# *Research Article*

# **The essential oil composition of** *Juniperus osteosperma* **foliar essential oil from southern Idaho**

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## **Abstract**



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#### *Keywords*

Utah juniper, Cupressaceae, chiral, enantiomer, multivariate analysis, gas chromatography.

*Article Information Juniperus osteosperma* (Utah juniper) is an abundant tree within its range in the Great Basin. It is an important source of food for birds and mule deer and has served as traditional medicine for Native Americans. The purpose of this work is to examine essential oils of *J. osteosperma* growing in the northern limits of its range and to evaluate the enantiomeric distribution of chiral monoterpenoids. Monoterpene hydrocarbons (23.7-31.3%) and oxygenated monoterpenoids (58.5-70.1%) made up the bulk of the essential oil compositions. The main constituents in *J. osteosperma* essential oils were bornyl acetate (9.3-27.0%), camphor (3.9-31.1%), terpinen-4-ol (7.9-15.3%), sabinene (4.3-12.3%), αpinene (3.9-9.6%), limonene (2.6-5.2%), borneol (1.6-4.2%), *trans*-verbenol (up to 10.2%), *p*-cymene (1.2-4.9%), α-elemol (1.2-6.3%), and γ-terpinene (1.4-2.0%). Chiral GC-MS revealed (+)-α-pinene (≥ 99.5%), (+)-camphene (≥ 93.0%), (+)-sabinene (100%), (+)-βpinene (100%), (+)-limonene ( $\geq$  97.7%), (+)-*cis*-sabinene hydrate ( $\geq$  93.7%), (+)-*trans*sabinene hydrate ( $\geq$  95.8%), (+)-camphor ( $\geq$  98.3%), (+)- $\alpha$ -terpineol (100%), and (+)verbenol (100%) to be the predominant enantiomers. The essential oil compositions of *J. osteosperma* from southern Idaho are similar to those from California, Nevada, and Utah, with bornyl acetate, sabinene, terpinen-4-ol and camphor dominating the essential oils. The reported biological activities of the major components are consistent with the Native American traditional uses of the plant.

# **1. Introduction**

*Juniperus osteosperma* (Torr.) Little, Cupressaceae (Utah juniper) is a conspicuous and abundant tree in the Great Basin. The tree grows to 4 m tall with scalelike leaves, female cones that turn blue when mature (6-12 mm), and bark that shreds (Fig. 1)  $[1]$ . The tree occurs occasionally in southern Idaho and southern Montana and ranges south through Nevada, Utah, western Colorado, and into Arizona and southeastern California (Fig. 2) [2]. Utah juniper is an important source of cover and food for wildlife, including bird species such as western screech owl (*Megascops kennicottii* Elliot), gray flycatcher (*Empidonax wrightii* S.F. Baird), Woodhouse's scrub

jay (*Aphelocoma woodhouseii* S.F. Baird), juniper titmouse (*Baeolophus ridgwayi* Richmond), chipping sparrow (*Spizella passerina* Bechstein), dark-eyed junco (*Junco hyemalis* Linnaeus), and warbling vireo (*Vireo gilvus* Vieillot) [2, 3]. Rodents such as desert woodrat (*Neotoma lepida* Thomas) and pinyon pine mouse (*Peromyscus truei* Shufeldt) rely on *J. osteosperma* for food [4–6]. The foliage is browsed by mule deer (*Odocoileus hemionus* Rafinesque) and elk (*Cervus canadensis* Erxleben) during deep snow when other food sources are scarce [7]. Several Native American tribes (e.g., Paiute, Shoshoni) have used Utah juniper to treat colds and coughs, rheumatism, and skin





**Figure 1.** *Juniperus osteosperma* from southern Idaho. **A:** Foliage (leaves, female and male cones). **B:** Bark.



**Figure 2.** Range map of *Juniperus osteosperma* (U.S. Geological Survey, Public domain, via Wikimedia Commons, https://commons.wikimedia.org/wiki/File: Juniperus\_osteosperma\_range\_map.jpg Accessed on 11 November 2023)

infections [8]. Samples from southern Idaho are near the northern limit of the range, so the purpose of this investigation is to compare the compositions from Idaho with essential oil samples from other geographical regions as well as to evaluate the distribution of enantiomeric monoterpenoids.

