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The influence of the isolation method on the composition of the essential oil of leaves and twigs of *Juniperus communis* L. var. *saxatilis* Pall. growing in Norway

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Abstract

Studies on the essential oil of leaves and terminal twigs of *Juniperus communis* L. var. *saxatilis* Pall., isolated by steam and hydro distillation using Clevenger type apparatuses showed marked differences in the composition depending on whether the plant material was either comminuted or not prior to the distillation, as well as depending upon the duration of the distillation connected herewith.

Keywords : *Juniperus communis* L. var. *saxatilis* Pall., *Cupressaceae*, essential leaf/twig oil, GC-MS

Introduction

The chemical composition of essential oils, especially their terpenoid pattern, has during the last two decades often been used for supplementary taxonomic purposes (1-28). An important condition for the conclusions to be drawn upon variations in the essential oil composition is, however, that the composition of the essential oil analysed, qualitatively and quantitatively is approximately the same as that of the living plant, which means a practically complete extraction of the essential oil and no alteration of the oil components during the isolation and analysis. Few systematic investigations seem to have been performed in this field.

A series of investigations on the essential leaf/twig oil of *Juniperus* species for taxonomic purposes has been performed with oil samples isolated by hydro or steam distillation using various Clevenger type equipment.

Since a relatively long lasting steam and hydro distillation of leaves and twigs of *Juniperus* species may cause transformations of sensitive constituents of the essential oil occurring in the plant material, we found it of interest to study some isolation techniques often applied: steam distillation of uncomminuted plant material using a Clevenger apparatus designed by Adams (6) and the commonly applied Clevenger circulary hydro distillation technique with uncomminuted and comminuted plant material.

A couple of kilos of leaves and terminal twigs of *Juniperus communis* L. var. *saxatilis* Pall. (29) growing in Norway about 1000 m above sea level were collected at one habitat in Valdres in Central Norway. The material was mixed well and divided in portions of 100

g, each of which was submitted to steam and hydro distillation under different conditions:

1) Hydro distillation of 100 g uncomminuted plant material in 800 ml of water for 2, 4 and 12 hours using a Clevenger type apparatus.
2) Hydro distillation of 100 g plant material in 800 ml water with 10 g calcium carbonate added and treated for one minute in a Warring Blender prior to the distillation using the same Clevenger type apparatus as above. Duration of distillation 15 min, 30 min and 60 min.
3) Steam distillation of 100 g uncomminuted plant material for 2 and 4 and 12 hours using the Clevenger type apparatus designed by Adams (6).

According to Adams (6) there is no need with juniper to cut up the leaves prior to the steam distillation. He found that steam distillation for 2 h removed about 35 % of the volatile oil in juniper and 24 h distillation about 95 %. For chemotaxonomic studies he used the 2 h fraction.

Results and discussion

Our studies on the influence of comminuation of the plant material upon the recovery of essential oil and its composition gave the results presented in Table 1 for plant material collected in the month of February. The shrubs were covered by about 1 m of snow. The total amount of essential oil in the material was determined by hydro distillation of comminuted plant material using the Clevenger hydro distillation technique, as mentioned above, for 1 h.

Table 1. Influence of comminution or no comminution of the plant material and of the duration of the distillation on the recovery of essential oil from 100 g leaves and twigs of *J. comm.*var. *saxatilis* collected in February.

Hydro distillation - Clevenger apparatus - uncomminuted material

Duration of distillation	2 h	4 h
Oil yield (%)	0.7	1.2

Hydro distillation - Clevenger apparatus - comminuted material

Duration of distillation	15 min	30 min	60 min
Oil yield (%)	1.6	2.0	2.3

Steam distillation - Adams technique - uncomminuted material

Duration of distillation	2 h	4 h
Oil yield (%)	0.6	0.9

A comparison of the oil composition (%) from plant material collected in the month of February and isolated by Clevenger hydrodistillation of uncomminuted and comminuted material, as described above , is shown in Table 2.

Table 2. Oil composition (some main components) of oil samples from uncomminuted and comminuted plant material.

