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## Essential Oil of *Portenschlagiella ramosissima* from Croatia, a Rich Source of Myristicin

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**Abstract.** Isolation of the essential oil from the ripe fruits of *Portenschlagiella ramosissima* (*Apiaceae*) by hydrodistillation yielded 1.2 % (vol./w). By GC-FID and GC-MS analyses on fused silica capillaries with polar and non-polar stationary phases, respectively, 19 compounds representing 98.0 % of the total peak area could be identified. Major constituents were  $\gamma$ -terpinene (41.0 %) followed by myristicin (25.3 %,  $257.2 \pm 2.70$  mg per gram of oil) and *p*-cymene (14.5 %).

As a conclusion, in the sample of *P. ramosissima* collected in Middle Dalmatia a high content of myristicin could be detected which is in contradiction to previous investigations by Harborne *et al.*

**Keywords:** *Portenschlagiella ramosissima*, essential oil, fruits, chemical composition, myristicin, GC-MS

### INTRODUCTION

*Portenschlagiella ramosissima* (Port.) Tutin [syn. *Athamanta ramosissima* Port., *Portenschlagia ramosissima* (Port.) Vis., non Tratt.] belongs to the family *Apiaceae* (*Umbelliferae*). This aromatic plant is an Illyric-Appennines species with a very limited distribution in mediterranean and mediterranean-montane regions of Croatia, Bosnia and Herzegovina, Montenegro, Albania, Campania and Basilicata (Italy). It grows in the fissures of limestone rocks in different types of vegetation.<sup>1–3</sup>

Previous investigations of the essential oil are limited<sup>4,5</sup> and afforded contradicting results concerning phenylpropanoids in the oil obtained from the fruits. Hence it seemed to be of interest to analyse the essential oil composition of a sample collected in Croatia in detail with special attention to myristicin and other phenylpropanoids.

Aside from the psychotropic effect of myristicin, the major compound in nutmeg oil, additional effects such as a hepato-protective activity which could be partly attributed to inhibition of TNF- $\alpha$  release from macrophages<sup>6</sup> and an inhibition of benzo[a]pyrene-induced tumorigenesis<sup>7</sup> by myristicin have been reported.

### EXPERIMENTAL

The ripe fruits of *P. ramosissima* were collected at Mt. Mosor, Croatia, at ca. 900 m above sea level on 17 August 2002. Voucher specimen has been deposited in the Herbarium Croaticum (ZA) under collection No. 563-1.

Three hundred grams of dried fruits were subjected to two hours of hydrodistillation using an apparatus according to the *European Pharmacopoeia*.<sup>8</sup>

Quantitative GC-FID analysis was performed on an autosystem gas chromatograph (Perkin Elmer Inc., Waltham Massachusetts, USA). HP5-MS column was used; 60 m x 0.25 mm i.d., film thickness 0.25  $\mu$ m. Working conditions for the column: injector temperature 250 °C, detector temperature 270 °C, linear oven temperature program from 70 to 250 °C with 3 °C min<sup>-1</sup>. The carrier gas was helium 5.6 (column head pressure 200 psi at 70 °C). Quantification of the relative amounts of individual components was done according to the area percent method, disregarding calibration factors. Additionally, myristicin was quantified in the essential oil (diluted 1:1000 in methanol) using a calibration by myristicin standards (Fluka, Buchs, Switzerland), 45, 90 and 180  $\mu$ g ml<sup>-1</sup>, and eugenol (Roth,

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Karlsruhe, Germany) at a concentration of  $106 \mu\text{g ml}^{-1}$  as internal standard in samples and calibration solutions. The calibration line ( $y = 0.064x - 0.872$ ;  $r^2 = 0.9996$ ) was calculated by linear regression analysis using Microsoft Excel (Version 2003).

