# Essential oil and glycosidically bound volatile compounds from the needles of common juniper ( *Juniperus communis* L.)

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CROATICA CHEMICA ACTA CCACAA **73** (2) 585–593 (2000)

ISSN-0011-1643
*CCA-2668* *Original Scientific Paper*

# Essential Oil and Glycosidically Bound Volatile Compounds from the Needles of Common Juniper (*Juniperus communis* L.)[#]

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Received March 25, 1999; revised September 6, 1999; accepted September 20, 1999

The essential oil was isolated by hydrodistillation and glycosides were extracted with ethyl acetate from the needles of common juniper. The obtained essential oil was fractionated on microcolumn with solvents of different polarity. All fractions were analysed by gas chromatography-mass spectrometry (GC-MS) on two columns with different polarity of the stationary phases. Sixty-three compounds were identified. The main components of juniper needle oil were: α-pinene (16.9%), sabinene (12.1%), terpinene-4-ol (7.7%), β-phellandrene (7.3%), widdrene (6.4%), γ-terpinene (5.9%), β-terpinene (4.3%), α-terpinene (3.8%), and other compounds in smaller quantities. After isolation, final purification of the glycosides and enzymatic hydrolysis, the liberated aglycones were analysed in the same way as fractions of the essential oil. Twenty-two aglycones were identified. The main aglycones were: 3-phenyl-2-propen-1-ol, (32.8%), 2-phenylpropanol (6.0%), thymoquinone (4.6%), 3,4,5-trimethoxybenzaldehyde (4.3%), 3-methyl-2-buten-1-ol (2.8%), 2-phenylethanol (2.8%), 3-phenyl-2-propenal, (2.7%), methyl-3-hydroxybenzoate (2.6%), and *p*-cymen-8-ol, *o*-methoxybenzyl alcohol, methyl salycilate, α-methylbenzyl alcohol, 1-octen-3-ol, and other compounds.

*Key words: Juniperus communis* L., needle essential oil, fractionation of essential oil, GC-MS analysis, glycosidically bound volatiles

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[#] Partially presented at the 29th International Symposium on Essential Oils, Frankfurt am Main, September 1998.

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## INTRODUCTION

*Juniperus communis* L. (*Cupressaceae*), common juniper, is a very well-known aromatic and medicinal herb. It is an evergreen shrub that grows wild in many parts of the world. Extracts and essential oils of this plant are used to prepare alcoholic and nonalcoholic beverages, frozen desserts, baked goods, meat and meat products. Berries, needles, essential oil and extracts are used as folk remedies for many diseases.[1,2] Essential oil of *Juniperus communis* has been intensively investigated,[3–7] but glycosidically bound volatiles were not sufficiently investigated.[8] The aim of this work was to isolate and identify glycosidically bound volatile compounds and to establish if there is a similarity between them and free volatile compounds present in the essential oil. These glycosides can be found as polar compounds in tea and plant extracts. Thus, the study of the free and glycosidically bound volatile compounds can be of pharmacological interest as well as for the food and perfume industry.

## EXPERIMENTAL

### *Material and Methods*

*Plant Material: Juniperus communis* trunks were collected from several female plants in the continental part of Croatia, near Perušić, in May 1997. The plant material was dried at room temperature in a shaded place. Dried needles were separated from the trunks and berries. The weight of dried needles was approximately 1.5 kg. The voucher specimens are deposited at the Department of Organic Chemistry, Faculty of Chemical Technology, Split, Croatia.

*Isolation of Essential Oil:* 100 g of dried needles and 500 mL water were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for three hours. The obtained essential oil was separated, successively dried over anhydrous sodium sulfate and stored under an argon sealed vial at –20 °C until required.

