11-*n*-C_{39}-diol; 10,12-*n*-C_{39}-diol; 8,10-*n*-C_{40}-diol; 9,11-*n*-C_{40}-diol; 10,12-*n*-C_{40}-diol; 8,10-*n*-C_{41}-diol; 8,11-*n*-C_{41}-diol; 10,12-*n*-C_{41}-diol; 10,13-*n*-C_{41}-diol; 8,11-*n*-C_{43}-diol; 10,12-*n*-C_{43}-diol; 10,13-*n*-C_{27}-diol	Jetter, 2000
Papaver alpinum - leaves	1,7-*n*-C_{26}-diol; 1,9-*n*-C_{28}-diol; 1,11-*n*-C_{30}-diol	Jetter *et al*., 1996
Papaver nudicaule - leaves	1,7-*n*-C_{26}-diol; 1,9-*n*-C_{28}-diol; 1,11-*n*-C_{30}-diol	Jetter *et al*., 1996
Papaver orientale - leaves	1,3-*n*-C_{22}-diol; 1,3-*n*-C_{24}-diol; 1,7-*n*-C_{26}-diol	Jetter *et al*., 1996
Papaver somniferum – leaves	1,7-*n*-C_{26}-diol; 1,9-*n*-C_{28}-diol; 1,10-*n*-C_{29}-diol; 1,11-*n*-C_{30}-diol	Jetter *et al*., 1996
Picea abies - leaves	10,13-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 4,10-*n*-C_{29}-diol; 3,10-*n*-C_{29}-diol	Percy *et al*., 2009
Picea glauca - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol	Tulloch, 1987
Pinus radiata - leaves	5,10-*n*-C_{25}-diol; 5,10-*n*-C_{27}-diol; 5,10-*n*-C_{28}-diol; 10,13-*n*-C_{29}-diol; 10,16-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol; 6,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 4,10-*n*-C_{29}-diol; 10,13-*n*-C_{31}-diol;	Franich *et al*., 1979
Ricinus communis - leaves	1,3-(*n*-C_{22}-*n*-C_{28})-diols	Vermeer *et al*., 2003
Taxus baccata - leaves	4,10-*n*-C_{29}-diol; 5,10-*n*-C_{29}-diol; 3,10-*n*-C_{29}-diol; 6,10-*n*-C_{29}-diol; 7,10-*n*-C_{29}-diol; 10,13-*n*-C_{29}-diol	Wen *et al*., 2006
Taxus baccata - leaves	1,5-(*n*-C_{28}-*n*-C_{38})-diols	Wen and Jetter, 2007

Table 6

Composition of diterpenes in the needles of the common juniper (*Juniperus communis*)

Chemical name†	Compound class	Concentration (mg/kg dry weight) and (%)
Pimaric acid ≡ pimara-8(14),15-dien-19-oic acid	Pimarane-type	2174.2 (7.0)
Ferruginol ≡ Abieta-8,11,13-trien-12-ol	Abietane-type	241.6 (0.8)
Sandaracopimaric acid	Pimarane-type	3038.5 (9.7)
trans-Communic acid	Labdane-type	1302.2 (4.2)
Dehydroabietol ≡ Abieta-8,11,13-trien-18-ol	Abietane-type	105.8 (0.3)
Isopimaric acid ≡ 13-Methyl-17-norabieta-7,15-diene-18-oic acid	Pimarane-type	1347.8 (4.3)
Totarol = 14-Isopropylpodocarpa-8,11,13-trien-13-ol	Abietane-type	0.5 (0.002)
Palustric acid ≡ 13-isopropylpodocarpa-8,13-dien-15-oic acid	Abietane-type	53.5 (0.2)
Levopimaric acid	Pimarane-type	0.5 (0.0015)
Dehydroabietic acid ≡ Abieta-8,11,13-trien-18-oic acid	Abietane-type	1.0 (0.003)
Nor-16-imbricatolic acid‡	**Labdane-type**	**107.2 (0.3)**
Imbricataloic acid ≡ 15-oxo-8(17)-labden-19-oic acid	Labdane-type	8463.1 (27.1)
Pisiferol ≡ 20-hydroxyferruginol	Abietane-type	37.2 (0.1)
Isoagatholal	Labdane-type	273.8 (0.9)
Hinokiol ≡ 3-hydroxyferruginol	Abietane-type	10.2 (0.03)
Nor-16-acetylimbricatolic acid	**Labdane-type**	**69.6 (0.2)**
Sugiol ≡ 7-ketoferruginol	Abietane-type	64.0 (0.2)
Imbricatolic acid ≡ 15-hydroxy-8(17)-labden-19-oic acid	Labdane-type	11775.2 (37.7)
Isocupressic acid ≡ 15-hydroxylabda-8(17),13E-dien-19-oic acid	Labdane-type	1320.1 (4.2)
Acetylimbricatolic acid	**Labdane-type**	**503.4 (1.6)**
Dihydroagathic acid ≡ labda-8(17)-ene-15,19-dioic acid	Labdane-type	306.9 (1.0)
Acetylisocupressic acid ≡ 15-acetoxy-8(17),*E*- 13-labdadien-19-oic acid	Labdane-type	15.5 (0.05)
Total		**31211.8 (100)**

†Compounds of each class are ordered by increasing retention time, in elution order in GC

‡In bold are marked new labdane-type diterpenoids

§Underlined are indicated the two most abundant

Table 7

Summary of the chemical composition of cuticular waxes contained i*n* the needles of *Juniperus communis*

Class of compound	Concentration (mg/kg dry weight)	Percentage (%)
n-Alkanes	101.1	0.3
n-Alkanols	53.9	0.2
n-Alkanoics	126.5	0.4
n-Alkenoics	49.2	0.1
n-ω-Hydroxyalkanoics	17.2	0.05
n-Secondary alcohols (10-ols)	484.2	1.4
n-secondary/secondary alkanediols	392.8	1.1
Tocopherols	148.4	0.4
Sterols	426.0	1.2
Phenolic acids and phenols	244.1	0.7
Flavonoids	281.2	0.8
Lignans	99.1	0.3
Monoterpenes	40.8	0.1
Sesquiterpenes	1802.2	5.1
Diterpenes[†]	**31211.8**	**88.0**
Monoglycerides	6.8	0.02
Phytol	9.9	0.03
Squalene	95.3	0.3
Total	35590.5	100

[†]In bold is indicated the most abundant class of compounds from the aerial parts of *J. communis*.

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Supplementary data

Labdane-type diterpenoids in the needles from *Juniperus communis*

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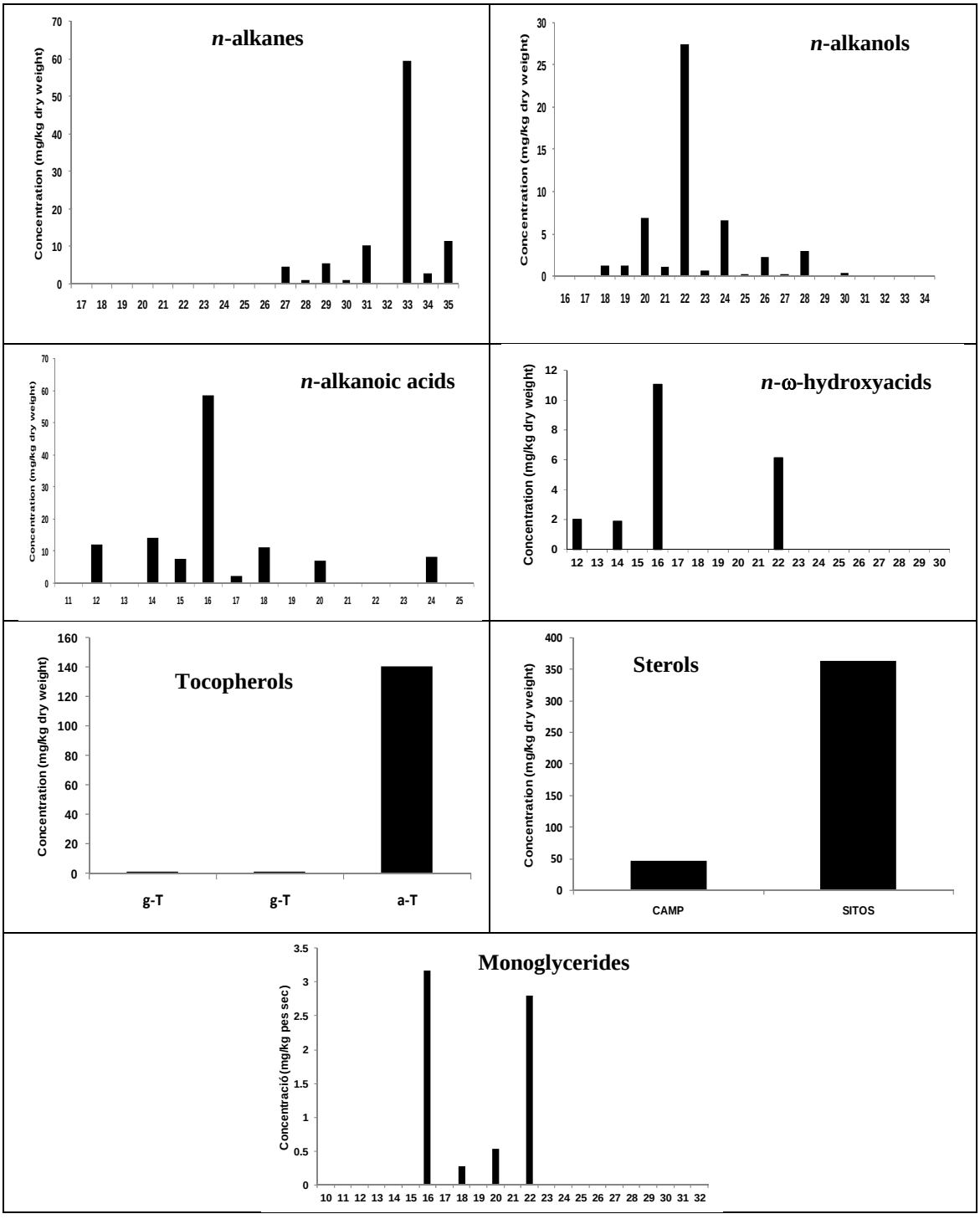
Supplementary Data Contents:

S1. Histogram of *n*-alkanes, *n*-alkan-1-ols, *n*-alkanoic acids, *n*-ω-hydroxy acids, tocopherols, sterols and monoglycerides isolated from the needles of the common juniper (*Juniperus communis* L). The numbers in the abscissas indicate the carbon chain length. γ-T, γ-Tocopherol and α-T, α-Tocopherol. CAMP, campesterol and SITOS, sitosterol.



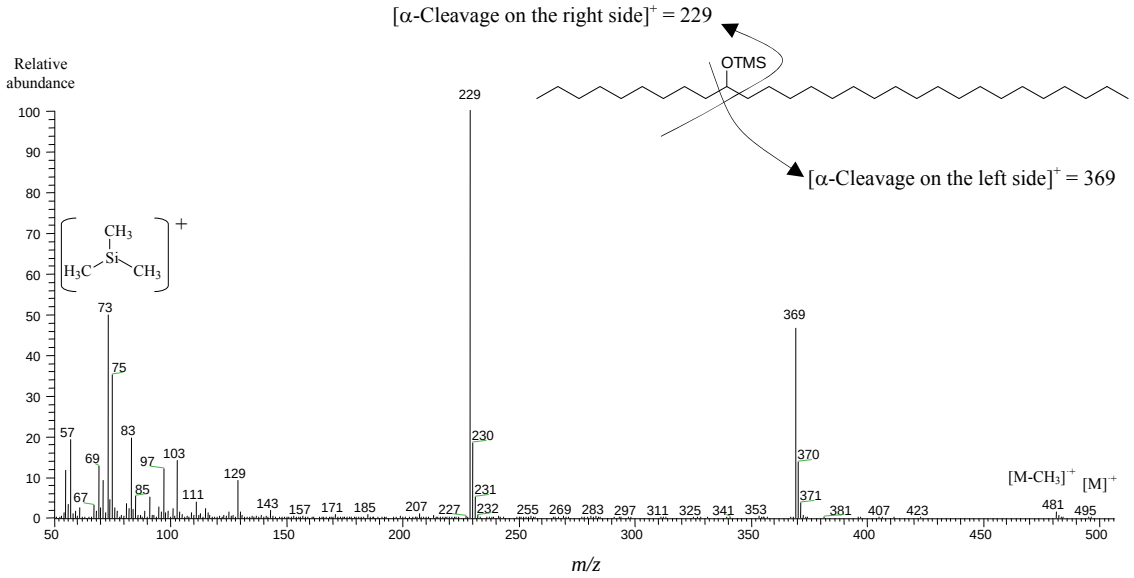
S2. Secondary alcohols from the members of the *Juniperus* genus (Cupressaceae) previously described in the literature.

S2

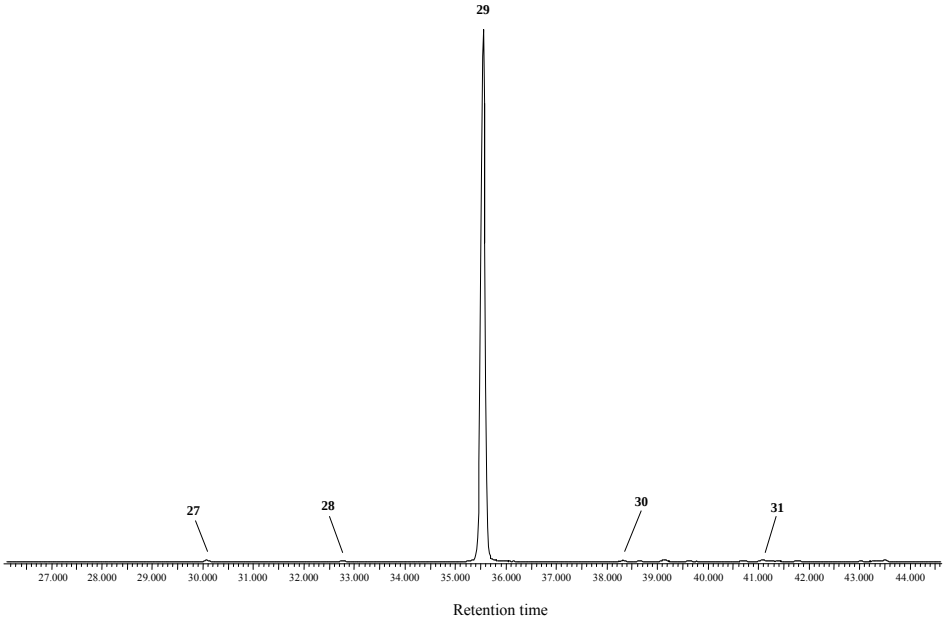
Secondary alcohols previously identified in other studies