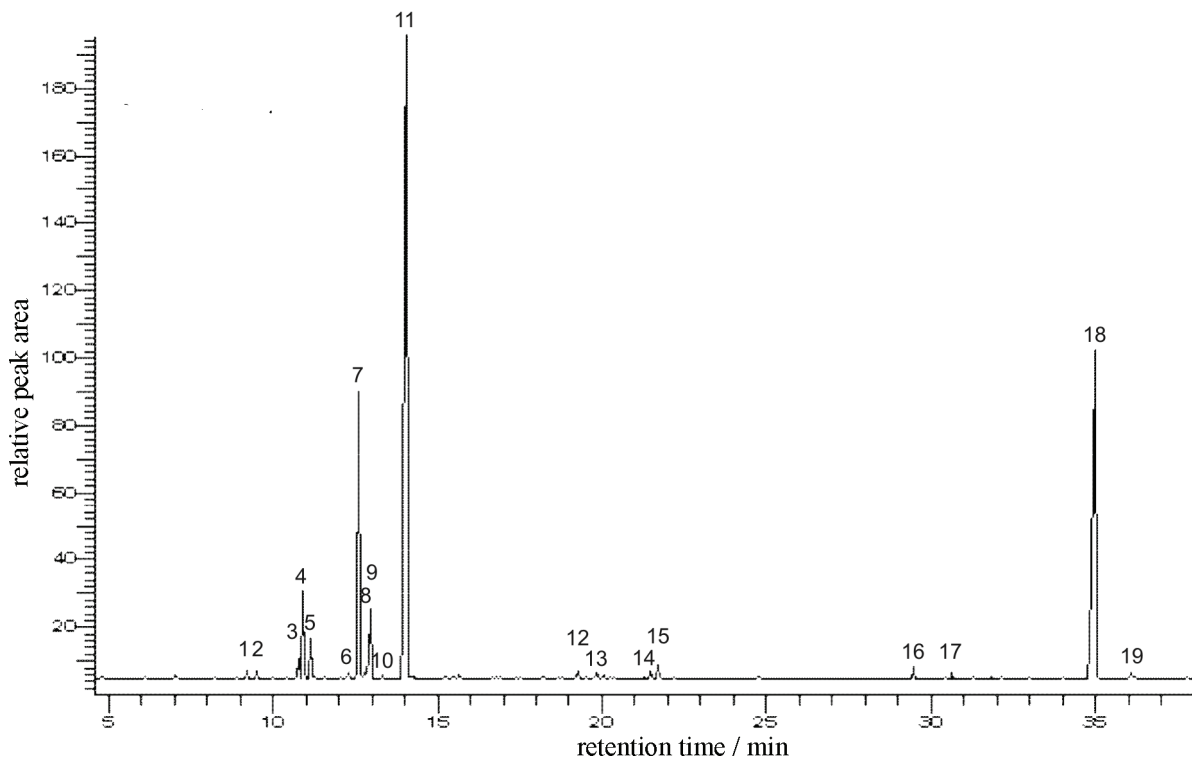
Qualitative analyses of volatile compounds were run on a GC-MS system: 5890 Series II Plus gas chromatograph interfaced to 5989 B mass spectrometer (both Hewlett-Packard Company, Palo Alto, USA). Two columns of different polarity were used: a HP5-MS column (30 m x 0.25 mm i.d.; film thickness 0.50  $\mu\text{m}$ ) and a HP-Innowax (60 m x 0.25 mm i.d., film thickness 0.25  $\mu\text{m}$ ). Linear oven temperature program was from 70 to 250  $^{\circ}\text{C}$  with 3  $^{\circ}\text{C min}^{-1}$ . The carrier gas was helium 5.6 (0.8  $\text{ml min}^{-1}$ ). The mass spectrometer operated in the electron ionization mode (70 eV), ion source temperature was 250  $^{\circ}\text{C}$ . The compounds were identified upon comparison with reference compounds, mass spectra library search and retention index references.<sup>9-12</sup> Linear retention indices<sup>13</sup> were calculated by using a series of  $\text{C}_8$ - $\text{C}_{22}$  *n*-alkanes according to the following equation:  $\text{RI} = 100 n_c + 100 [(t'_R)_x - (t'_R)_c / (t'_R)_{c+1} - (t'_R)_c]$ ; RI = retention index;  $t'_R$  = retention times corrected by the dead time;  $x$  = compound;  $n_c$  = number of carbons of the standard eluting before the compound;  $c$  = standard peak eluting before the compound;  $c+1$ : standard peak eluting after the compound.

## RESULTS AND DISCUSSION

The hydrodistillation of the ripe fruits of a Croatian sample of *P. ramosissima* yielded 1.2 % (vol./w) of an almost colourless oil (herbaceous odour with a terpentine-like note). The oil was analysed by GC-FID and GC-MS. The results of these analyses are presented in Table 1 as a relative peak area of each constituent. A representative chromatogram of GC-FID analysis is shown in Figure 1.

Only 19 compounds amounted to 98.0 % of the total peak area. Monoterpene hydrocarbons dominated the oil and  $\gamma$ -terpinene represented the major constituent (41.0 %). As a characteristic feature of the fruit oil, myristicin was present in a high yield (25.3 %) whereas other phenylpropanoids were present only in minor quantities (eugenolmethyl ether 0.7 %, elemicin 0.2 %). Phenolic compounds were also represented by the methyl ethers of thymol (0.4 %) and carvacrol (0.5 %). Strikingly, the respective non methylated parent compounds (eugenol, thymol and carvacrol) could not be detected in the oil. Myristicin was also quantified using an authentic standard which resulted in  $257.2 \pm 2.70$  mg per gram of oil (25.7 percent mass fraction). Sesquiterpene hydrocarbons were present in trace amounts and only *trans*- $\beta$ -caryophyllene (0.2 %) could be identified.