*Isolation of Glycosides:* The glycosides were isolated by exhaustive percolation with ethyl acetate from 100 g of ground plant material at room temperature. 500 μg of octyl-β-D-glucoside was added to ethyl acetate for percolation as internal standard.[9] After percolation, pooled extracts were concentrated to dryness in a rotating evaporator under reduced pressure. The residue was dissolved in ethanol and purified by selective precipitation of ballast compounds with water and amonia-ethanol.[10,11] Finally, purification was performed by flash chromatography on a silica gel column as in previous paper.[11] The pooled glycosidic fractions were concentrated to dryness and dissolved in a citrate buffer (pH 5.5; 5 mL). The aqueous solution was washed with 5 × 5 mL of pentane-dichloromethane 2:1 *V/V* and with 5 × 5 mL of pure pentane, to remove possibly existing free terpenes and other hydrophobic compounds before enzymatic hydrolysis. After being concentrated to a few drops, the last pentane extract (5mL) was tested by TLC and GC and found to be without traces of free terpenes and other hydrophobic compounds.

*Fractionation of the Essential Oil:* 20 μL of the essential oil was fractionated on a microcolumn of silica gel (500 mg, 30–60 μm) and four fractions were obtained. 10 mL pentane was used for fractionation (fraction I) and a mixture of pentan-ether: 5 mL 5% ether (fraction II), 5 mL 10% (fraction III) and 5 mL 50% (fraction IV) . All fractions were concentrated to 0.5 mL and tested by thin layer chromatography (TLC). The first fraction contained only nonpolar monoterpene and sesquiterpene hydrocarbons. The other fractions contained polar, oxygenated compounds according to the increasing polarity from fraction II to fraction IV. These results were also confirmed by GC-MS analysis.

*Hydrolysis of Glycosides:* β-Glucosidase from almonds (»Fluka«, 20 mg ) was added to the glycosidic solution along with 3 mL pentane for trapping liberated aglycones. Hydrolysis was carried out at 30 °C for 72 hours, with the mixture being shaken occasionally. After hydrolysis, the pentane layer was separated. The remaining aglycones were extracted from the aqueous layer with pentane (10 × 2 mL). The combined pentane extracts were dried over anhydrous sodium sulphate, concentrated to a final volume of 0.5 mL, and 1 μL was used for GC-MS analysis.

*Gass Chromatography-Mass Spectrometry (GC-MS):* The essential oil, all fractions of the essential oil and volatile aglycones were analyzed by gas chromatography-mass spectrometry (Hewlett-Packard, model 5890, with a mass selective detector, model 5971A). Two columns with different polarity of stationary phases, HP-20M and HP-101, were used.

GC operating conditions: column HP-20M (Carbowax 20M), 50 m × 0.2 mm, i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 4 minutes, then increased to 180 °C at a rate of 4 °C $min^{-1}$; column HP-101 ( Dimethylpolysiloxane fluid), 25 m × 0.2, mm i.d., film thickness 0.2 μm; column temperature programmed from 70 °C isothermal for 2 minutes, then increased to 200 °C at a rate of 3 °C $min^{-1}$. Carrier gas: helium flow rate: 1 mL $min^{-1}$; injector temperature: 250 °C ; volume injected: 1 μL; split ratio: 1:50.

MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: 30–300 mass units.

*Identification and quantitative determination:* Individual peaks were identified by comparison of their retention indices with those of authentic samples, as well as by comparison of their mass spectra with those stored in the database (Wiley library). The percentage composition of the samples was computed from the GC peak areas without using correction factors. The contents of aglycones were calculated from the GC-peak areas related to the GC-peak area of 1-octanol (liberated from octyl-β-D-glucoside). Preliminary GC-MS analysis showed the absence of 1-octanol as potential aglycone. In the same way, menthol was used as internal standard for determination of fraction masses of the essential oil.

