CHEMOSYTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION II

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ABSTRACT

The essential oils of leaves of *J. virginiana* tree were collected and analyzed as fresh vs. air dried and stored at ambient conditions (21º C) for up to 16 months before extraction. ANOVA of the 58 components revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies. *Phytologia 93(1)51-62 (April 1, 2011).*

KEY WORDS: *Juniperus,* oils from dried leaves, storage tests, chemosystematics.

In a previous study (Adams, 2010), leaves of *Juniperus pinchotii* Sudw. and *J. virginiana* L. were air dried (as per specimens) and analyzed as fresh and stored (ambient lab conditions, 21º C) for up to 8 months before extraction. The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of the 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but with considerable intermixing of samples.

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22º C, 16 days) leaves of *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. and found only small differences.

The purpose of the present study is to report on changes in the composition of the steam distilled leaf oil of *J. virginiana* from specimens stored for 16 months.

MATERIALS AND METHODS

Plant material - *J. virginiana, Adams11768,* cultivated, nw corner of Gruver City Park, Hansford Co. TX, initial bulk collection: 23 Apr 2009. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20º C until analyzed. The extracted leaves were oven dried (48h, 100º C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principle

Components Analysis (PCA) as formulated by Veldman (1967) was performed to examine correlations between components.

RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oil of *J. virginiana* and a comparison of components over the 16 month storage period. Perhaps most non-intuitive is that the percent oil yield did not decline (significantly) throughout the 16 month study (Table 1). It would seem that the loss of volatiles from dry leaves over such a period would be significant. Shanjani et al. (2010) reported that α-pinene (the major and most volatile component) declined from 23.9 to 14.2% when the foliage of *J. excelsa* was air dried. Achak et al. (2008) found oil yields to be greater from fresh than air dried leaves from 2 populations of *J. thurifera* var. *africana*, but with a lower yield in another population. Later, Achak et al. (2009) reported lower oil yields in dried leaves of *J. thurifera* var. *africana* and *J. oxycedrus*, but a much higher yield from dried leaves of *J. phoenicea*.

The compounds (as percent total oil) are remarkably stable during the drying and storage tests for the first 8 months but there are major changes between 8 and 16 months storage tests. In the tests up to 8 months storage, only 9 compounds significantly differed, and only 4 compounds differed highly significantly (Adams, 2010). However, distillation of leaves stored for 16 months revealed 4 significant and 19 highly significant differences (Table 1). Several compounds had major declines in concentration from 8 to 16 month: sabinene (17.6, 13.3), limonene (14.6, 11.7), β-phellandrene (9.7, 8.0) and germacrene D-4-ol (3.8, 3.4). In contrast, several compounds increased: safrole (9.9, 11.1), methyl eugenol (2.2, 2.5), elemol (5.8, 8.8) and 8-α-acetoxyelemol (10.7, 12.4). Figure 1A shows the major compounds that declined. Notice that sabinene, limonene, and β-phellandrene show similar patterns. Pregeijerene B shows a gradual decline from 1 month to 16 months.

The patterns for 4 of the major components that increased during the study are shown in figure 1B. Safrole and methyl eugenol

(both from the phenyl propanoid pathway) show similar patterns along with elemol. However, 8-α-acetoxyelemol (dashed line, Fig. 1B) increased from fresh to week 1, then declined, then increased to 2 month, then declined and finally increased in the final, 16 month, sample.

