JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2011, 17(1), 54-57

Journal of Agroalimentary Processes and Technologies

# Caryophyllene from *Juniperus communis* and *Juniperus virginiana* Romanian extracts

**Carmen Manuela Pleşa[1*], Nicoleta Gabriela Hădărugă[3], Daniel Ioan Hădărugă[1], Valentin Laurenţiu Ordodi[4], Alexandra Teodora Gruia[5], Alina Gharibeh Branic[2], Aurel Ardelean[2], Alfa Xenia Lupea[1,2]**

[1] *"Politehnica" University of Timişoara, Faculty of Industrial Chemistry and Environmental Engineering, P-ţa Victoriei 2, 300006-Timişoara, Romania*
[2] *"Vasile Goldiş" West University of Arad, Faculty of Natural Sciences, 310025-Arad, Bd. Revoluţiei 81, Romania*
[3] *Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Food Processing Technology, Food Quality Departament, C. Aradului 119, 300645-Timişoara, Romania.*
[4] *Department of Biology, University of Medicine and Pharmacy "Victor Babeş" Timişoara, 2A E. Murgu Square, RO-300041, Timişoara, Romania*
[5] *Regional Center for Transplant Immunology, Emergency Clinical County Hospital Timişoara, 10 Iosif Bulbuca Bv., RO-300736, TEL/FAX: 0356433211, Timişoara, Romania*

**Received: 27 December 2010; Accepted: 11 March 2011**

***Abstract***

This paper presents the study of Juniperus communis and Juniperus virginiana extracts. Several extractions were carried out using different solvents (tetrahydrofuran, chloroform, hexane, ethyl acetate and toluene) and all extracts were analyzed using Gas Chromatography and Mass Spectroscopy. The identification of compounds was done using NIST database.

The twigs with ripe berries, the berries and branches of Juniperus communis and Juniperus virginiana were collected from three habitats of Romania. The extracts were obtained from dry berries, berries and branches by refluxing extraction into different solvents. Caryophyllene was found in all analyzed extracts (max. 4.49%). Chloroform extract from branches contained a greater amount of this sesquiterpenoid. The GC-MS analyses of extracts also revealed that the caryophyllene percentage depends on the Juniper species, on the parts of plant extracted and solvents used.

***Keywords***: *Juniperus communis*, *Juniperus virginiana*, extract, caryophyllene

## 1. Introduction

The genus *Juniperus* (Cupressaceae) consists of 55 species, all of which occur throughout the northern hemisphere of the world. Evergreen shrubs and trees in this conifer genus are slow growing and long lived [1].

The chemical composition of the berries [2,3], needles [3,4] and branches [3] from *Juniperus communis* and of needles [3,5] from *Juniperus virginiana* were previously reported, but not from Romania two *Juniperus* species.

J. *virginiana* L. (Family Cupressaceae) commonly called eastern red cedar is a widely distributed species in the USA and parts of Canada. *Juniperus virginiana* heartwood is well-known for its use of durable, termite resistant and insect resistant heartwood (redwood).

The heartwood is also used for commercial production of essential oil, commonly termed cedar wood oil [5].

Corresponding author: *e-mail:* nico_hadaruga@yahoo.com

*Juniperus communis* L., common juniper, is an evergreen shrub and grows in Apuseni's mountains and Banat's upper hills. Essential oils from section Juniperus are generally simple, being predominantly composed of monoterpenoid hydrocarbons (approximately 70 to 90% [3]).

The Romanian berries, needles and branches extraction by using five solvents (tetrahydrofurane, chloroform, ethyl acetate, hexane and toluene) upon the use of refluxing extraction have never been studied. So it seemed of interest to investigate caryophyllene from the berries, needle and branches extracts.

The extracts and essential oils of the plant are used for preparing alcoholic and nonalcoholic beverages, frozen desserts, baked goods, meat and meat products [6].

The aim of this study was to identified the highest percentage of caryophyllene from three anatomical part of two Juniper's species by refluxing solvent extraction.