## RESULTS AND DISCUSSION

*Essential Oil:* The yield of the essential oil obtained by hydrodistillation of dried needles was 0.18% (*w/w*). The chemical composition and content of the essential oil of *Juniperus communis* are given in Table I. The essential

TABLE I

Percentage composition of the juniper needle essential oil (*Juniperus communis* L.) isolated by hydrodistillation and prefractionated by column chromatography

| No. Compound | % area | Method of identification |
|---|---|---|
| Hydrocarbons | | |
| 1. α-pinene | 16.9 | $I_1$, $I_2$, MS |
| 2. sabinene | 12.1 | $I_1$, $I_2$, MS |
| 3. β-terpinene | 4.3 | $I_1$, $I_2$, MS |
| 4. β-thujene | 3.6 | $I_1$, –, MS |
| 5. α-terpinene | 3.8 | – $I_2$, MS |
| 6. limonene | 0.2 | $I_1$, $I_2$, MS |
| 7. bornylene | 1.7 | $I_1$, –, MS |
| 8. β-phellandrene | 7.3 | $I_1$, –, MS |
| 9. γ-terpinene | 5.9 | $I_1$, $I_2$, MS |
| 10. *p*-cymene | 2.5 | $I_1$, –, MS |
| 11. terpinolene | 2.9 | $I_1$, $I_2$, MS |
| 12. cembrene | 0.7 | $I_1$, –, MS |
| 13. β-caryophyllene | 0.3 | $I_1$, $I_2$, MS |
| 14. widdrene | 6.4 | $I_1$, $I_2$, MS |
| 15. γ-elemene | 0.7 | –, $I_2$, MS |
| 16. α-humulene | 0.3 | $I_1$, $I_2$, MS |
| 17. β-cubebene | 0.7 | $I_1$, $I_2$, MS |
| 18. β-selinene | 0.2 | –, $I_2$, MS |
| 19. aristolene | 0.5 | $I_1$, – , MS |
| 20. β-himachalene | 0.5 | $I_1$, –, MS |
| 21. δ-cadinene | 1.4 | $I_1$, $I_2$, MS |
| Oxygen containing compounds | | |
| 22. acetic acid | t | $I_1$, –, MS |
| 23. α-thujone | 0.1 | $I_1$, –, MS |
| 24. α-campholene aldehyde | 0.1 | $I_1$, –, MS |
| 25. camphor | 0.1 | $I_1$, $I_2$, MS |

TABLE I (continued)

| No. Compound | % area | Method of identification |
|---|---|---|
| 26. 2-pentanol | 0.7 | $I_1$, –, MS |
| 27. *cis*-sabinenehydrate | 1.1 | $I_1$, –, MS |
| 28. linalool | t | $I_1$, $I_2$, MS |
| 29. *trans*-sabinenehydrate | 0.6 | $I_1$, –, MS |
| 30. bornyl acetate | 0.7 | $I_1$, –, MS |
| 31. terpinen-4-ol | 7.7 | $I_1$, –, MS |
| 32. citronellyl acetate | 0.1 | $I_1$, $I_2$, MS |
| 33. myrtenyl acetate | 0.2 | $I_1$, –, MS |
| 34. borneol | 0.3 | $I_1$, $I_2$, MS |
| 35. α-terpineol | 1.1 | –, $I_2$, MS |
| 36. 1-*p*-menthen-8-yl-acetate | 0.9 | $I_1$, $I_2$, MS |
| 37. phellandral | 0.1 | $I_1$, –, MS |
| 38. *cis*-piperitol | 0.3 | $I_1$, –, MS |
| 39. β-citronellol | 0.1 | $I_1$, –, MS |
| 40. *p*-isopropylbenzaldehyde | 0.1 | $I_1$, –, MS |
| 41. phellandrene epoxide | 0.1 | $I_1$, –, MS |
| 42. 1-phenyl-1-butanol | 0,1 | $I_1$, –, MS |
| 43. myrtenol | 0.2 | $I_1$, –, MS |
| 44. 2,4-decadienal | 0.1 | $I_1$, –, MS |
| 45. *p*-mentha-2,5-dien-7-ol | 0.3 | $I_1$, –, MS |
| 46. *trans*-carveol | 0.2 | $I_1$, –, MS |
| 47. hexanoic acid | t | $I_1$, –, MS |
| 48. *p*-cymen-8-ol | 0.2 | $I_1$, $I_2$, MS |
| 49. β-ionone | 0.1 | $I_1$, –, MS |
| 50. 2,4-decadien-1-ol | 0.1 | $I_1$, –, MS |
| 51. nerolidol | 0.1 | $I_1$, $I_2$, MS |
| 52. octanoic acid | t | $I_1$, –, MS |
| 53. elemol | 0.1 | $I_1$, –, MS |
| 54. capnellane-8-on | 0.2 | $I_1$, –, MS |
| 55. α-cedrol | 0.3 | $I_1$, $I_2$, MS |
| 56. spathulenol | 0.4 | $I_1$, –, MS |

