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Composition of the Essential Oils from Rocky Mountain Juniper (Juniperus scopulorum), Big Sagebrush (Artemisia tridentata), and White Sage (Salvia apiana)

Theodore T. Borek, James M. Hochrein, and Adriane N. Irwin

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Composition of the Essential Oils from Rocky Mountain Juniper (*Juniperus scopulorum)*, Big Sagebrush (*Artemisia tridentata)*, and White Sage (*Salvia apiana)*

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Abstract

The essential oils of *Juniperus scopulorum, Artemisia tridentata,* and *Salvia apiana* obtained by steam extraction were analyzed by GC-MS and GC-FID. For *J. scopulorum,* twenty-five compounds were identified which accounts for 92.43% of the oil. The primary constituents were sabinene (49.91%), α-terpinene (9.95%), and 4-terpineol (6.79%). For *A. tridentata,* twenty compounds were identified which accounts for 84.32% of the oil. The primary constituents were camphor (28.63%), camphene (16.88%), and1,8-cineole (13.23%). For *S. apiana*, fourteen compounds were identified which accounts for 96.76% of the oil. The primary component was 1,8-cineole (60.65%).

We would like to thank Native Scents of Taos, New Mexico for supplying the oils and Regional and Small Business Partnering group for making this research possible.

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INTRODUCTION

Over the past decade, interest in the therapeutic application of essential oils has increased enormously as consumers search for relatively inexpensive, natural ways to boost their health. Since 1994, sales of aromatherapy products in the United States has increased by more than 40 percent and stirred major corporations like Colgate-Palmolive and Nu Skin to change their product line to include merchandise inspired by floral, citrus, and wood essences.[1-3] With claims to relieve pain, headaches, nausea and vomiting, and emotional stress and anxiety, essential oils have created a market where both business and consumer hope to capitalize.[1] However, many worry about the risks surrounding the use of essential oils because the chemical composition of many oils is still unknown. They argue that essential oils provide no real health benefit while simultaneously exposing the consumer to possible toxic compounds.[4] Using GC/MS and GC/FID, we have attempted to determine the chemical composition and identify the possible toxic compounds in the essential oil of *Juniperus scopulorum, Artemisia tridentata,* and *Salvia apiana.*

EXPERIMENT

Essential Oil Samples

All essential oils analyzed are products of Native Scents of Taos, New Mexico. The oils were extracted through the steam distillation of the branches and leaves of the plant. Essential oil samples were obtained from the plants *J. scopulorum*, *S. apiana,* and *A. tridentata*.

J. scopulorum, more commonly referred to as the Rocky Mountain juniper, is scattered through most of west North America with high concentrations found the upper elevations of the Rocky Mountains.[5] Standing between six and forty-eight feet in height, it is easily identified by its red bark and soft berries, both of which contain identifiable amount of oil.[6] Identified as a diuretic in the 17th century, juniper oil has historically been used to treat congestive heart failure, bladder infections, arthritis, intestinal cramps, and numerous other diseases and infections.[7]

S. apiana, also referred to as the White sage, is scattered throughout most of southwestern North America with the highest concentrations found throughout southern California.[8] The essential oil, found primarily within the plant's leaves, is highly aromatic and acts as a natural insecticide against caterpillars, grasshoppers, and other small herbivores. Historically, it is the sage used most frequently used in Native American rituals and when applied, can cool inflammation, particularly for throat and respiratory infections.[9]

A. tridentata, also referred to as the Big sagebrush, is found through most of the lower elevations western United States although it is mostly known for its ability to survive in the "cold desert" of the Great Basin.[6,10] The Big Sagebrush has distinctive three-toothed leaves up to an inch or so long that aid in the synthesis of an essential oil rich in terpenoids. Just as the oil of the *S. apiana*, the oil of the Big sagebrush acts as a natural insecticide and is also used for treating colds and coughs.[11]

Experimental Techniques

Most of the primary constituents of the oils were identified by gas chromatography/ mass spectrometry (GC/MS). Reference materials were purchased from Sigma Aldrich and used for retention time and mass spectral matching. Further comparison was carried out using the gas

chromatography with flame ionization detection (GC/FID). For the constituents in which a reference was unavailable, the mass spectra were compared to published literature values and the NIST library and checked by computerized library matching.