Species – part of the plant	10-ol-Homologues detected	Reference
Abies balsamea - needles	10-*n*-C_{29}-ol	Beri and Lemon, 1970
Abies balsamea – leaves	10-*n*-C_{29}-ol	Tulloch, 1987
Adenophora tetraphlla – roots	10-*n*-C_{29}-ol	Yao *et al.*, 2007
Agathis australis – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{31}-ol	Holloway *et al*., 1976
Aquilegia alpinum – leaves	*n*-C_{29}-ol	Holloway *et al*., 1976
Arabidopsis thaliana – stems	14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol	Hannoufa *et al*., 1993
Arabidopsis thaliana – leaves and stems	13-*n*-C_{27}-ol; 14-*n*-C_{27}-ol; 14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol; 15-*n*-C_{31}-ol; 16-*n*-C_{31}-ol	Rashotte *et al*., 2001
Brassica oleracea	14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol	Netting and Macey, 1971
Brassica oleracea – leaves	13-*n*-C_{27}-ol; 13-*n*-C_{28}-ol; 13-*n*-C_{29}-ol; 13-*n*-C_{30}-ol; 13-*n*-C_{31}-ol; 14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol	Holloway *et al*., 1976
Chamaecyparis lawsoniana	9-*n*-C_{27}-ol; 9-*n*-C_{28}-ol; 9-*n*-C_{29}-ol; 9-*n*-C_{31}-ol; 10-*n*-C_{29}-ol; 11-*n*-C_{29}-ol	Holloway *et al*., 1976
Chelidonium majus – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol	Holloway *et al*., 1976
Cirsium arvense – not reported	10-*n*-C_{29}-ol	Tulloch and Hoffman, 1982
Clarkia elegans – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{31}-ol	Holloway *et al*., 1976
Cocculus hirsutus – not reported	10-*n*-C_{29}-ol	Ahmad *et al*., 1987
Crataegus sp. – fruits and leaves	14-*n*-C_{29}-ol	Wollrab, 1969
Encephalartos spp. – leaves	10-*n*-C_{25}-ol; 10-*n*-C_{26}-ol; 10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol	Osborne and Stevens, 1996
Exochorda racemosa – leaves	7-*n*-C_{27}-ol; 8-*n*-C_{27}-ol; 8-*n*-C_{28}-ol; 9-*n*-C_{27}-ol; 9-*n*-C_{28}-ol; 9-*n*-C_{29}-ol; 9-*n*-C_{30}-ol; 9-*n*-C_{31}-ol; 10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol ; 11-*n*-C_{29}-ol; 11-*n*-C_{31}-ol	Holloway *et al*., 1976
Fragaria ananassa, F. ovalis and *F.chiloensis* – leaves	9-*n*-C_{31}-ol; 10-*n*-C_{31}-ol; 11-*n*-C_{31}-ol; 12-*n*-C_{31}-ol; 10-*n*-C_{33}-ol; 11-*n*-C_{33}-ol; 12-*n*-C_{33}-ol; 13-*n*-C_{33}-ol; 14-*n*-C_{33}-ol	Baker and Hunt, 1979
Ginkgo biloba – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol	Holloway *et al*., 1976
Ginkgo biloba – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 13-*n*-C_{29}-ol	Casal and Moyna, 1979
Juniperus macropoda - berries	10-*n*-C_{29}-ol	Siddiqui and Sen, 1971
Juniperus pinchotti	10-*n*-C_{29}-ol	Kircher, 1982
Juniperus scopulorum – leaves	10-*n*-C_{29}-ol	Tulloch and Bergter, 1981
Lonicera hypoleuca – leaves	10-*n*-C_{29}-ol	Khan and Shoeb, 1985
Malus domestica - cuticle fruit	10-*n*-C_{29}-ol	Veraverbeke *et al.*, 2001
Papaver bracteatum – leaves	10-*n*-C_{29}-ol	Theuns *et al*., 1985
Papaver somniferum – leaves	13-*n*-C_{27}-ol; 13-*n*-C_{29}-ol; 13-*n*-C_{30}-ol; 14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol	Holloway *et al*., 1976
Picea abies – needles	10-*n*-C_{29}-ol, 6-*n*-C_{29}-ol, 10-*n*-C_{31}-ol	Percy *et al*., 2009
Picea glauca – leaves	10-*n*-C_{29}-ol	Tulloch, 1987
Picea mariana – needles	10-*n*-C_{29}-ol	Beri and Lemon, 1970
Picea pungens – leaves	*n*-C_{29}-ol	Holloway *et al*., 1976
Picea sitchensis – leaves	8-*n*-C_{27}-ol; 9-*n*-C_{27}-ol; 9-*n*-C_{28}-ol; 9-*n*-C_{29}-ol; 9-*n*-C_{30}-ol; 9-*n*-C_{31}-ol; 10-*n*-C_{27}-ol; 10-*n*-C_{29}-ol; 11-*n*-C_{29}-ol	Holloway *et al*., 1976
Pinus canariensis – needles	10-*n*-C_{29}-ol	Stabentheiner *et al*., 2004
Pinus halepensis – needles	10-*n*-C_{29}-ol	Matas et *al*., 2003
Pinus sylvestris – pollen	10-*n*-C_{29}-ol	Caldicott and Eglinton , 1975
Piper atenuatum – leaves	8-*n*-C_{31}-ol	Sumathykutty and Rao, 1991
Pisum sativum – leaves	16-*n*-C_{31}-ol; 15-*n*-C_{31}-ol	Macey and Barber, 1970
Pisum sativum – leaves	13-*n*-C_{29}-ol; 14-*n*-C_{29}-ol; 15-*n*-C_{29}-ol; 13-*n*-C_{30}-ol; 14-*n*-C_{30}-ol; 15-*n*-C_{30}-ol; 12-*n*-C_{31}-ol; 13-*n*-C_{31}-ol; 14-*n*-C_{31}-ol; 15-*n*-C_{31}-ol; 16-*n*-C_{31}-ol; 14-*n*-C_{32}-ol; 15-*n*-C_{32}-ol; 16-*n*-C_{32}-ol; 13-*n*-C_{33}-ol; 14-*n*-C_{33}-ol; 15-*n*-C_{33}-ol; 16-*n*-C_{33}-ol; 17-*n*-C_{33}-ol	Wen *et al*., 2006a
Prunus avium – fruit	10-*n*-C_{29}-ol	Peschel *et al*., 2007
Prunus domestica – fruit	10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol	Ismail *et al*., 1977
Prunus domestica – leaves	7-*n*-C_{27}-ol; 8-*n*-C_{27}-ol; 8-*n*-C_{28}-ol; 9-*n*-C_{27}-ol; 9-*n*-C_{28}-ol; 9-*n*-C_{29}-ol; 9-*n*-C_{30}-ol; 9-*n*-C_{31}-ol; 10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol ; 11-*n*-C_{29}-ol; 11-*n*-C_{31}-ol	Holloway *et al*., 1976
Pyrus malus – fruit	10-*n*-C_{21}-ol; 10-*n*-C_{22}-ol; 10-*n*-C_{23}-ol; 10-*n*-C_{24}-ol; 10-*n*-C_{25}-ol; 10-*n*-C_{26}-ol; 10-*n*-C_{27}-ol; 10-*n*-C_{28}-ol; 10-*n*-C_{29}-ol	Dodova-Anghelova and Ivanov, 1973
Raspberry apple – cuticle fruit	10-*n*-C_{29}-ol	Wollrab, 1969
Rhus cortinus atropurpurea	11-*n*-C_{29}-ol	Holloway *et al*., 1976
Rose – flowers	7-*n*-C_{29}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{31}-ol	Wollrab, 1969
Taxus baccata – leaves	10-*n*-C_{29}-ol	Wen *et al.*, 2006b
Tropaeolum majus – leaves	10-*n*-C_{27}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{29}-ol; 10-*n*-C_{30}-ol; 10-*n*-C_{31}-ol	Holloway *et al*., 1976
Tropaeolum majus – leaves	10-*n*-C_{29}-ol; 2-*n*-C_{29}-ol; 3-*n*-C_{28}-ol	Koch *et al*., 2009
Tulipa gesneriana – leaves	9-*n*-C_{27}-ol; 9-*n*-C_{28}-ol; 9-*n*-C_{29}-ol; 9-*n*-C_{30}-ol; 9-*n*-C_{31}-ol; 10-*n*-C_{29}-ol; 11-*n*-C_{29}-ol	Holloway *et al*., 1976

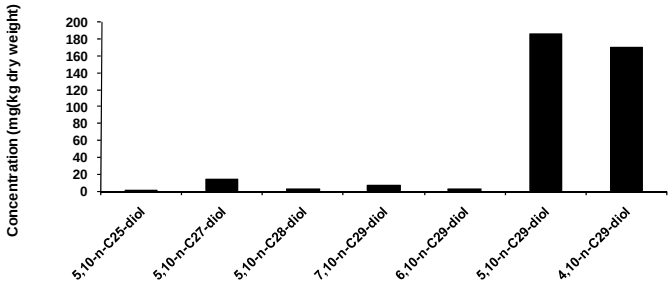
S3. Mass spectra of the secondary alcohol silylated 10-*n*-nonacosanol identified in the leaves of common juniper (*Juniperus communis* L.). O-TMSi-10-*n*-nonacosanol GC/MS (70 eV) *m/z* (rel. Int.): 495 (0.3), 481 (2), 369 (48), 229 (100), 129 (10), 103 (13), 83 (21), 75 (35), 73 (50).



S4. Fragmentogram (*m/z* = 229) of *n*-secondary alcohols identified in the needles from common juniper (*Juniperus communis* L.).



S5. Histogram of secondary/secondary alkanediols identified in the leaves of common juniper (*Juniperus communis* L.).



S6. 5,10-nonacosanediol mass spectrum interpretation

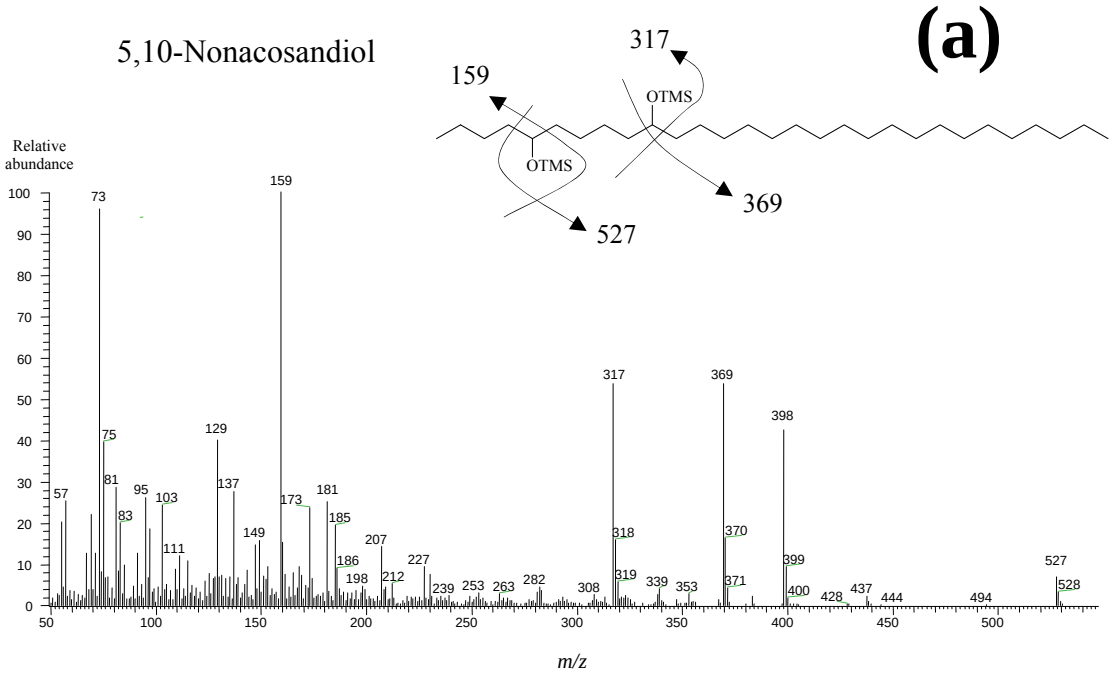
Mass spectrum of 5,10-nonacosanediol (**S6. (*Cont.*)**) shows the result of a mainly four ways fragmentation pattern:

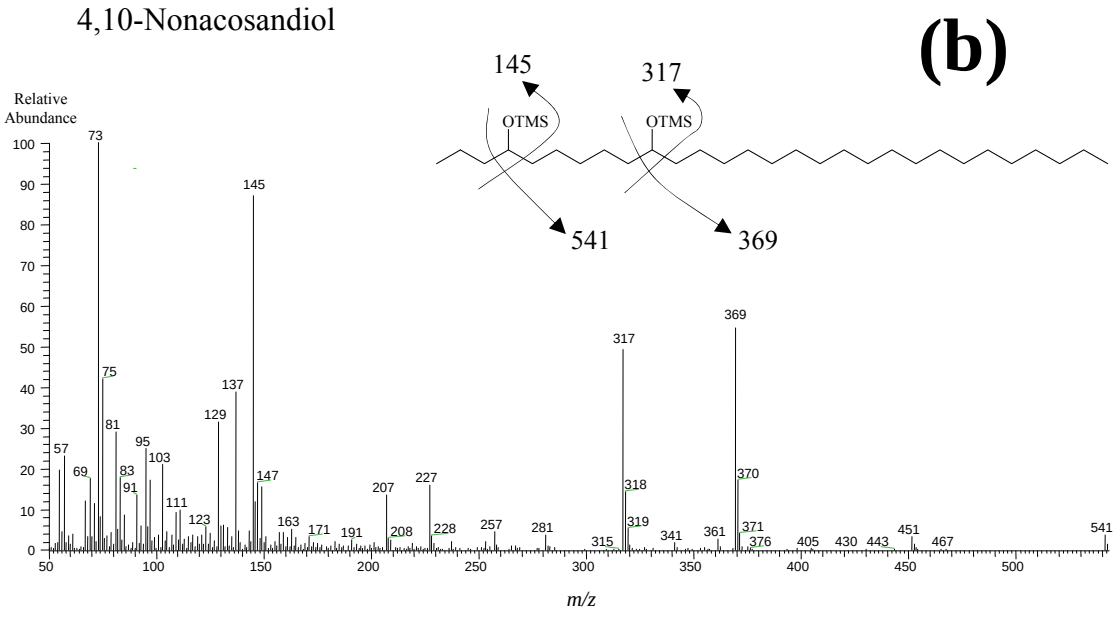
1. A first group of three ions the parent of which was at *m/z* 527 corresponding to the fragment that contains 2 OTMS groups after the α-cleavage in the left side of the methine which supports the first hydroxyl group at C-5 and then two other minor daughter ions at *m/z* 437 and 347 corresponding to the loss of one and two TMSOH respectively from that first parental ion.
2. A second group formed by two ions the first of which was at *m/z* 369 corresponding to the fragment that contains a OTMS group also after the left α-cleavage respect to the methine attached to the second hydroxyl group at C-10 and other minor daughter ion at *m/z* 279 which was the loss of the silylated functional group together with a proton from that second parental ion.
3. A third group of two ions the parent of which was the base peak at *m/z* 159 corresponding to the fragment that contains a OTMS group after the α-cleavage in the right side of the methine which supports the first hydroxyl group at C-5 and other minor daughter ion at *m/z* 69 delivered after the loss of the TMSOH from that third parental ion.
4. Finally, A fourth group of three ions the parent of which was at *m/z* 317 corresponding to the fragment that contains 2 OTMS groups after the α-cleavage in the right side of the carbon atom which supports the second hydroxyl group at

C-10 and then two other minor daughter ions at *m/z* 227 and 137 corresponding to the loss of one and two TMSOH respectively from that fourth parental ion.

Daughter ions resulting from the α-rupture of the original silylated molecule at the left side of the carbon atom that bear the functional groups at C-5 and C-10 (527(8)-437(2)-347(2)-369(43)-279(1)) were less abundant than those resulting from the cleavage at the right chain side (317(44)-227(8)-137(28)-159(100)-69(21)). The same is true for the 4,10-diol (541(4)-451(4)-361(3)-369(52)-279(0)) << (317(48)-227(14)-137(40)-145(8%)-55(18)) and for the 7,10-isomer (499(2)-409(25-exception)-319(3)-369(40)-279(1) << 317(9)-227(84)-137(4)-187(88)-97(24)). These results can be interpretated in the sense than the ions released after the cleavage at the right side of the carbon bearing the hydroxyl group are more stable than the released after the electron interaction with the C-C_α bond on the right side. Molecular ion and M-15 fragment were missing from all the diols MS. Other significant ions were at *m/z* 73 > 129 > 103 > 95 > 57.

S6 (*Cont.*). Mass spectra of representative bis TMSi ether derivatives alkanediols contained in the needle wax of common juniper (*Juniperus communis* L.). Nonacosane-5,10-diol **(a)** and nonacosane-4,10-diol **(b)**.





S7. Spectrometric (EIMS-70 eV) distinctive characteristic (*m/z* (rel. int.)) of the members of the homologue series of alkanediols identified in the needles of *J. communis*.

S7

Spectrometric (EIMS-70 eV) distinctive characteristic (*m/z* (rel. int.)) of the members of the homologue series of secondary alkanediols identified in the needles of *J. communis*.

Homologue	Ret. time (min)[†]	$[M]^+$ (%)	Key fragment ions (GC/MS *m/z* (Relative intensity)[‡,†]
5,10-Pentacosanediol	29.886		
5,10-Hexacosanediol	32.786		
5,10-Heptacosanediol	35.269	556(-)	541($[M-CH_3]^+$, missing); **499(3)**; **409(-)**; **341(18)**; **319(-)**; **317(15)**; 281(9); 253(6); **25179(1)**; **227(4)**; 207(33); **159(36)**; 149(10); 147(11); **137(12)**; 129(18); 103(14); 97(11); 95(18); 91(9); 83(12); 81(16); 75(18); 73(100); **69(18)**; 67(13); 57(19); 55(19)
5,10-Octacosanediol	37.970		
7,10-Nonacosanediol	39.620	584(-)	569($[M-CH_3]^+$, missing); **499(2)**; **409(25)**; **369(40)**; **319(3)**; **317(9)**; 281(12); **279(1)**; 253(6); **227(84)**; 207(48); **187(88)**; 155(18); 149(16); 147(22); **137(4)**; 129(51); 115(12); 103(18); **97(24)**; 95(22); 91(11); 83(22); 81(20); 75(31); 73(100); 69(24); 67(16); 57(38); 55(38)
6,10-Nonacosanediol	40.120	584(-)	569($[M-CH_3]^+$, missing); **513(-)**; **423(-)**; **369(4)**; **333(-)**; **317(8)**; 281(25); **279(-)**; 253(11); **227(3)**; 207(100); 191(15); **173(17)**; 149(12); 147(14); **137(5)**; 129(10); 105(17); 95(22); 91(27); 81(26); 75(28); 73(86); 69(30); 67(22); 57(29); 55(35)
5,10-Nonacosanediol	40.720	584(-)	569($[M-CH_3]^+$, missing); **527(right fragment second α-cleavage, 8)**; **437(daughter ion from *m/z* 527-TMSOH, 2)**; **369(right fragment first α-cleavage, 43)**; **347(daughter ion from *m/z* 527-2TMSOH, 2)**; **317(left fragment second α-cleavage, 44)**; 282(3); **279(daughter ion from *m/z* 369-TMSOH, 1)**; 230(6); **227(daughter ion from *m/z* 317-TMSOH, 8)**; 212(5); 207(11); 185(18); 173(21); **159(left fragment first α-cleavage, 100)**; 149(15); 147(13); **137(daughter ion from *m/z* 317-2TMSOH, 28)**; 129(40); 115(11); 111(12); 103(22); 95(26); 91(11); 81(20); 75(40); 73(97); **69(daughter ion from *m/z* 159-TMSOH, 21)**; 67(13); 57(25); 55(20)
4,10-Nonacosanediol	41.403	584(-)	569($[M-CH_3]^+$, missing); **541(4)**; **451(4)**; **369(52)**; **361(3)**; **317(48)**; 281(3); **279(missing)**; 257(3); **227(14)**; 207(11); **145(85)**; 149(13); 147(16); **137(40)**; 129(31); 111(10); 103(20); 95(22); 91(11); 81(25); 75(40); 73(100); 69(15); 67(13); 57(22); **55(18)**

[†]Order of elution are given on the DB-5 nonpolar column

[‡]Compounds after silylation with BSTFA (bis-TMSi ether derivatives)

[†]In bold are indicated the ions which belong to the four main groups derived from the fragmentation of the molecular ion and the sec./sec. 5,10-*n*-C_{29}-diol its explanation is included

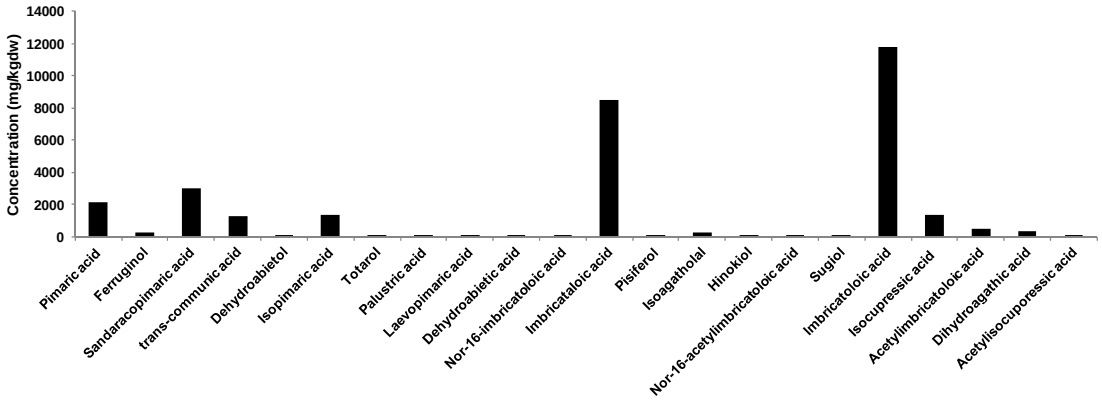
S8. Phytochemical analysis of diterpenoids from the members of the *Juniperus* genus (Cupressaceae) as described in the literature.