TABLE I (continued)

| No. Compound | % area | Method of identification |
|---|---|---|
| 57. nonanoic acid | t | $I_1$, –, MS |
| 58. T-cadinol | 0.3 | $I_1$, –, MS |
| 59. T-muurolol | 0.3 | $I_1$, –, MS |
| 60. carvacrol | 0.1 | $I_1$, –, MS |
| 61. α-bisabolol | t | $I_1$, –, MS |
| 62. decanoic acid | t | $I_1$, –, MS |
| 63. dodecanoic acid | t | $I_1$, –, MS |
| Hydrocarbons | 72.9 | |
| Oxygen containing compounds | 17.7 | |
| Total | 90.6 | |

$I_1$ = retention indices on HP-20M; $I_2$ = retention indices on HP-101; MS = mass spectra; t = trace < 0.1%

oil contains 72.9% hydrocarbons (61.2% monoterpene and 11.7% sesquiterpene hydrocarbons) and 17.7% oxygenated compounds. The sixty-three identified compounds represent 90.6% of the total oil. Several compounds (9.4% of the essential oil) remained unidentified. The main components of the essential oil were: α-pinene (16.9%), sabinene (12.1%), terpinen-4-ol (7.7%), β-phellandrene (7.3%), widdrene (6.4%), γ-terpinene (5.9%), β-terpinene (4.3%) and α-terpinene (3.8%). This essential oil also contains smaller amounts of β-thujene, terpinolene, *p*-cymene, limonene, δ-cadinene, and other compounds. In contrast, only twenty-eight compounds were identified in the essential oil without fractionation. Namely, the GC analysis of a total oil does not usually give a complete separation of all components because peaks of some compounds are often overlapping. On the other hand, some compounds are in too low concentration in the essential oil for identification. It is obvious that fractionation facilitates qualitative and quantitative analysis of the essential oil.

Gelsomini *et al.*[6] identified only 14 components, mainly monoterpene hydrocarbons, from pentane extracts of *Juniperus communis* L. needles. Comparison of these results and our results shows significant similarity in qualitative composition.

Vernin *et al.*[7] reported that the juniper needle oil from Provence (France) contained about 70 compounds with sabinene (48.4%), α-pinene (16.5%), myrcene (3.5%) and *p*-cymene (3.2%) as the main components. In contrast to this report, our oil contains a lower percent of sabinene (12.1%) and a higher percent of β-phellandrene (7.3%), γ-terpinene (5.9%), β-terpinene (4.3%) and α-terpinene (3.8%). On the other hand, our essential oil contains almost the same amount of α-pinene (16.9%). It is likely that variations in the composition of the essential oil reflect the geographical variation.

*Glycosidically Bound Volatiles:* The content of glycosidically bound volatile compou nds in dried needles was 48.6 mg $kg^{-1}$. Among the aglycones aliphatic alcohols, terpene compounds, derivatives of phenylpropanes and $C_{13}$-norisoprenoids were identified. Twenty-two aglycones were identified. The results are shown in Table II. The main aglycones were: 3-phenyl-2-propen-1-ol (32.8%), 2-phenylpropanol (6.0%), thymoquinone (4.6%), 3,4,5-trimethoxybenzaldehyde (4.3%), 3-methyl-2-buten-1-ol (2.8%), 2-phenylethanol (2.8%), 3-phenyl-2-propenal (2.7%), methyl-3-hydroxybenzoate (2.6%), and *p*-cymen-8-ol, *o*-methoxybenzyl alcohol, methyl salycilate, α-methylbenzyl alcohol, 1-octen-3-ol, 3-(3-hydroxybutyl)-2,4,4-trimethyl-2-cyclohexen-1-on, dihydro-β-ionone, and other compounds. In contrast, J. M. A. van den Dries and A. B. Svendsen[8] identified only geraniol, linalol, α-terpineol, 1-hexanol, 3-hexene-1-ol, and 1-octen-3-ol as aglycones in *Juniperus communis* needles.