#### **2. Materials and methods**

#### *2.1. Plant material*

Foliage was obtained from six individual trees growing near Juniper, Idaho, in April 2023. For comparison, one sample from southern Utah (near Toquerville, Utah) was also collected (see Table 1). The trees were identified in the field by W.N. Setzer using a field guide [9] and verified by comparison with herbarium samples from the Southern Utah University Herbarium [10]. A voucher specimen (WNS-Jo-7049) has been deposited in the University of Alabama in Huntsville herbarium. The fresh foliage was frozen (–20 °C) until hydrodistillation.

#### *2.2. Essential oil*

For each sample, the foliage (leaves, no "berries") was hydrodistilled for four hours using a Likens-Nickerson apparatus with continuous extraction of the distillate with dichloromethane to give pale yellow essential oils (Table 1).

#### *2.3. Gas chromatographic analyses*

The foliar essential oils of *J. osteosperma* were analyzed by gas chromatography as previously described [11]. GC-MS: Shimadzu GCMS-QP2010 Ultra instrument (Shimadzu Scientific Instruments, Columbia, MD, USA), electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 atomic mass units, scan rate = 3.0 scans/s, and GC-MS solution software, ZB-5ms column (Phenomenex, Torrance, CA, USA, 60 m length, 0.25 mm inner diameter, 0.25 μm film thickness), He carrier gas (column head pressure = 208.2 kPa, flow rate = 2.0 mL/min, injector temperature =  $260^{\circ}$ C, ion source temperature =  $260^{\circ}$ C; GC oven temperature program (50 °C initial temperature, increased at a rate of  $2^{\circ}$ C/min to  $260^{\circ}$ C, then held at 260 °C for 5 min. For each essential oil sample,  $0.1 \mu L$  (5% w/v solution in dichloromethane) was injected, splitting mode = 24.5:1. Retention index (RI) values were calculated according to the linear equation of van den Dool and Kratz [12]. The *J. osteosperma* components were identified by comparing their RI values (within 10 RI units) and their MS fragmentation patterns (> 80% similarity) with those reported in the Adams [13], FFNSC3 [14], NIST20 [15], and Satyal [16] databases. GC-FID: Shimadzu GC 2010 instrument with FID detector (Shimadzu Scientific Instruments, Columbia, MD, USA), ZB-5 GC column (Phenomenex, Torrance, CA, USA,  $60 \text{ m} \times 0.25 \text{ mm} \times$ 0.25 μm film thickness), same operating conditions as above for GC-MS. The component percentages were calculated from raw peak integration without standardization. Chiral GC-MS: Shimadzu GCMS-QP2010S instrument (Shimadzu Scientific



**Table 1.** Collection and essential oil extraction details of *Juniperus osteosperma*.

Sample numbers with an asterisk (\*) had abundant female cones (berries). Sample #7\*(sU) was collected in southern Utah

Instruments, Columbia, MD, USA), Restek B-Dex 325 column (Restek Corp., Bellefonte, PA, USA, 30 m 0.25 mm diameter  $\times$  0.25 µm film thickness), injector and detector temperatures = 240 °C. He carrier gas (column head pressure  $= 53.6$  kPa, flow rate  $= 1.00$ mL/min); GC oven temperature program (50 °C initial temperature held for 5 min, increased to 100 °C at a rate of 1.0 °C/min, then increased to 220 °C at a rate of 2 °C/min). For each sample, 0.3 μL (5% w/v solution in dichloromethane) was injected, splitting mode = 24.0:1. The enantiomers were determined by comparison of RI values with authentic samples (Sigma-Aldrich, Milwaukee, WI, USA), which are compiled in our own in-house database; enantiomer ratios were calculated from raw peak areas.

#### *2.4. Multivariate analyses*

Multivariate analyses were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). Hierarchical cluster analysis (HCA) was carried out using the concentrations of the 12 most abundant components (bornyl acetate, camphor, terpinen-4-ol, sabinene, α-pinene, limonene, borneol, *trans*verbenol, *p*-cymene, α-elemol, γ-terpinene, and βphellandrene) from this study as well as previously reported compositions from the literature [17–21]. Dissimilarity was used to determine clusters considering Euclidean distance and Ward's method was used to define agglomeration. Principal component analysis (PCA, type Covariance) was used to verify the similarity of essential oil samples based on the HCA analysis.

# **3. Results and discussion**

#### *3.1. Chemical composition*

Hydrodistillation of the foliage of *J. osteosperma* collected from southern Idaho gave pale yellow essential oils in yields ranging from 0.873% to 2.738%.