S8

Phytochemical analysis of diterpenoids from the members of the *Juniperus* genus (Cupressaceae) as described in the literature.

Species – part of the plant	Component	Reference
J. ashei - Leaves	Abieta-7,13-dien-3-one > sempervirol > manoyl oxide > 4-epi-abietol > *trans*-ferruginol >>> *trans*-totarol, abietatriene, abietadiene	Adams *et al*., 2013
J. brevifolia – Leaves	3β–Hydroxy-abieta-8,11,13-trien-7-one, 18-hydroxy-sandaracopimara-8(14),15-dien-7-one, sandaracopimara-8(14),15-dien-18-yl formate, abieta-8,11,13-trien-18-yl hexadecanoate, 7-oxoabieta-8,11,13-trien-18-yl hexadecanoate, sandaracopimara-8(14),15-dien-18-yl hexadecanoate, sugiol, *E*-communic acid, *Z*-communic acid, hinokiol, pomiferin A, abieta-8,11,13-trien-18-yl formate, sandaracopimara-8(14),15-dien-18-ol, sandaracopimaric acid, methyl ester of 15-agathic acid, sandaracopimara-8(14),15-diene, 7-oxoabieta-8,11,13-trien-18-ol, 15,16-bisnor-13-oxo-labda-8(17),11*E*-dien-19-oic	Seca *et al*., 2008
J. brevifolia - Bark	Dehydroabietane, 6,7-dehydroferrugin-12-methyl ether, 6-7-dehydroferruginol, ferruginol, sandaracopimaric acid, totarol, 6-oxoferruginol, dehydroabietic acid, 11-hydroxy-6,7-dehydroferruginol, sugiol, totarol-3-one, 6.7-dehydrohinokiol, totarol-1,3-dione, hinokiol	Seca and Silva, 2008
J. brevifolia – Leaves	Abieta-8,11,13-triene, sandaracopimara-8(14),15-diene, 6,7-dehydroferruginol methyl ether, 18-hydroxydehydroabietane, sandaracopimara-8(14),15-dien-18-ol, sugiol	Seca and Silva, 2010
J. chinensis – Leaves	Sandaracopimaric acid, isocupressic acid, sempervirol, totarol, ferruginol, hinokiol, abieta-8,11,13-trien-7α-ol, abieta-8,11,13-trien-7β-ol, abieta-8,11,13-trien-7-one, abieta-8,11,13-trien-12,19-diol, 4-epiabietinal, 4-epiabietic acid, juniperolide, juniperal, norjuniperolide, secojuniperolide and chinanoxal	Fang *et al*, 1993
J. chinensis – Bark	Sandaracopimaric acid; ferruginol; 7-dehydroabietanone; sugiol; 6α-Hydroxy-7-oxoferruginol, 6,7-secoferruginol-6,7-dial; totarol; totarolone; *cis*-communic acid; *trans*-communic acid; isocupressic acid; agathic acid; 12,15-dihydroxylabda-8(17),13*E*-dien-19-oic acid; 12,15-dihydroxylabda-8(17),13*Z*-dien-19-oic acid; 2α-hydroxy-*trans*-communic acid; 2α-hydroxy-*cis*-communic acid; 12,13-epoxylabda-8(17),14-dien-19-oic acid; 12,13-dihydroxylabda-8(17),14-dien-19-oic acid; 15,16-bisnor-8,17-epoxy-13-oxolabd-11*E*-en-19-oic acid	Fang *et al*., 1993
J. chinensis – leaves	18-Norpimara-8(14),15-dien-4-ol, 18-norabieta-8,11,13-trien-4-ol, 19-norabieta-8,11,13-trien-4-ol, 19-norabieta-8,11,13-trien-4-yl formate, 18-norabieta-8,11,13-triene-4-hydroperoxide, 19-norabieta-8,11,13-triene-4-hydroperoxide, 4-hydroxy-18-norabieta-8,11,13-trien-7-one, 4-hydroxy-19-norabieta-8,11,13-trien-7-one, 4-hydroperoxy-19-norabieta-8,11,13-trien-7-one, 7α-hydroxy-19-norabieta-8,11,13-triene-4-hydroperoxide, 19-norabieta-7,13-dien-4-ol, 13β,14β-epoxy-4-hydroxy-19-norabiet-7-en-6-one.	Lee *et al*.. 1995
J. chinensis – Bark	14,15-Dihydroxy-8(17),12E-labdadien-19-oate, *trans*-communic acid, *cis*-communic acid, 15,16-bisnor-13-oxo-8(17),11E-labdadien-19-oic acid, sandaracopimaric acid, 7-oxosandaracopimaric acid, sugiol, 7-oxototarol,	Chang *et al*., 2008
J. communis – Leaves	7-oxo-13-*epi*-pimara-8,15-dien-18-oic acid, 7α-hydroxysandaracopimaric acid, (14*S*)-14,15-dihydroxylabda-8(17),13(16)-dien-19-oic acid, *trans*-communic acid, isopimaric acid, sandaracopimaric acid, imbricataloic acid, isocupressic acid, 13,14-epoxyimbricatolic acid, 7α-hydroxydehydroabietic acid	De Pascual Teresa *et al*, 1980
J. communis - Leaves	15-O-palmitoyl isocupressic acid (5), isocupressic acid (1a) (32.8%), isopimaric acid (3a) (43.6%), imbricataloic acid (6a), imbricatoloic acid (11a)	San Feliciano *et al*., 1991
J. communis – Aerial parts	Isocupressic acid, *cis*-communic acid, *trans*-communic acid	Carpenter et al., 2012
J. conferta – Heartwood	Totarol, 3-oxototarol, Δ^1-3-oxototarol, xanthoperol, 1,3-dioxototarol, sugiol, sandaracopimaric acid and isopimaric acid	Doi and Shibuya, 1972
*J. formosana –B*bark	Sugiol methyl ether, D5-dehydrosugiol methyl ether, sugiol, totarolone, totarolenone, cis-communic acid, trans-communic acid, secoabietanedialdehyde, sandaracopimaric acid, enantio-oliveric acid and cupressic acid.	Kuo and Yu, 1996
J. macropoda - Berries	Sugiol	Siddiqui and Sen, 1971
J. macrocarpa – Leaves and seed cones	Leaves: Abietadien > abietatriene > manoyl oxide Seed cones: manoyl oxide > abietadien	Lesjak *et al*., 2014
J. nana – Berries	Imbricatoloic acid, isocupressic acid, isopimaric acid, sandaracopimaric acid, *trans*-communic acid, myrceocommunic acid and *cis*-communic acid	Sakar *et al*., 2002
J. osteosperma -Leaves -Bark	15-Hydroxy8(17),11,13-triene-19-oic acid, isocupressic acid Agathic acid	Gardner *et al*., 2009
J. phoenicea and *J. thurifera* – Leaves	12-oxo-8α,15-dihydroxyabiet-13-en-19-oic acid, 12-oxo-8α-hydroxyabiet-13-en-19-oic acid, 15-hydroperoxy-8α,12α-epidioxyabiet-13-en-19-oic acid, 15-hydroxy-8α,12α-epidioxyabiet-13-en-19-oic acid, 15-hydroperoxy-8α,14α,12α,13α-diepoxyabietan-13-en-19-oate, 7α,12β-dihydroxysandaracopimaric acid, abietic acid; 4-*epi*-abietic acid; abietinal; dehydroabietinal; abietinol; 15-hydroxy-9α,13α-epidioxyabiet-8(14)-en-18-oic acid, *cis*-communic acid; *trans*-communic acid; sandaracopimaric acid; isopimaric acid; pimara-8(14),15-dien-18-ol; pimara-7,15-dien-18-ol, 3β-hydroxysandaracopimaric acid, 3β-hydroxyisopimaric acid, pimara-8(14),15-dien-3β,18-diol, pimara-7,15-dien-3β,18-diol	Barrero *et al*., 2004
J. thurifera – Leaves	Labd-12E-8,15-diol, 8,15-dihydroxy-labd-13E-en-19-al, 8,15-.dihydroxy-labda-13(16),14-dien-19-yl trans-coumarate, 8-hydroxy-14-oxo-15-norlabd-13(14)-en-19-oic, sclareolic acid, episclareolic acid, 8,15-dihydroxy-14-oxo-labd-13(16)-en-19-oic, 8,15-dihydroxy-labd-13E-en-19-oic, (14R)-8,14,15-trihydroxy-labd-13(16)-en-19-oic acid, (14S)-8,14,15-trihydroxy-labd-13(16)-en-19-oic acid	San Feliciano *et al*., 1992

S9. Histogram of diterpenoid compounds identified in the needles of the common juniper (*Juniperus communis ssp. communis var. communis*). In order to respect the real relative amounts of each homologue the concentration of the minor constituents was not virtually rescaled.