Early workers have postulated that glycosidically bound aglycones are involved in essential oil metabolism and serve as a transport form of essential oil compounds. If the glycosidically bound aglycones are compared with the corresponding free compounds of the essential oil of the same plant, only limited similarity turns out, as was shown by B. Svendsen's group in a study on 20 Coniferous species, and by E. Stahl-Biskup's group investigations of six *Lamiaceae* species.[12]

Some compounds are very common among volatile aglycones of different plants, such as aliphatic alcohols, 2-phenylethanol, benzyl alcohol, eugenol, linalool, geraniol, nerol, α-terpineol and terpinen-4-ol. It is interesting that eugenol, which is a ubiquitous aglycone and was found to be the main aglycone in eight plants of family *Lamiaceae* (our previous papers),[11,13] was not identified among the aglycone compounds from *Juniperus communis*. On the other hand, 3-phenyl-2-propen-1-ol, which is not a common compound occurring as aglycone, was identified as the main aglycone in *Cupressus arizonica* Greene var. *glauca* Woodal[14] and *Cupressus sempervirens* L.[15] (plants belonging to family *Cupressaceae*). Only myrtenol and *p*-cymen-8-ol were present in both the essential oil and the volatile aglycone fraction. This observation may confirm Svendsen's hypothesis that volatile aglycones seem

TABLE II

Percentage composition of glycosidically bound volatile compounds in dried needles of *Juniperus communis* L., isolated by ethyl acetate extraction, purified by column chromatography and hydrolized by means of β-glucosidase

| No. Compound | % area | Method of identification |
|---|---|---|
| 1. 3-hexen-1-ol | 1.3 | $I_1$, –, MS |
| 2. 1-octen-3-ol | 1.3 | $I_1$, $I_2$, MS |
| 3. thymoquinone | 4.6 | $I_1$, –, MS |
| 4. methyl salicylate | 1.0 | $I_1$, $I_2$, MS |
| 5. myrtenol | 0.7 | $I_1$, –, MS |
| 6. α-methylbenzyl alcohol | 0.8 | $I_1$, –, MS |
| 7. benzyl alcohol | 2.0 | $I_1$, –, MS |
| 8. methyl-3-hydroxybenzoate | 2.6 | $I_1$, –, MS |
| 9. 2-phenylethanol | 2.8 | $I_1$, –, MS |
| 10. perilla alcohol | t | $I_1$, –, MS |
| 11. 3-phenyl-2-propenal | 2.7 | $I_1$, $I_2$, MS |
| 12. 2-phenylpropanol | 6.0 | $I_1$, –, MS |
| 13. *p*-cymen-8-ol | 2.2 | $I_1$, –, MS |
| 14. *o*-methoxybenzyl alcohol | 1.5 | $I_1$, $I_2$, MS |
| 15. thymol | t | $I_1$, –, MS |
| 16. 3-phenyl-2-propen-1-ol | 32.8 | $I_1$, –, MS |
| 17. isoeugenol | 1.0 | $I_1$, $I_2$, MS |
| 18. 3-methyl-2-buten-1-ol | 2.8 | $I_1$, –, MS |
| 19. 3,4,5-trimethoxybenzaldehyde | 4.3 | $I_1$, –, MS |
| 20. 4-(3-hydroxy-1-butenyl)-3,5,5--trimethyl-2-cyclohexen-1-on | 2.4 | $I_1$, –, MS |
| 21. 3-(3-hydroxybutyl)-2,4,4-trimethyl--2-cyclohexen-1-on | 2.2 | $I_1$, –, MS |
| 22. dihydro-β-ionone | 1.8 | $I_1$, –, MS |
| Total | 76.8 | |