	Uncomminuted			Comminuted	
Sample	A	B	C	D	E
Distillation time	2h	4h	12h	15min	30min
α-Thujene	2,7	3,3	4,8	3,9	4,0
α-Pinene	7,5	7,9	12,4	12,7	13.2
Sabinene	40,1	36,2	26,1	57,2	49,5
Myrcene	3,2	3,4	4,5	4,5	4,3
α-Phellandrene	0,6	0,5	0,7	0,4	0,5
δ_3-Carene	0,8	0,8	1,0	0,9	1,0
α-Terpinene	3,6	4,3	6,1	0,6	1,2
p-Cymene	0,8	0,6	0,5	0,3	0,4
Limonene/β-Phellandrene	4,7	4,6	5.5	4,6	4,6
γ-Terpinene	6,2	7,2	10,1	1,5	2,4
Cis sabinene hydrate	0,3	0,3	0.2	0,4	0,4
Terpinolene	3,1	3,2	4,2	2,2	2,4
Trans sabinene hydrate	0,8	1,0	0,8	0,0	0,2
Trans sabinol	0,6	0,7	0,6	0,1	0,1
Terpinen-4-ol	15,6	17,2	15,9	1,9	3,6
α-Terpineol	0,7	0,7	0.8	tr	0
α-Terpinyl acetate	0,8	0,8	0,5	0,5	0,7
Germacrene D	0,2	0,1	0,2	1,2	1,4
δ-Cadinene	1,0	0,7	0,6	0,7	1,9
Germacrene B	0,2	0,1	tr	1,5	1,6

A comparison of the oil composition in percentages from plant material collected in the month of May and isolated by Clevenger hydro distillation of comminuted material and by the Adams steam distillation technique with uncomminuted material is given in Table 3.

Table 3. The percentage composition of some compounds of the juniper oil isolated from comminuted and uncomminuted leaves and twigs by a 30 min Clevenger hydro distillation and a 2 h Adams steam distillation.

Plant material	Comminuted	Uncomminuted
Distillation method / time	Hydro-30 min	Steam - 2h
α-Thujene	3.2	2.6
α-Pinene	24.0	16.9
Sabinene	41,9	36,2
Myrcene	4,1	3,5
δ_3Carene	2,7	2,2
α-Terpinene	0,6	2,0
p.Cymene/Limonene	0,5	0,9
γ-Terpinene	1,3	3,5
β-Phellandrene	5,6	6.0
Terpinolene	1,9	2,4
Cis sabinene hydrate	0,3	0,4
Trans sabinene hydrate	0,2	0,4
Trans sabinol	0,1	0,4
Terpinen-4-ol	2.0	9.8
α-Terpineol	0,1	0,7
β-Elemene	0,7	0,3
Caryophyllene	0,6	0,2
α-Humulene	0,5	0,2
Germacrene D	2,2	0,8
δ-Cadinene	0,8	0,8
α-Cadinene	0,8	0,4
Germacrene B	1,1	0,4

The results obtained seem to indicate clearly that an isolation of the essential oil from leaves and twigs of *Juniperus communis* var. *saxatilis* via a relatively long lasting hydro or steam distillation of uncomminuted plant material, 2h or more, lead to transformations of some of the compounds occurring in the living plant. When compared with the oil samples recovered from comminuted material, there is a clear decrease in the percentage of sabinene in the oil recovered by hydro or steam distillation of uncomminuted plant material (2 h, 4 h, 12 h) and a corresponding increase of terpinen-4-ol, α-terpinene, γ-terpinene and to some extent of terpinolene. The results are in good agreement with those presented in previous papers, obtained with Soxhlet extraction of the plant material with diethyl ether-pentane followed by a Likens-Nickerson hydro distillation-solvent extraction and GC as well as head space gas chromatography (5,7) . So, a short hydro distillation, 15 - 30 min of comminuted leaves and twigs of juniper gives probably better informations about the oil composition in the plant than the longer lasting hydro and steam distillations of uncomminuted material.

For chemotaxonomic studies on junipers, relatively long lasting hydro or steam distillation methods with uncomminuted plant material, should preferably be replaced by short lasting distillation methods with comminuted material and GC or head space gas chromatography.

Experimental

Plant material

Leaves and young twigs were collected at selected habitats in Valdres, Central Norway, about 1000 m above sea level. The plant material was either submitted to hydro or steam distillation immediately or kept at - 20° C until distillation.

Isolation of essential oil

1) Samples of 100 g plant material were mixed with 10 g calcium carbonate and 800 ml distilled water and cut very finely in a Warring Blender for 60 seconds. The mixture obtained was submitted to hydro distillation for 15 or 30 minutes, using a Clevenger type apparatus. Calcium carbonate was added to prevent interaction of the plant acids present.
2) Samples of 100 g uncomminuted plant material were submitted to hydro distillation for 2, 4 and 12 h using a Clevenger type apparatus as described by Adams (6)
3) Samples of 100 g uncomminuted plant material were submitted to steam distillation for 2 and 4 h using a Clevenger type apparatus described by Adams (6).

Liquid-solid chromatography (LSC)

To facilitate the analysis of the essential oil, 20 μl of the oil was prefractionated by LSC over silica gel, using Sep-Pak Vac 3 cc (500 mg) Silica cartridges, by elution with 10 ml of pentane and 10 ml diethyl ether in succession. This afforded two fractions (hydrocarbons and oxygen containing compounds respectively), which were each concentrated under reduced pressure at 0°C to ca 1 ml. 1 μl of each fraction was used for GC and GC-MS analyses.