S10. Mass spectrometry data for the diterpenoids investigated in this study.

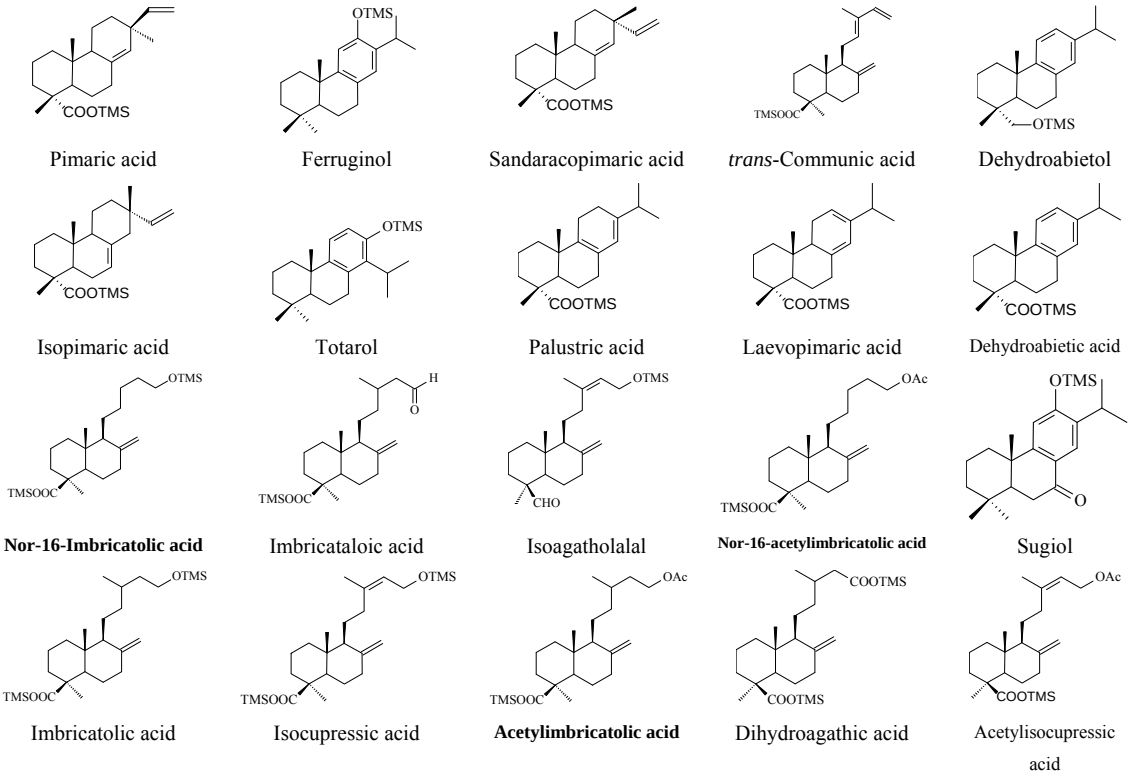
S10

Mass spectrometric (EIMS-70 eV) data from diterpenoid described in the text.

Chemical name	Ret. time[‡]	$[M]^+$ (%)	Diagnostic ions *m/z* (%)
Pimaric acid	16,818	374(3)	359 ($[M-CH_3]^+$, 20); 257($[M-COOTMS]^+$, 32); 256($[M-HCOOTMS]^+$, 25); 241($[M-HCOOTMS-CH_3]^+$, 20); 189(19); 187(17); 173(12); 163(18); 161(17); 143(22); 133(30); 123(17); 121(52); 107(40); 93(48); 81(51), 73(100)
Ferruginol	16,868	358(4)	343($[M-CH_3]^+$, 2); 273(1), 261(1), 247(1), 73(100)
Sandaracopimaric acid	17,368	374(14)	359($[M-CH_3]^+$, 11); 257($[M-COOTMS]^+$, 8); 256($[M-HCOOTMS]^+$, 7); 241($[M-HCOOTMS-CH_3]^+$, 4); 239(5); 187(10); 161(17); 148(15); 143(12); 135(24); 121(52); 107(25); 105(22); 94(21); 91(20); 73(100); 69(15); 67(13); 55(18)
trans-Communic acid	17,535	374(7)	359($[M-CH_3]^+$, 8); 257($[M-COOTMS]^+$, 7); 256($[M-HCOOTMS]^+$, 8); 241($[M-HCOOTMS-CH_3]^+$, 7); 239(3); 187(5), 161(12); 148(13); 143(10); 135(13); 134(15); 133(15); 121(32); 107(18); 105(20); 93(18); 91(18); 81(26); 73(100); 69(11); 67(9); 55(17)
Dehydroabietol	17,668	358(7)	343(3); 253(100); 239(22); 225(9), 211(9); 197(8); 185(21); 173(92); 159(14); 143(12); 131(15); 103(8); 91(11); 81(10); 75(15); 73(49); 69(6; 67(5); 55(15)
Isopimaric acid	18,001	374(11)	359($[M-CH_3]^+$, 13); 257($[M-COOTMS]^+$, 11); 256($[M-HCOOTMS]^+$, 13); 241($[M-HCOOTMS-CH_3]^+$, 12); 187(8); 175(21); 161(14); 148(16); 143(12); 134(20); 121(24); 119(30); 105(24); 93(22); 91(20); 81(41); 79(32); 73(100); 69(12); 67(10); 55(17)
Totarol	18,201	358(4)	343($[M-CH_3]^+$, 10);
Palustric acid	18,268	374(5)	359($[M-CH_3]^+$, 21); 256($[M-HCOOTMS]^+$, 62); 241($[M-HCOOTMS-CH_3]^+$, 90); 227(14); 201(20); 185(17); 173(14); 159(12); 143(21); 119(22); 109(25); 105(30); 91(28); 81(29); 75(30); 73(100); 69(18); 67(17); 55(29)
Laevopimaric acid	18,601	374(4)	359($[M-CH_3]^+$, 9); 257($[M-COOTMS]^+$, 6); 256($[M-HCOOTMS]^+$, 5); 241($[M-HCOOTMS-CH_3]^+$, 7); 187(10); 175(6); 161(11); 159(80); 143(22); 129(22); 121(30); 119(17); 105(20); 93(24); 91(30); 81(31); 79(20); 73(100); 69(25); 67(20); 55(39)
Dehydroabietic acid	19,051	372(6)	357($[M-CH_3]^+$, 8); 255((7); 239(100); 173(19); 159(14); 143(17); 121(29); 91(21); 73(30); 69(25); 55(36)
Nor-16-imbricatoloic acid	19,818	452(3)	437($[M-CH_3]^+$, 8); 362($[M-TMSOH]^+$, 1); 334($[M-HCOOTMS]^+$, 12); 319($[M-HCOOTMS-CH_3]^+$, 1); 293($[M-TMSO-C5H_{11}-CH_3]^+$, 5); 244($[M-HCOOTMS-TMSOH]^+$, 6); 229($[M-HCOOTMS-TMSOH-CH_3]^+$, 5); 189(5); 175(7);161(14); 143(31); 121(49); 107(17); 93(17); 81(12); 75(35); 73(100); 69(15); 67(10); 55(16)
Imbricataloic acid	20,252	392(8)	377($[M-CH_3]^+$, 21); 374($[M-18]^+$, 2); 364($[M-28]^+$, 5); 302($[M-90]^+$, 2); 274($[M-HCOOTMS]^+$, 22); 256($[M-HCOOTMS-18]^+$, 13); 247(11); 241(10); 201(7); 189(7); 175(11); 161(32); 159(20); 149(28); 143(31); 133(20); 121(100); 109(73); 93(45); 81(71); 73(99); 69(44); 67(32); 55(46)
Isoagatholal	21,168	376(1)	361($[M-CH_3]^+$, 1); 348(1); 286($[M-TMSOH]^+$, 5); 271($[M-TMSOH-CH_3]^+$, 6); 257(14); 253(13); 243(13); 229(4); 215(5); 201(8); 187(17); 175(10); 169(10); 161(15); 156(13); 147(17); 143(17); 135(18); 121(24); 119(23); 109(25); 107(32); 93(30); 91(20); 81(46); 75(50); 73(100); 69(10); 67(24); 55(29)
Nor-16-acetylimbricatolic acid	21,868	422(3)	407($[M-CH_3]^+$, 4); 304($[M-HCOOTMS]^+$, 20); 189(4); 175(7); 173(7); 161(16); 143(16); 121(60); 109(25); 93(21); 81(27); 73(100); 69(22); 67(16); 55(25)
Sugiol	21,952	372(13)	357($[M-CH_3]^+$, 22); 315(5); 289(6); 275(7); 287(3)
Imbricatoloic acid	22,252	466(10)	451($[M-CH_3]^+$, 16); 376($[M-TMSOH]^+$, 4); 348($[M-HCOOTMS]^+$, 32); 308($[M-TMSO-(CH_2)_2-CH(CH_3)-CH_2)]^+$, 2); 293($[M-TMSO-(C_5H_{10})-CH_3]^+$, 8); 259(20); 258($[M-HCOOTMS-TMSOH]^+$, 16); 238($[M-TMSOH-C_9H_{13}]^+$, 18); 189(16); 177(12); 175(16); 161(44); 149(32); 147(36); 143(84); 121(100); 107(56); 95(48); 81(78); 73(98); 69(42); 55(38).
Isocupressic acid	23,185	464(1)	449($[M-CH_3]^+$, 451); 374($[M-TMSOH]^+$, 4); 359($[M-TMSOH-CH_3]^+$, 3); 346($[M-HCOOTMS]^+$, 3); 331($[M-HCOOTMS-CH_3]^+$, 2); 307($[M-CH_2-C(CH_3)=CH-CH_2-TMSO]^+$, 2); 257(11); 256($[M-HCOOTMS-TMSOH]^+$, 13); 241($[M-HCOOTMS-TMSOH]^+-CH_3$, 13); 227(5); 189(12); 175(5); 161(10); 159(10); 147(15); 143(11); 133(10), 121(28); 107(15); 95(10); 93(12); 81(24); 75(30); 73(100); 69(8); 67(7); 55(9).
Acetylimbricatoloic acid	20,902	436(4)	421 ($[M-CH_3]^+$, 2); 376($[M-60]^+$, 1); 346([M-90]); 318($[M-HCOOTMS]^+$, 20); 259($[M-HCOOTMS-OOCCH_3]^+$, 7); 238(5); 189(6); 175(8);161(16); 149(10); 143(13); 121(92); 109(28); 107(26); 95(20); 93(18); 81(36); 75(20); 73(100); 69(20); 67(14); 55(25)
Dihydroagathic acid	24,102	480(1)	465(7); 362($[M-HCOOTMS]^+$, 15); 347($[M-HCOOTMS-CH_3]^+$, 5); 293(4); 273(3); 272(3); 239(3); 189(3); 175(6); 161(15); 159(12); 143(14); 121(45); 109(15); 95(10); 93(12); 81(18); 75(34); 73(100); 69(15); 67(6); 55(13)
Acetylisocupressic acid	24,335	434(-)	419 ($[M-CH_3]^+$, -); 3746($[M-60]^+$, 3); 316($[M-HCOOTMS]^+$, 2); 257($[M-HCOOTMS-OOCCH_3]^+$, 12); 241(7); 189(10); 175(5);161(12); 147(12); 143(13); 133(11); 121(37); 109(18); 107(21); 95(18); 93(24); 81(29); 75(30); 73(100); 69(18); 67(14); 55(24)