Gas chromatographic analysis led to the identification of 106 chemical components comprising 98.5-99.4% of the essential oil compositions (Table 2). The major components in *J. osteosperma* essential oils were bornyl acetate (9.3-27.0%), camphor (3.9-31.1%), terpinen-4 ol (7.9-15.3%), sabinene (4.3-12.3%), α-pinene (3.9- 9.6%), limonene (2.6-5.2%), borneol (1.6-4.2%), *trans*verbenol (up to 10.2%), *p*-cymene (1.2-4.9%), α-elemol (1.2-6.3%), and  $\gamma$ -terpinene (1.4-2.0%).

Adams and co-investigators have previously examined *J. osteosperma* leaf essential oils from Nevada, northern Utah, southern California, and Arizona [17-20]; Wilson and co-workers have also examined the essential oils of *J. osteosperma* from Utah, including trunk, limb, leaf [21], and "berries" [22]. Based on the main components in the essential oils from this study and those from the previous reports, a hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out to place the chemical compositions into perspective. The HCA reveals four well-defined chemical groupings (Fig. 3): A camphor/bornyl acetate group, a camphor/terpinen-4-ol/*trans*-verbenol group, a bornyl acetate/sabinene group, and a single camphor-rich sample. The PCA (Fig. 4) agrees with the HCA and shows the three closely related groupings correlating with camphor, bornyl acetate, and terpinen-4-ol, and the lone camphor-rich sample. Based on the multivariate analyses, there does not seem to be any correlation with geographical location or the presence/absence of juniper "berries". That is, samples from Idaho, Utah, Nevada, and California are found in the camphor/bornyl acetate group; samples from Idaho, and Utah are found in the camphor/terpinen-4-ol/*trans*-verbenol group; and samples from Idaho, Utah, Arizona, and Nevado occupy the bornyl acetate/sabinene group. Likewise,



**Figure 3.** Hierarchical cluster analysis (HCA) of *Juniperus osteosperma* foliar essential oils. Samples #1-#7 are from this work, samples with an asterisk had abundant female cones (berries); samples NV (Nevada), nUT (northern Utah), sCA (southern California), AZ(12) (Arizona, 2012) are from Adams, 2012 [17]; samples wNV (western Nevada) are from Adams, 2013 [18]; sample AZ(14) (Arizona, 2014) is from Adams et al. 2014 [19]; samples Utb (Utah, browsed) and Utnb (Utah, not browsed) are from Adams et al., 2016 [20]; sample Utah is from Wilson et al. 2019 [21].



**Figure 4.** Principal component analysis (PCA) of *Juniperus osteosperma* foliar essential oils. Samples #1-#7 are from this work, samples with an asterisk had abundant female cones (berries); samples NV (Nevada), nUT (northern Utah), sCA (southern California), AZ(12) (Arizona, 2012) are from Adams, 2012 [17]; samples wNV (western Nevada) are from Adams, 2013 [18]; sample AZ(14) (Arizona, 2014) is from Adams et al. 2014 [19]; samples Utb (Utah, browsed) and Utnb (Utah, not browsed) are from Adams et al., 2016 [20]; sample Utah is from Wilson et al. 2019 [21].

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## **Table 2.** Chemical composition (percent of total) of the foliar essential oils of *Juniperus osteosperma*.



## **Table 2.** *(Continued)*



#### **Table 2.** *(Continued)*



RIcalc = Retention index determined using a homologous series of *n*-alkanes on a ZB-5ms column. RIdb = Reference retention index from the databases. Sample numbers with an asterisk (\*) had abundant female cones (berries). Sample #7(sU) was collected in southern Utah. tr = trace (< 0.05%). - = not detected. a MS(EI): 164(20%), 140(100%), 91(44%), 77(10%), 65(7%), 51(7%), 43(8%) (the MS looks like either thymyl methyl ether or carvacryl methyl ether, but the RI is too high).

samples with and without "berries" were distributed in all three groups.

Several of the major components have demonstrated relevant biological activities. Bornyl acetate [23–25], sabinene [26], camphor [27], borneol [28], terpinen-4 ol [29–31], (+)-α-pinene [32,33], (+)-limonene [34,35], and *p*-cymene [36] have shown anti-inflammatory activities. Camphor [27], (+)-limonene [37], and *p*cymene [36, 38] have analgesic properties. Camphor has demonstrated antitussive effects in rodent models [39, 40] and has been used as a home treatment for colds and as a topical analgesic [41]. (+)- $\alpha$ -Pinene, sabinene, (+)-limonene, (–)-borneol, and bornyl acetate have shown antimicrobial activities against several pulmonary and dermal pathogens [26,42]. The anti-inflammatory, analgesic, antitussive, and antimicrobial activities of the major components of *J. osteosperma* essential oil are consistent with the Native American traditional uses of the plant to treat coughs and colds, rheumatism, and skin infections.