As a conclusion, our results confirmed the occurrence of high amounts of myristicin in *P. ramosissima*



**Figure 1.** GC-FID analysis of the essential oil of the fruits of *Portenschlagiella ramosissima* on a HP5-MS column (for list of compounds see table 1).

**Table 1.** Composition (relative peak area, RPA), retention indices and mode of identification of the essential oil of the fruits of *Portenschlagiella ramosissima*

| No. | Compound                        | RPA <sup>(a)</sup> | RI <sup>(b)</sup> | Identification <sup>(c)</sup> |
|-----|---------------------------------|--------------------|-------------------|-------------------------------|
| 1   | $\alpha$ -Thujene               | 0.3                | 930               | 1,2,3                         |
| 2   | $\alpha$ -Pinene*               | 0.3                | 939               | 1,2,3                         |
| 3   | Sabinene                        | 0.9                | 979               | 1,2,3                         |
| 4   | $\beta$ -Pinene*                | 5.7                | 985               | 1,2,3                         |
| 5   | $\beta$ -Myrcene                | 1.1                | 992               | 1,2,3                         |
| 6   | $\alpha$ -Terpinene             | 0.3                | 1022              | 1,2,3                         |
| 7   | <i>p</i> -Cymene*               | 14.5               | 1026              | 1,2,3                         |
| 8   | Limonene                        | 0.2                | 1030              | 1,2,3                         |
| 9   | <i>cis</i> - $\beta$ -Ocimene   | 5.4                | 1037              | 2,3                           |
| 10  | <i>trans</i> - $\beta$ -Ocimene | 0.2                | 1048              | 2,3                           |
| 11  | $\gamma$ -Terpinene*            | 41.0               | 1064              | 1,2,3                         |
| 12  | Terpinen-4-ol                   | 0.5                | 1183              | 1,2,3                         |
| 13  | $\alpha$ -Terpineol             | 0.3                | 1196              | 2,3                           |
| 14  | Thymolmethyl ether              | 0.4                | 1232              | 2,3                           |
| 15  | Carvacrolmethyl ether           | 0.5                | 1238              | 2,3                           |
| 16  | Eugenolmethyl ether*            | 0.7                | 1407              | 1,2,3                         |
| 17  | <i>trans</i> -Caryophyllene     | 0.2                | 1434              | 2,3                           |
| 18  | Myristicin*                     | 25.3               | 1524              | 1,2,3                         |
| 19  | Elemicin*                       | 0.2                | 1560              | 2,3                           |

\*Compounds also previously identified (by Ref. 4).

<sup>(a)</sup> Non calibrated data (GC-FID).

<sup>(b)</sup> Linear retention index<sup>13</sup> relative to C<sub>8</sub>-C<sub>22</sub> alkanes on HP5-MS column.<sup>9-12</sup>

<sup>(c)</sup> Mode of identification: 1 – co-GC with reference compounds; 2 – MS library search (NIST05, Wiley 138K, Adams Essential Oil Library (Mass spectral library 2205)<sup>12</sup> and laboratory own database); 3 – comparison of linear retention indices (with Ref. 9–12).

fruits which is in agreement with investigations of Bohannon *et al.*<sup>4</sup> who found even higher amounts (70 %, relative peak area, non calibrated data) of myristicin in the mature fruits. The major compounds (except for *cis*- $\beta$ -ocimene) were commensurate with our results. However, our data are in contradiction to the study of Harborne *et al.*<sup>5</sup> on myristicin occurrence in *Apiaceae* fruits which reports that myristicin could not be detected in seeds (fruits) of *P. ramosissima*. The essential oil was not analysed in detail by these authors.

Further studies including samples collected from different geographical regions seem to be necessary to clarify the question of different chemotypes within this species.

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## SAŽETAK

### Eterično ulje vrste *Portenschlagiella ramosissima* iz Hrvatske, bogat izvor miristicina

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Postupkom hidrodestilacije izolirano je 1,2 % (vol./w) eteričnog ulja iz zrelih plodova vrste *Portenschlagiella ramosissima* (Apiaceae). GC-FID i GC-MS postupcima na dvije kolone razičite polarosti utvrđena je prisutnost 18 sastavnica, koje su činile 97,8 % ulja. Najzastupljenije sastavnice eteričnog ulja bile su  $\gamma$ -terpinen (41,0 %), miristicin (25,3 %,  $257.2 \pm 2.70$  mg po gramu ulja) i *p*-cimen (14,5 %). Eterično ulje izolirano iz uzorka vrste *P. ramosissima* skupljenog u srednjoj Dalmaciji sadržavalo je visoku količinu miristicina, koji uopće nije dokazan u istraživanjima Harborne i sur.