[‡] GC retention time (min) on a DB-5ms column

S11. Chemical structures of diterpenoid components identified in the needles of *Juniperus communis*. In bold the two most abundant compounds in the total extract of this plant. In bold are marked new labdane-type diterpenoids.



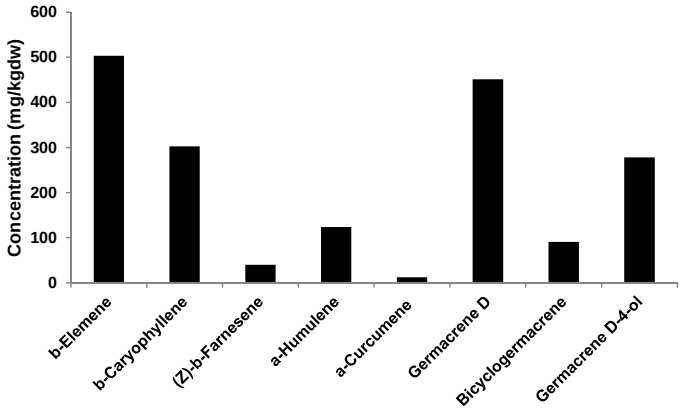
S12. Phytochemical analysis of monoterpenes and sesquiterpenes from *Juniperus communis* L. (Cupressaceae) essential oil in the literature.

S12

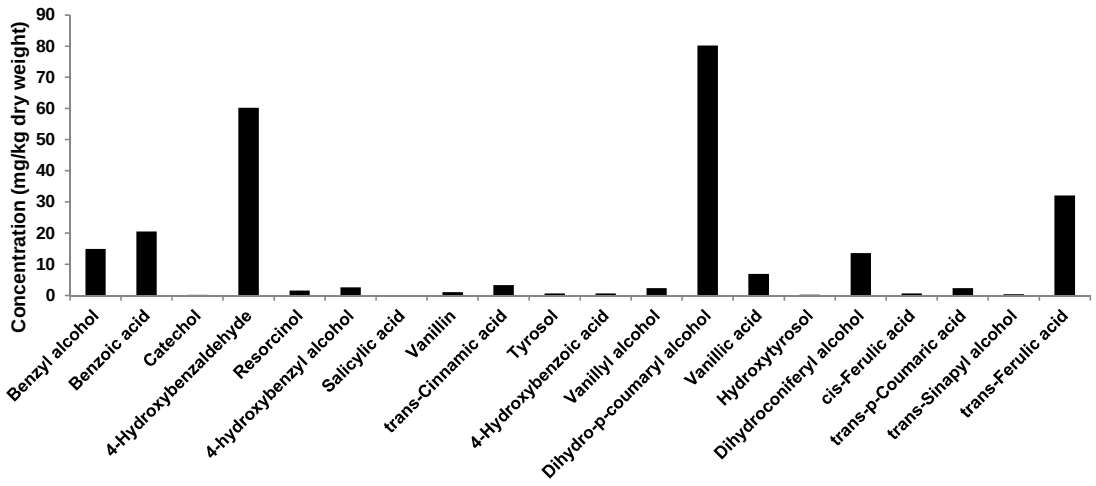
Phytochemical analysis of monoterpenes and sesquiterpenes from *Juniperus communis* L. (Cupressaceae) essential oil in the literature.

Morphological part of the plant	Component (Amount)	Reference
Berries	α-Pinene (28%), germacrene D (13%), sabinene (10.5%), myrcene (10.4%), β-caryophyllene (6.7%)	Chatzopoulou and Katsiotis, 1995
	α-Pinene (78%), myrcene (14.1%), β-pinene (4.4%), limonene (1.4%), camphene (0.7%)	Ochocka *et al*., 1997
	Sabinene (36.8), α-pinene (20%), limonene (10.6%), germacrene D (8.1%), myrcene (4.8%), terpinen-4-ol (3.4%)	Shahmir *et al*., 2003
	α-Pinene (52.2%), myrcene (15.3%), sabinene (5.6%), limonene (3.1%), β-Pinene (2.9%), terpinen-4-ol (1.5%)	Angioni *et al*., 2003
	α-Pinene (21%), sabinene (12%), γ-cadinene (12%), terpinen-4-ol (4.6%), β-caryophyllene (4.2%), β-myrcene (5%)	Barjaktarovic *et al*., 2005
	Limonene (49.3%), α-Pinene (22.1%), germacrene D (2%), δ-cadinene (1.2%), β-elemene (1.1%)	Gonny *et al.,* 2006
	α-Pinene (38%), sabinene (17%), myrcene (12%), limonene (4.5%), β-caryophyllene (3%), terpinen-4-ol (2.4%)	Glisic *et al*., 2007
	α-Pinene (25%), terpinen-4-ol (10%), limonene (4.2%), β-myrcene (4%), *p*-cymrene (3.5%), γ-terpinene (3.4%)	Vichi *et al*., 2007
	α-Pinene (46.6%), α-cedrol (12.4%), Δ^3-carene (9.8%), α-terpinolene (4.6%), terpinen-4-ol (2.9%)	Rezvani *et al*., 2009
	α-Pinene (48%), germacrene D (3.7%), β-myrcene (3.4%), *p*-mentha-1,5-dien-8-ol (2.9%), α-campholenal (2.4%)	Orav *et al*., 2010
Needles	α-Pinene (78%), β-pinene (5%), myrcene (4%), Δ^3-carene (1.8%), limonene (1%), methyl citronellate (0.7%)	Von Rudloff and Sood, 1969
	Sabinene (48.4%), α-pinene (16.5%), myrcene (3.5%), *p*-cymene (3.2%), limonene (2.0%), α-terpinolene (1.6%)	Vernin *et al*., 1988
	α-Pinene (41%), sabinene (17%), limonene (4%), terpinen-4-ol (2.7%), myrcene (2.6%), β-pinene (2%)	Chatzopoulou and Katsiotis, 1993
	α-Pinene (64%), β-phellandrene (19%), myrcene (9.5%), β-pinene (2.1%), Δ^3-carene (1.8%), limonene (1.5%),	Ochocka *et al*., 1997
	Sabinene (48.8%), α-pinene (6.2%), endofenchyl acetate (5.8%)	Pande and Mathela, 2000
	Sabinene (40.7%), α-pinene (12.5%), terpinen-4-ol (12.3%), γ-terpinene (3.8%), limonene (3.7%), α-thujene (3.1%)	Shahmir *et al*., 2003
	Sabinene (61.1%), terpinen-4-ol (10.7%), α-pinene (6.4%), γ-terpinene (3.3%), myrcene (2.6%), limonene (2.5%)	Angioni *et al*., 2003
	Limonene (31%), α-Pinene (24.4%), β-phellandrene (12.6%), α-terpinyl acetate (6%), α-terpineol (2.4%)	Gonny *et al.,* 2006
	Germacrene D-4-ol (20.3%), germecrene D (20.3%), α-Pinene (14.3%), α-humulene (5.2%), β-caryophyllene (5%)	Martz *et al*., 2009
	α-Pinene (36.4%), (*E*)-β-caryophyllene (8.1%), α-humulene (6.3%), β-phellandrene (6.3%), germacrene D (4.8%)	Orav *et al*., 2010
Roots	Cedrol (38%), longifolene (11.5%), longiborneol (8.2%), α-cedrene (6.7%), valencene (1.7%)	Gonny *et al.,* 2006
Wood	α-Pinene (64%), Δ^3-carene (10.5%), β-phellandrene (7.9%), myrcene (5.2%), limonene (3.1%), terpinolene (3.1%)	Ochocka *et al*., 1997
Wood	Limonene (19%), α-terpinyl acetate (9.1%), β-phellandrene (8.9%), α-terpineol (8.4%), α-Pinene (7.5%),	Gonny *et al.,* 2006