#### *3.2. Enantiomeric distribution*

The *J. osteosperma* essential oils in this study were subjected to chiral GC-MS in order to evaluate the enantiomeric ratios of chiral monoterpenoid components (Table 3). The dominant enantiomers were (+)- $\alpha$ -pinene ( $\geq$  99.5%), (+)-camphene ( $\geq$  93.0%),

(+)-sabinene (100%), (+)-β-pinene (100%), (+) limonene ( $\geq$  97.7%), (+)-*cis*-sabinene hydrate ( $\geq$  93.7%), (+)-*trans*-sabinene hydrate ( $\geq$  95.8%), (+)-camphor ( $\geq$ 98.3%), (+)- $\alpha$ -terpineol (100%), and (+)- verbenol (100%). The enantiomeric distribution in terpinen-4-ol was less extreme with (+)-terpinen-4-ol (64.0-70.3%) predominating over (–)-terpinen-4-ol. Unfortunately, it was not possible to distinguish the enantiomers for  $\alpha$ -thujene (although only one peak was observed in each essential oil sample, the RI values are too similar for the two reference enantiomers), β-phellandrene (although only one peak was observed in the essential oils, the observed RI value was in between the RI values for the reference enantiomers), borneol (although only one peak was observed in the essential oils, the observed RI value was in between the RI values for the reference enantiomers), or bornyl acetate (the concentrations of bornyl acetate were very large so separation of enantiomers was not likely possible).

Although previous investigations of *J. osteosperma* essential oils did not include enantioselective GC-MS, there have been several reports on enantiomeric distributions in other *Juniperus* species (Table 4) [43– 46]. There does not seem to be enantiomeric consistency in  $\alpha$ -pinene, camphene, sabinene,

**Table 3.** Enantiomeric distribution of terpenoid constituents in *Juniperus osteosperma* essential oils.



RIdb = Retention index from our in-house database. RI<sub>calc</sub> = Calculated retention index based on a homologous series of *n*-alkanes on a Restek B-Dex 325 capillary column. - = compound not detected.





<sup>a</sup> Averages. <sup>b</sup> The sum of enantiomers does not add up to 100%.

terpinen-4-ol or  $\alpha$ -terpineol. Interestingly, the major enantiomer was (+)-limonene in all *Juniperus* essential oils. In fact, in members of the Cupressaceae, (+) limonene seems to predominate over (–)-limonene, including essential oils of *Chamaecyparis*, *Cupressus*, and *Thuja*, as well as *Juniperus* [47–53], while (–) limonene predominates in members of the Pinaceae (*Abies*, *Picea*, *Pinus*) [48, 51–54].

# **4. Conclusions**

This work presents the foliar essential oil compositions of six individual *J. osteosperma* from southern Idaho. In comparison with essential oils of *J. osteosperma* from California, Nevada, and Utah, the dissimilarity is slight, with bornyl acetate, sabinene, terpinen-4-ol and camphor dominating the essential oils. The reported biological activities of the major essential oil components are consistent with the Native American traditional uses of the plant. Additionally, the enantiomeric distribution of chiral monoterpenoids has been assessed in *J. osteosperma*. The (+)-enantiomers dominated the distributions for α-pinene, β-pinene, camphene, limonene, *cis*- and *trans*-sabinene hydrate, camphor, α-terpineol, and verbenone, and add to our understanding of the volatile components of *Juniperus* species. Although this work expands our understanding of *J. osteosperma* essential oils to include southern Idaho, additional information on essential oils from Wyoming and Arizona would fill in some gaps. Additional work is needed on other members of the Cupressaceae to further delineate compositional trends in the family.

#### **Authors' contributions**

Conceptualization, W.N.S.; Methodology, P.S. and W.N.S.; Software, P.S.; Validation, W.N.S., Formal Analysis, A.P., P.S., and W.N.S.; Investigation, K.S., A.P., P.S., and W.N.S.; Resources, P.S. and W.N.S.; Data Curation, W.N.S.; Writing – Original Draft Preparation, W.N.S.; Writing – Review & Editing, K.S., A.P., P.S., and W.N.S.; Project Administration, W.N.S.

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# **Availability of data and materials**

All data will be made available on request according to the journal policy.

## **Conflicts of interest**

The authors declare no conflict of interest.

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