The essential oils were analyzed using GC/MS and GC/FID. The GC/MS analysis was performed on a Finnigan Mat SSQ710 with an A200S autosampler using a DB5MS column. The conditions included: column specifications, 60 m x 0.25 mm x 1 μm df; carrier gas, He at 37 psi; and injector, 280°C. The temperature program used for the GC analysis was as follows: the oven began at 50°C for 5 min, heated to 300°C at a rate of 25°C/min, and then held at 300°C for 10 min. The conditions for the MS analysis were as follows: source temp, 150°C; manifold temp, 70°C; mass range, 33-350; scan time, 0.5 sec. The method is further summarized in the appendix as Fig 1.

The GC-FID analysis was preformed on an HP5890 with a HP 7673A injector using a AT-1 column: column specifications, 30 m x 0.32 mm x 5 μm df; carrier gas, He at 15 psi; injector, 245°C; split, 200:1. The temperature programs used for GC analysis was: the oven initiated at 50°C for 2 min, heated to 280°C at a rate of 20°C/min, then held for 2 min. The same dilutions were used for the GC-FID as the GC-MS. The method is further as Fig 2.

The essential oil samples of *S. apiana*, A. *tridentate*, and standard solutions of 1,8 cineole and para-cymene were diluted into hexane at a ratio of 100:1 and 1,000:1 volume to volume. The essential oil of *J. scopulorum* and all other standard solutions were diluted into acetonitrile at the same ratios.

RESULTS

J. scopulorum

Table 1 summarizes the compounds found using the SSQ710 GC/MS. The constituents are listed by retention time and labeled with mode of identification. Using a 0.20% area threshold, thirty-six constituents were visible. A 0.20% area threshold was used to differentiate the essential oil components from the background noise. Twenty-five were identified to account for 92.43% of the oil. In addition, six components present in trace amounts (>0.20%) were also identified. The results show that the *J. scopulorum* is rich in a variety of monoterpinenes that collectively account for 70.37% of the essential oil. Primary constituents include sabinene (49.91%), α-terpinene (9.95%), and 4-terpineol (6.79%). The *J. scopulorum* essential oil is also rich in other forms of hydrocarbons including sesquiterpenes (2.18%), oxygenated monoterpenes (15.85%), and esters (1.29%). The total ion chromatograph can be viewed in Fig 3 and a 5% baseline threshold can be view in Fig 4.

S. apiana

Table 2 summarizes the properties of the compounds identified using the SSQ710 in order of their retention times. The constituents are listed by retention time and labeled with mode of identification. Using a 0.10% area threshold, eleven of the twenty-seven constituents were identified to account for 96.76% of the oil. Unlike the oil of *J. scopulorum*, the background noise for the chromatogram of *S. apiana* was minimal. This allowed for a 0.10% area threshold to be utilized and a more accurate integration. The result showed that the *S. apiana* is rich in a variety of oxygenated monoterpenes that collectively account for 64.00% of the essential oil. The primary components were 1,8-cineole (60.65%), β-pinene (10.68%), and

α-pinene (10.14%). The *S. apiana* essential oil is also rich in other forms of hydrocarbons including sesquiterpenes (1.72%) and monoterpinenes (31.04%). The total ion chromatograph can be viewed in Fig 5 and a 5% baseline threshold can be viewed in Fig 6.

A. tridentata

Table 3 summarizes the compounds found using the SSQ710 in order of their retention times. The constituents are listed by retention time and labeled with mode of identification. Using the same 0.10% area threshold from the *S. apiana* integration, forty-two constituents were visible. Twenty were identified to account for 84.32% of the oil. The result showed the *A. tridentate* was also rich in a variety of oxygen monoterpinenes that collectively account for 48.22% of the essential oil. The primary components were camphor (28.63%), camphene (16.88%), and 1,8-cineole (13.23%). The *A. tridentate* essential oil was also rich in other forms of hydrocarbons including sesquiterpenes (1.55%) and monoterpinenes (22.54%). The total ion chromatogram can be viewed in Fig 7 and a 5% baseline threshold can be view in Fig 8.