Gas chromatography (GC)

The isolated oils and fractions obtained by LSC were analysed on a DB-5 fused silica capillary column (J & W Scientific) 30 m x 0.25 mm i.d., film thickness 0.25 μm on a Shimadzu GC-14 A, equipped with FID and connected with a chromatographic data processor, Chromatopac C-R3A (Shimadzu). The conditions were as follows. Columns: fused silica, 30 m and 60 m

To facilitate the analysis of the essential oil, 20 μl of x 0.25 mm i.d., film thickness 0.25 μm:

Oven temperature: 60°-240°C at 3°C /min.

Carrier gas: Helium.

The samples were injected using the split sampling technique, ratio ca 1:20, sample size: 1.0 μl of dilutions in pentane.

The percentage composition of the sample was computed for the GC peaks without using corrections factors.

Gas chromatography-mass spectrometry (GC-MS)

Mass spectra were recorded on a Shimadzu GCMS-QP5050 system using a fused silica column, 30 m x 0.25 mm i.d., coated with DB-5, film thickness 0.25 μm. Oven temperature 60°-240°C at 3°C/min. Carrier gas Helium, velocity linear 0.8 ml/min, split injection at 1/10.

References

1. Karlsen, J. and Baerheim Svendsen, A. (1965), Medd. Nor. Farm, Selsk., 27, 165
2. Karlsen, J. and Baerheim Svendsen, A. (1966), Medd. Nor. Farm. Selsk., 30, 1
3. Banthorpe, D.V., Davies, H.S., Gatford, C. and Williams, R. (1973). Planta, 23,64
4. Hörster, H. (1974). Planta, 25, 73
5. Baerheim Svendsen, A., Scheffer, J.J.C. and Looman, A. (1985) Sci. Pharm., 53, 159
6. Adams, R.P. (1991), In Modern Methods of Plant Analysis: Oils and Waxes, Edited by H.F. Linskens and J.F. Jackson, Springer-Verlag, Berlin, p. 131.
7. Adams, R.P., Thappa, R.K., Agarwal, S.G., Kapahi, B.K. and Sarin, Y.K. (1992), J. Ess. Oil Res., 4, 143
8. Adams, R.P., Zhang Shao Zhen and Chu Ge-lin (1993), J. Ess. Oil Res., 5, 571.
9. Chatzopoulos, P.S. and Katsiotis, S.T. (1993), J. Esas. Oil Res., 5, 603
10. Adams, R.P., Zhang Shao-Zhen and Zhu Gen-lin (1993), J. Ess. Oil Res., 5, 675
11. Adams, R.P., Chu Ge-lin and Zhang Shao-Zhen (1994), J. Ess. Oil Res., 6, 17
12. Adams, R.P., Shatar, S. and Dembitsky, A.D. (1994), J. Ess. Oil Res., 6, 217
13. Caramiello, R., Bocco, A., Buffa, G. and Maffei, M. (1995), J. Ess. Oil Res., 7, 133
14. Adams, R.P., Chu Ge-lin and Zhang Shao-Zhen (1994), J. Ess. Oil Res., 6, 149
15. Adams, R.P. and Chaudhary, R.P. (1996), J. Ess. Oil Res., 8, 677
16. Adams, R.P., Shao-Zhen Zhang and Ge-lin Chu (1996), J. Ess. Oil Res., 8,53
17. Adams, R.P., Barrero, A.F. and Lara, A. (1996), J. Ess. Oil Res., 8, 367
18. Adams, R.P. (1997), J. Ess. Oil Res., 9, 641
19. Adams, R.P., Dembitsky, A.D. and Shatar, S. (1998), J. Ess. Oil Res., 10, 489
20. Adams, R.P., Thappa, R.K., Agarwal, S.G., Kapahi, B.K., Srivastava, T.N. and Chaudhary, R.P. (1998), J. Ess. Oil Res., 10, 21
21. Adams, R.P. (1999), Biochem. Syst. Ecol., 27, 709
22. Adams, R.P. (2000), Biochem. Syst. Ecol., 28, 149
23. Le Duc, A., Adams, R.P. and Zhong, M. (2000), Hort Science, 35, 157
24. Adams, R.P. (2000), Biochem. Syst. Ecol., 28, 515
25. Adams, R.P. (2000), Biochem. Syst. Ecol., 28, 529
26. Adams, R.P. (2000), Biochem. Syst. Ecol., 28, 599
27. Adams, R.P. (2000), Biochem. Syst. Ecol., 28, 975
28. Adams, R.P. (2001), Biochem. Syst. Ecol., 29, 609
29. Høeg, O.A. (1981) Eineren i norsk natur og tradisjon. Norsk Skogbruksmuseum særpublikasjon nr. 5, Elverum, Norway

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