## 2. Materials and methods

*Plant material and solvents.*The plant material was collected from wild growing *Juniperus communis* shrubby trees from Lipova and Albac regions, whereas *Juniperus virginiana* from "Macea" Botanical Garden in October 2008. Three kinds of samples were selected: black mature berries, needles and branches.

The solvents used for refluxing extraction were chloroform, ethyl acetate, tetrahydrofurane and toluene – Chimopar products, and hexane – Sigma-Aldrich product. For extract drying, anhydrous $Na_2SO_4$ (Fluka) is used.

*Refluxing by using different solvents.* The plant material was dried stored at room temperature. 2g of dried berries, needles and branches were ground with grinding mill, then treated with 15mL of each solvent (tetrahydrofurane, chloroform, ethyl acetate, hexane and toluene). The refluxing time was 30 min.

The extract was dried over anhydrous $Na_2SO_4$ and stored, in glass containers of 10mL each, under refrigeration (-4$^0$C) until analyzed by GC-MS.

*Gas chromatography and mass spectroscopy.* The extracts obtained by refluxing extraction were analyzed by GC-MS using HP6890 gas chromatograph. It was equipped with a splid-splitless injector and a DB5-MS fused silica column of 5% phenyl-methylpolysiloxane, 30m × 0.25mm, film thickness 0.25µm. The GC conditions used were: heating from 60 to 280$^0$C at 4$^0$C/min folowed by 30min under isothermal conditions at 280$^0$C. The injector was maintained at 250$^0$C. Helium was the GC carrier gas at 1.0mL1/min and the sample (1µL) was injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer, model HP 5973. MS conditions were the followings: ionization energy, 70eV; electronic impact ion source temperature, 200$^0$C; quadrupole temperature, 100$^0$C; scan rate 1.6scan/s; mass, 40-500 amu. The percentage composition of the volatile compounds of two species of juniper was computed from the GC peak areas. The qualitative analysis was based on the composition of the retention indexes and the mass spectra with corresponding data with the computer mass spectra libraries (NIST 98 (USA National Institute of Science and Technology software)).

## 3. Results and Discussion

We were collected *Juniperus communis* and *Juniperus virginiana* from three Romanian regions (Lipova, Albac and Macea). On *Juniperus virginiana* species were harvested three anatomical parts (needles, branches and berries), for *Juniperus communis* (Lipova) were selected just needles and branches, and from *Juniperus communis* (Albac) we analyzed only berries in all five solvents. The extraction method used was refluxing in five solvents for almost all extracts. The amount of caryophyllene is expressed as a percentage of the obtained peak area, compared with total area of all peaks from chromatogram and is presented on table 1.

The GC-MS (Figure 1 - Caryophyllene mass spectroscopyanalyses) showed that caryophyllene is presented in large amount in all three juniper parts, in special in branches. Caryophyllene is present in highest amount in Macea *Juniperus virginiana* branches chloroform (4.49%), hexane (4.07%) and ethyl acetate (3.1%) extracts, as we can see from Figure 2. On berries, the highest percentage were observed on *Juniperus communis* (Lipova), on chloroform (2.35%) and hexane (2.27%) extracts.

***Table 1.*** Percent of total area, retention time and solvent of extraction for caryophyllene from different *Juniper* anatomical part