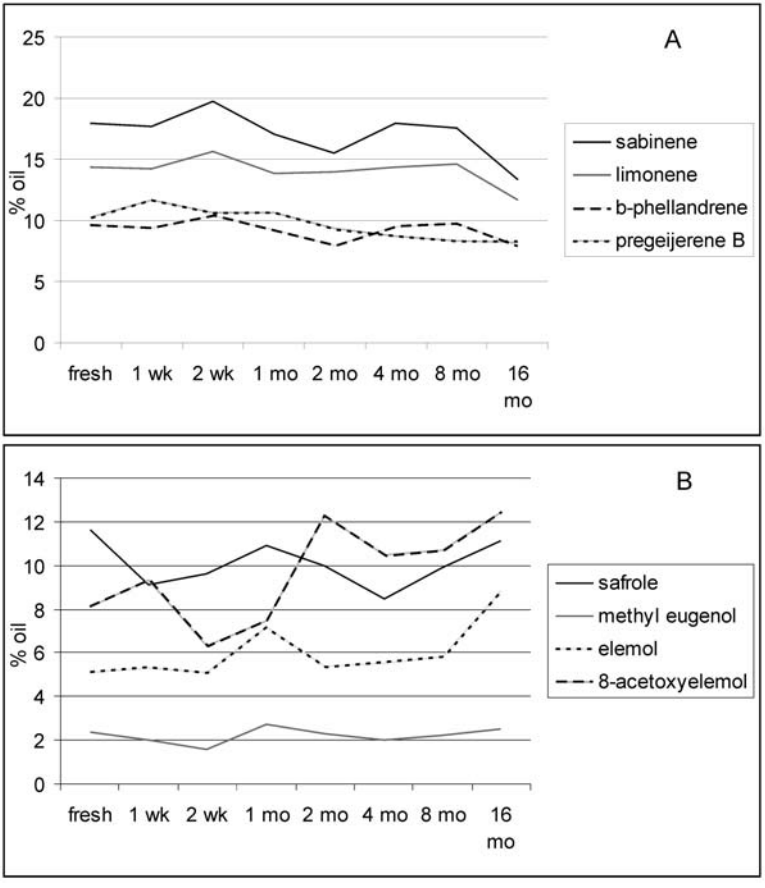

Figure 1. A (upper) Changes in concentration (% total oil) for four major components that declined during leaf storage. B (lower) Changes in concentration (% total oil) for four major components that increased during leaf storage.

The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle. So volatilization in this instance seems to be minimized by the intact glands and waxy cuticle.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (figure 2) shows the major trend is for the separation of the 16 months samples on axis 1 (21% of the variance among samples).

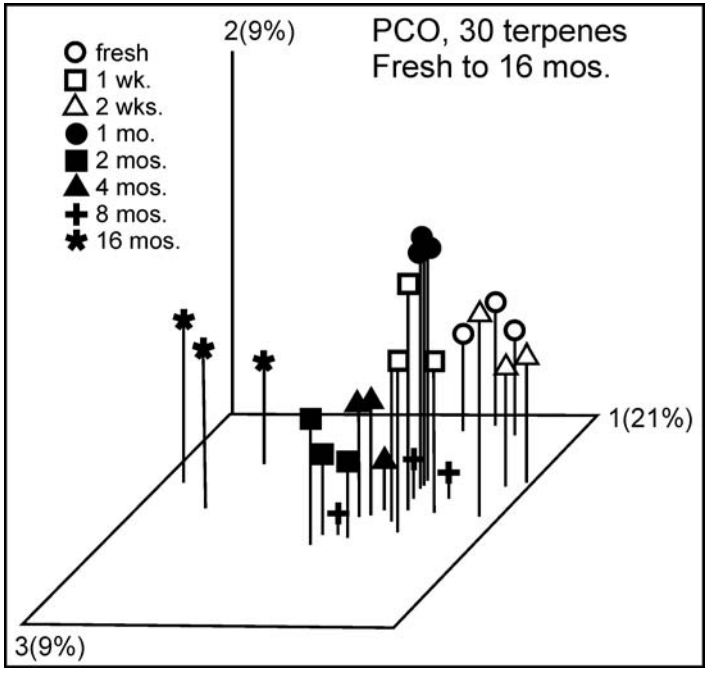

Figure 1. PCO of 8 sample sets ranging from fresh to storage for 16 months at ambient herbarium conditions (air conditioned, 21ºC).

Examination of the correlation between components was performed by PCA. Factoring the correlation matrix resulted in eigenroots that appeared to asymptote after 5 eigenroots. These accounted for 44.2, 11.3, 9.39, 6.64 and 6.45% of the variance among the components. PCA shows correlation patterns among various classes of terpenoids and phenolic compounds (Fig. 2). In general, the