$I_1$ = retention indices on HP-20M; $I_2$ = retention indices on HP-101; MS = mass spectra; t = trace < 0.1%

to occur all over the vegetable kingdom, independently of the essential oil occurrence.[8]

## REFERENCES


1. E. Guenther, *The Essential Oils*, Vol. VI, D. Van Nostrand Company, New York, 1952, p. 370.
2. J. A. Duke, *Handbook of Medicinal Herbs*, CRC Press, Boca Raton FL, 1987, p. 188.
3. Y. Masada, *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, John Wiley & Sons, New York, 1976, p. 283.
4. P. K. Koukos and K. I. Papadopoulou, *J. Ess. Oil Res.* **9** (1997) 35–39.
5. P. C. Chatzopoulou and S. T. Katsiotis, *Planta Med.* **59** (1993) 554–556.
6. N. Gelsomini, V. Vidrich, P. Fusi, and M. Michelozzi, *J. High Resolut. Chrom. Commun.* **11** (1988) 218–220.
7. G. Vernin, C. Boniface, J. Metzger, C. Chiglione, A. Hammoud, K. Suon, D. Fraisse, and C. Parkany, *Phytochemistry* **27** (1988) 1061–1064.
8. J. M. A. van den Dries and A. Baerheim Svendsen, *Flavour Fragr. J.* **4** (1989) 59–62.
9. M. Miloš, J. Mastelić, and A. Radonić, *Croat. Chem. Acta* **71** (1998) 139–145.
10. J. Mastelić and D. Kuštrak, *Acta Pharm*. **47** (1997) 133–138.
11. J. Mastelić, M. Miloš, D. Kuštrak, and A. Radonić, *Croat. Chem. Acta* **71** (1998) 147–154.
12. E. Stahl-Biskup, F. Intert, J. Holthuijzen, M. Stengele, and G. Schulz, *Flavour Fragr. J.* **8** (1993) 61–80.
13. J. Mastelić, *The Study of the Relations of Terpenes and Terpene Glycosides of the Aromatic Plants Belonging to the Family Lamiaceae (Labiatae)*, Ph. D. Thesis, University of Zagreb, 1995.
14. M. Miloš and J. Mastelić, *Flavour Fragr. J*. **13** (1998) 248–250.
15. M. Miloš and A. Radonić, *Acta Pharm.* **46** (1996) 309–314.


## SAŽETAK

### Eterično ulje i glikozidno vezani hlapljivi spojevi iglica borovice (*Juniperus communis* L.)

*Josip Mastelić, Mladen Miloš, Danica Kuštrak i Ani Radonić*

Iz iglica obične borovice izolirano je eterično ulje hidrodestilacijom, a glikozidi ekstrakcijom etil-acetatom. Dobiveno ulje frakcionirano je na mikrokoloni, s otapalima različite polarnosti. Sve su frakcije bile analizirane vezanim sustavom plinska kromatografija-spektrometrija masa (GC-MS) na dvije kolone različite polarnosti stacionarnih faza. Identificirana su 63 spoja. Glavne su komponente: α-pinen (16,9%), sabinen (12,1%), terpinen-4-ol (7,7%), β-felandren (7,3%), vidren (6,4%), γ-terpinen (5,9%), β-terpinen (4,3%), α-terpinen (3,8%), a drugi spojevi nalaze se u manjim količinama.

Poslije izolacije glikozida, konačnog pročišćavanja i enzimske hidrolize, oslobođeni aglikoni analizirani su na isti način kao frakcije eteričnog ulja. Identificirana su 22 aglikona. Glavni aglikoni bili su: 3-fenil-2-propen-1-ol (32,8%), 2-fenilpropanol (6,0%), timokinon (4,6%), 3,4,5-trimetoksibenzaldehid (4,3%), 3-metil-2-buten-1-ol (2,8%), 2-feniletanol (2,8%), 3-fenil-2-propenal (2,7%), metil-3-hidroksibenzoat (2,6%) i *p*-cimen-8-ol, *o*-metoksibenzil-alkohol, metil-salicilat, α-metilbenzil-alkohol, 1-okten-3-ol uz manje količine drugih spojeva.