| | Caryophyllene | | | | | |
|---|---|---|---|---|---|---|
| **No.** | **RT** | **% total area** | ***Juniper* species** | **Part of shrub** | **Sources** | **Solvent of extraction** |
| 1. | 17.401 | 0.56 | *Juniperus communis* | needles | Lipova | chloroform |
| 2. | 17.384 | 0.36 | *Juniperus communis* | needles | Lipova | toluene |
| 3. | 17.402 | 0.67 | *Juniperus communis* | needles | Lipova | hexane |
| 4. | 17.39 | 0.39 | *Juniperus communis* | needles | Lipova | ethyl acetate |
| 5. | 17.399 | 1.18 | *Juniperus virginiana* | needles | Macea | tetrahydrofurane |
| 6. | 17.397 | 1.46 | *Juniperus virginiana* | needles | Macea | chloroform |
| 7. | 17.396 | 1.70 | *Juniperus virginiana* | needles | Macea | hexane |
| 8. | 17.397 | 1.23 | *Juniperus virginiana* | needles | Macea | ethyl acetate |
| 9. | 17.397 | 0.90 | *Juniperus virginiana* | needles | Macea | toluene |
| 10. | 17.392 | 1.20 | *Juniperus virginiana* | berries | Macea | tetrahydrofurane |
| 11. | 17.405 | 2.13 | *Juniperus virginiana* | berries | Macea | chloroform |
| 12. | 17.39 | 2.09 | *Juniperus virginiana* | berries | Macea | hexane |
| 13. | 17.386 | 1.92 | *Juniperus virginiana* | berries | Macea | ethyl acetate |
| 14. | 17.381 | 0.91 | *Juniperus virginiana* | berries | Macea | toluene |
| 15. | 17.414 | 1.91 | *Juniperus communis* | berries | Albac | tetrahydrofurane |
| 16. | 17.422 | 2.35 | *Juniperus communis* | berries | Albac | chloroform |
| 17. | 17.397 | 2.27 | *Juniperus communis* | berries | Albac | hexane |
| 18. | 17.384 | 1.83 | *Juniperus communis* | berries | Albac | ethyl acetate |
| 19. | 17.386 | 1.36 | *Juniperus communis* | berries | Albac | toluene |
| 20. | 17.404 | 1.98 | *Juniperus virginiana* | branches | Macea | tetrahydrofurane |
| 21. | 17.391 | 4.49 | *Juniperus virginiana* | branches | Macea | chloroform |
| 22. | 17.385 | 4.07 | *Juniperus virginiana* | branches | Macea | hexane |
| 23. | 17.399 | 3.10 | *Juniperus virginiana* | branches | Macea | ethyl acetate |
| 24. | 17.388 | 1.13 | *Juniperus virginiana* | branches | Macea | toluene |
| 25. | 17.394 | 0.10 | *Juniperus communis* | branches | Lipova | tetrahydrofurane |
| 26. | 17.397 | 0.30 | *Juniperus communis* | branches | Lipova | chloroform |
| 27. | 17.402 | 0.75 | *Juniperus communis* | branches | Lipova | hexane |
| 28. | 17.401 | 0.50 | *Juniperus communis* | branches | Lipova | ethyl acetate |