S13. Histogram of *sesquiterpenoids* detected in the leaves of common juniper (*Juniperus communis* L.)



S14. Histogram of phenolic acids and phenols compounds contained in the leaves of common juniper (*Juniperus communis* L.).



S15. Phytochemical analysis of lignans from the members of the *Juniperus* genus (Cupressaceae) as described in the literature.

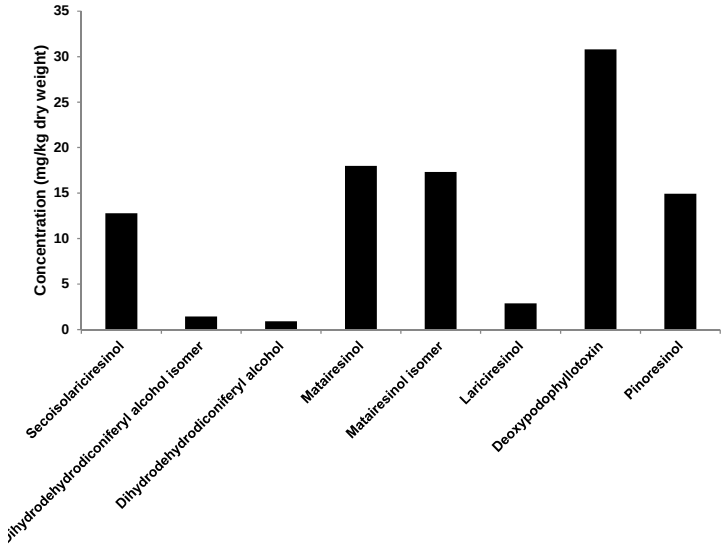
S15

Phytochemical analysis of lignans from the members of the *Juniperus* genus (Cupressaceae) as described in the literature

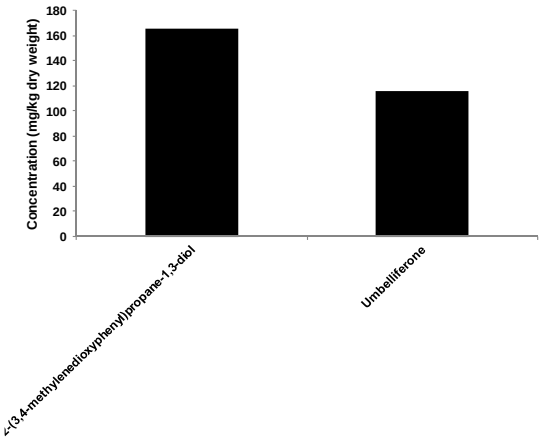
Species – part of the plant	Component	Reference
J. brevifolia - leaves	Rel-(7α,8β)-3-methoxy-4',7-epoxy-8,3'-oxyneolignan-4,9,9'-triol)	Seca and Silva, 2010
J. chinensis - leaves	**Umbelliferone**†, 2-(3,4-methylenedioxyphenyl)propane-1,3-diol, *meso*-secoisolariciresinol, 3,4-methylenedioxy-3',4'-dimethoxylignan-9',9-olide, hibalactone, isohibalactone, 7-oxohinokinin, 7-hydroxyhinokinin, 7-acetyloxyhinokinin, (+)-xanthoxylol, **dihydrodehydrodiconiferyl alcohol**, 3-methoxy-8,4'-oxyneoligna-3',4,7,9,9'-pentol, (8*S*)-3-methoxy-8,4'-oxyneoligna-3',4,9,9'-tetraol, (7*S*,8*S*)-3-methoxy-3',7-epoxy-8,4'-oxyneoligna- 4,9,9'-triol and (7*R*,8*S*)-3-methoxy-3',7-epoxy-8,4'-oxyneoligna-4,9,9'-triol	Fang *et al*., 1992
J. conferta - heartwood	Savinin	Doi and Shibuya, 1972
J. foetidissima - leaves	**Matairesinol**, **secoisolariciresinol**	Lesjak *et al*., 2013
J. nana - berries	**(-)-Desoxypodophyllotoxin**	Sakar *et al*., 2002
J. Sabina - leaves	β-Peltatin-B methyl ether, podorhizol acetate, 2'-methoxyepipicropodophyllotoxin, 2'-methoxypicropodophyllotoxin, picropodophyllotoxone, epipodophyllotoxin, (+)-dihydrosesamin, podorhizol, anhydropodorhizol, epipicropodophyllotoxin and 2'-methoxypodophyllotoxin	San Feliciano *et al*., 1990
J. Sabina - fruits	**Desoxypodophyllotoxin**	Gao *et al*., 2004
J. thurifera - leaves	(-)-dihydrosesamin, (-)sesamin, (-)-hinokinin, (-)-balactone, (-)-deoxypodorhizon, nemerosin, podorhizol, (-)-epi-podorhizol, deoxypodophyllotoxin, β-peltatin-A-methylether, podophyllotoxin, **deoxypodophyllotoxin**, picropodophyllotoxin, acetyl-epipicropodophyllotoxin	San Feliciano *et al*., 1989

†In bold are indicated the compounds which were identified in this study.

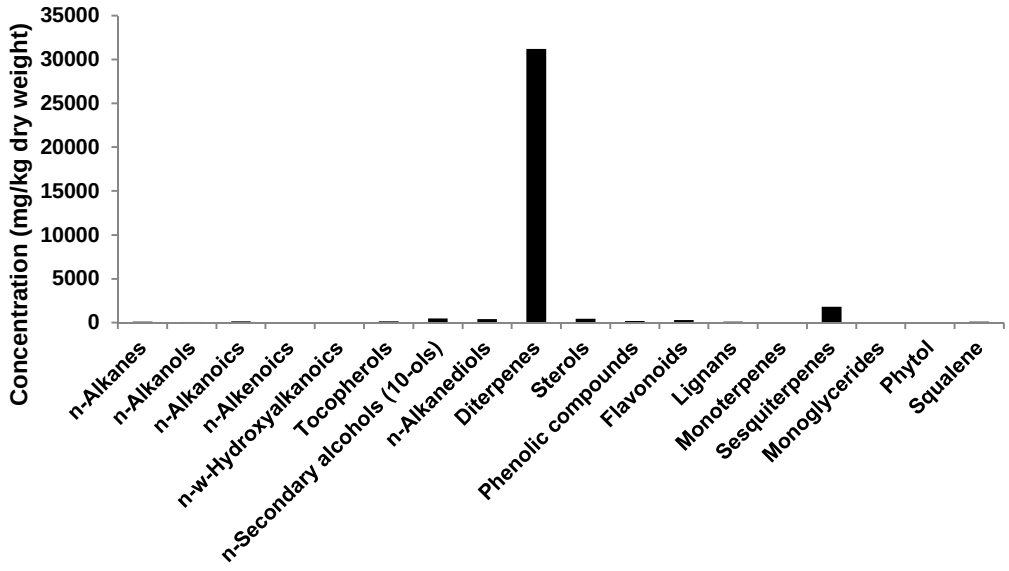
S16. Histogram of *lignans* identified in the leaves of common juniper (*Juniperus communis* L.)



S17. Histogram of *flavonoids* contained in the leaves of common juniper (*Juniperus communis* L.)



S18. Summary of the composition of cuticular waxes identified in the needles of the common juniper (*Juniperus communis*). Total concentration of major compound classes identified in the dichloromethane/pentane extract from the leaves of the common juniper.



S19. References of the supplementary data.

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