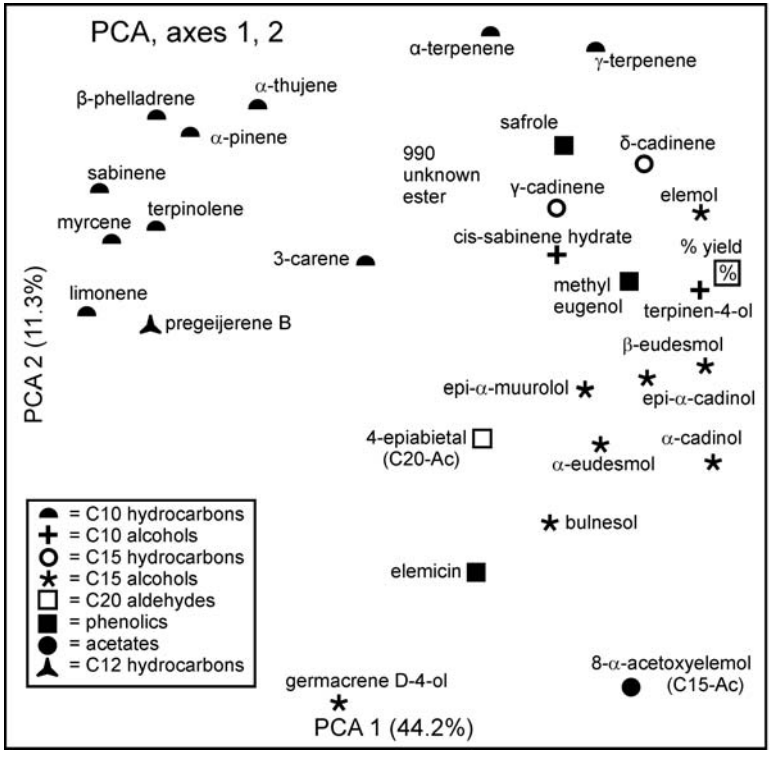

Figure 2. PCA of 39 components from J. virginiana samples stored from fresh to 16 months.

hydrocarbons are correlated (upper left, Fig. 2) and the sesquiterpene alcohols are clustered (middle right, Fig. 2). The phenolics (safrole, elemicin, methyl eugenol), from the phenyl propanoid pathway, are

somewhat scattered (Fig. 2). It appears that axis one is also separating components that increased (phenolics, sesquiterpene alcohols) from those that deceased (terpene hydrocarbons) during the 16 month study. It should be noted that only the first 2 axes are displayed, so separation of variables on the 3rd and succeeding axes is not accounted for in figure 2.

CONCLUSIONS

In this study, ANOVA revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies. If such studies were conducted among species with large differences in the essential oil compositions, the utilization of oils from both fresh and air dried leaves might still be valid. However, the present study raises concerns about the unexpected changes between 8 and 16 months of herbarium storage. It may be difficult to predict the stability of leaf essential oils in specimens over long periods of storage.

ACKNOWLEDGEMENTS

Thanks to Art Tucker and Billie Turner for reviews. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

LITERATURE CITED

Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2008. Effect of the leaf drying and geographic sources on the essential oil composition of *Juniperus thurifera* L. var. *africana* Maire from the Tensift -Al Haouz, Marrakech region. J. Essential Oil Res. 20: 200-204.

Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2009. Chemical studies of the leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). J. Essential Oil Res. 21: 337-341.

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 1991. Cedar wood oil - analysis and properties. In Modern Methods of Plant Analysis: Oils and Waxes. Edits., H. F. Linskins and J. F. Jackson, pp. 159 - 173, Springler-Verlag, Berlin, Germany.

Adams, R. P. 2007. Identification of essential oils by gas chromatography/ mass spectrometry, 4th edition. Allured Publ., Carol Stream, IL, USA.

Adams, R. P. 2010. Chemosystematics of *Juniperus*: Effects of leaf drying on essential oil composition. Phytologia 92: 186-198.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Shanjani, P. S., M. Mirza, M. Calagari and R. P. Adams. 2010. Effects of drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*. Industrial Crops and Products 32: 83-87.

Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21° C. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	18mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	0.55	ns
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	0.4	ns
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	0.5	**
945	α-fenchene	t	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	13.3	**
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	0.2	**
990	74,87,43,115	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3	**
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	0.4	**
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	nt
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	11.7	**
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	8.0	**
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	0.6	ns
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	ns
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.5	*
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	ns
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	1.0	ns
1100	n-nonanal	t	t	0.2	t	0.2	t	t	t	ns
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	t	nt

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	t	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	1.5	**
1186	α-terpineol	t	t	t	t	t	t	t	t	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	t	ns
1223	citronellol	0.2	t	t	t	0.2	0.2	t	t	ns
1261	152,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	ns
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	8.2	**
1285	safrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	11.1	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	2.5	**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	ns
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	0.2	t	0.2	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	ns
1500	α-muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.3	ns
1513	γ-cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	0.5	*
1522	δ-cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	1.0	**

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
1539	α-copaen-11-ol	t	0.3	t	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	8.8	**
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	0.7	ns
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	t	ns
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	3.4	**
1630	γ-eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	ns
1638	epi-α-cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.9	**
1638	epi-α-muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	0.8	*
1649	β-eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	0.7	**
1652	α-eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	0.9	ns
1652	α-cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	1.4	**
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	0.5	*
1688	shyobunol	t	t	t	t	0.2	0.2	t	t	ns
1746	8-α-11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	t	ns
1761	iso to 8-α-acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	ns
1792	8-α-acetoxy-elemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	12.4	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	0.4	nt
2087	abietadiene	t	t	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	0.5	**

AI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	F sig
2312	abieta-7,13-dien-3-one	t	t	t	t	t	t	t	0.1	nt

AI = Arithmetic Index on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.