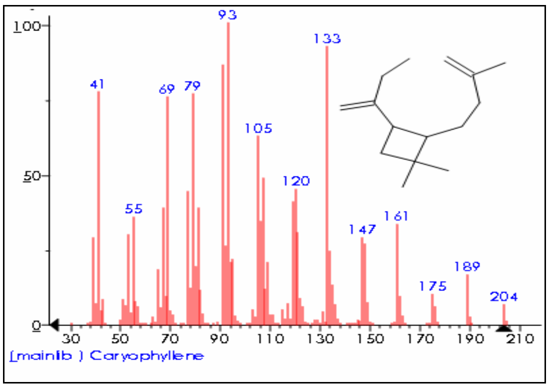


***Figure 1.*** Caryophyllene mass spectroscopy

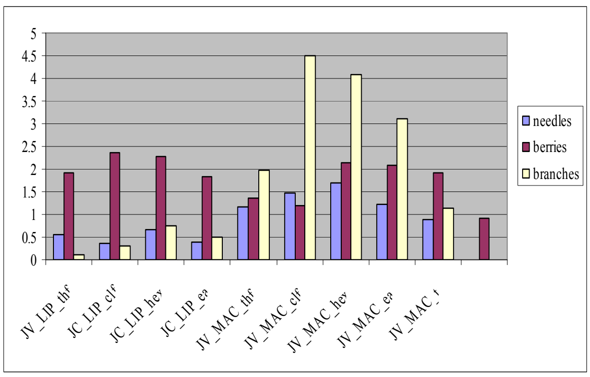


***Figure 2.*** Caryophyllene on analyzed juniper parts

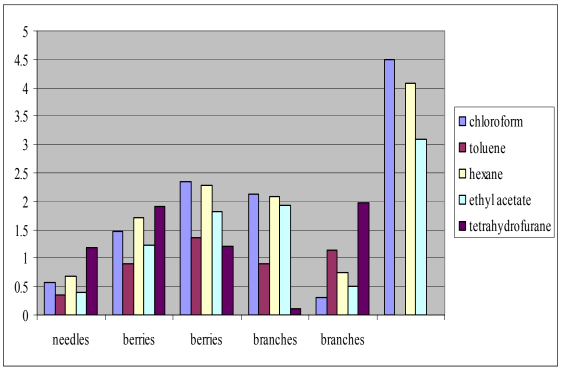


***Figure 3.*** Percentage of caryophyllene depending on solvents and juniper's part

On comparing the solvent used and juniper's anatomical parts (Figure 3), we can observe that the best solvent that extract the highest percentage of caryophyllene was chloroform, followed by hexane (in special from *Juniperus virginiana* branches).

## 4. Conclusion

On comparing the anatomical parts of *Juniperus communis* and *Juniperus virginiana*, we could say the highest caryophyllene amount was on berries.

If we compare the solvents, the best solvent was chloroform followed by hexane.


### Acknowledgements

This work was partially supported by the Grant POSDRU/21/1.5/G/13798, inside POSDRU Romania 2007-2013, and by the strategically Grant POSDRU 6/1.5/S/13 (2008) of the Ministry of Labor, Family and Social Protection (Romania) both co-financed by the European Social Fund – Investing in People.


**Abbreviations:** GC-MS - Gas Chromatography - Mass Spectroscopy, JC – *Juniperus communis,* JV – *Juniperus virginiana,* LIP – Lipova, ALB – Albac, MAC – Macea, clf – chloroform, ea – ethyl acetate, thf – tetrahydrofurane, hex – hexane, t – toluene.

### References


1. Unlu M., Unlu G., Vural N., Donmez E., Cakmak O., Composition and antimicrobial activity of juniperus excelsa essential oil, *Chemistry of Natural Compounds* **2008**, *44* (1), 129-131, doi: 10.1007/s10600-008-0040-x
2. Tunalier Z., Kirimer N., Baser K. H. C., The composition of essential oils from various parts of Juniperus foetidissima, *Chemistry of Natural Compounds* **2002**, *38*(1), 43-47, doi: 10.1023/A:1015725630556
3. Jo Gyu-Gap, Kim Jong-Hee, Infraspecific Variabiliy of Juniperus (Cupressaceae) Base don Monoterpenoid Compositions, *Journal of Plant Biology* **2005**, *48*(2), 155-158, doi: 10.1007/BF03030403
4. Svetomir Ž, Stojanović T. D., Palić R., Lazić M. L., Veljković V. B., Kinetics of distillation of essential oil from comminuted ripe juniper (Juniperus communis L.) berries, *Biochemical Engineering Journal* **2008,** *39*(3), 547-553, doi:10.1016/j.bej.2007.10.017
5. Gawde A.J., Cantrell Ch.L., Zheljazkova V. D., Dual extraction of essential oil and podophyllotoxin from Juniperus virginiana, *Industrial Crops and Products* **2009**, *30*(2), 276–280, doi:10.1016/j.indcrop.2009.05.005
6. Mastelić J., Miloš M., Kuštrak D., Radonić A., Essential Oil and Glycosidically Bound Volatile Compounds from the Needles of Common Juniper (*Juniperus communis* L.), *Croatica Chemica ACTA (CCACAA)* **2000,** *73*(2), 585-593.