DISCUSSION

All three essential oils were primarily composed of various forms of hydrocarbons including monoterpinenes, sesquiterpenes, acetates, and oxygenated monoterpinenes. Most contained an aromatic or cyclic ring with simple changes in the number of carbon, oxygen, and hydrogen molecules in the adjoining groups. Many of the same compounds were present in two or more of the oils. As a result, the differences between the essential oils did not arise from differing constituents, but instead from a differing concentration of those components. For example, comparing S. *apiana* (White Sage) and *A. tridentate* (Big Sagebrush), *S. apiana* had a fairly simple composition with only twenty-seven compounds in greater abundance that 0.10% and one compound, 1,8 cineole, accounting for over sixty percent of the oil while *A. tridentata* was relatively complex with forty-seven compounds and three compounds accounting for 10% or more of the oil.

The data can be further analyzed by comparison of our essential oil with that of other species of the same genus. For the *J. scopulorum* essential oil, the results were also compared to the essential oil analysis of the juniper berry and the needles of the common juniper (*J. communis)*. While the concentration of constituents varied throughout the different species, our order of elution was consistent with the literature values published.[12,13] Moreover, the concentrations of the *J. scopulorum* are similar to those of the *J. oblonga*. Both species demonstrated high concentrations of the compounds α-pinene, sabinene, and 4-terpineol and contained several uncommon constituents such as linalool and verbenene.[14] Although our data was reasonably consistent with the literature, differences in the concentration of mycrene were observed. For the genus *Juniperus,* myrcene typically composes between five and ten percent of the total oil.[14] Our sample of *J. scopulorum* oil contained only trace amounts co-eluting with sabinene. Myrcene was only later identifiable by retention time matching using a standard solution.

For the *A. tridentata*, our results were consistent with results published for the species *Artemisia roxbughiana* as the authors also name 1,8-cineole, camphor, and α-thujone as the primary constituents.[15] For the *S. apiana*, our results were consistent with those identifying 1,8-cineole as the primary constituent.[16-18] By comparing the chromatograms of *J. scopulorum*

with *S. apiana* and A. *tridentata,* it was observed that hexane provided a better separation and mass spectral response for the sesquiterpenes.

By identifying the chemical components for each oil, it is possible to consider any toxic side effects. The major components of *J. scopulorum* oil are sabinene, α-terpinene, and 4-terpineol. All three components are classified non-toxic and non-sensitizing, although 4-terpineol can be mildly irritating for individuals with sensitive skin.[4,19] The essential oil of *S. apiana* is composed mostly of 1,8-cineole. 1,8-cineole is regarded as safe in the amounts traditionally found in essential oils. However, a 1 mL oral ingestion can cause transient coma and poisoning can cause gastrointestinal and central nervous system damage.[4] Finally, the primary components of *A. tridentata* are camphor, camphene, and 1,8-cineole. Camphene is regarded as non-toxic, non-irritant, and non-sensitizing. Camphor, however, is toxic. Exposure can result from ingestion or absorption through the skin and mucous membranes and can result in nausea, vomiting, vertigo, confusion, delirium, convulsions, coma, respiratory failure, and death.[4,20] The only other compound classified as toxic that was found in greater abundance than 1.0% was thujone. The alpha and beta forms of thujone may cause convulsions and fat degeneration of the liver.[4,21] Although all of the oils contain some toxic chemical components, only the oil of *A. tridentata* should be regarded as potentially hazardous. It has identifiable amounts of both camphor and thujone that collectively account for 34.99% of the total oil.

References

1. C. Powell, Making scents of aromatherapy, in *The Seattle Times*. 2 April 2003, 7/24/2003, <seattletimes.nwsource.com/html/healthscience/13466619_aromatherapy020.html>

2. Colgate cites new U.S. products for 10% gain in profits, in *Chicago Sun Times*, 23 July 2003, 7/24/03, <www.suntimes.com/output/business/cst-fin-profits23.html>

3. Nu Skin unveils new product line, in *The Star,* 8 Jan. 2003, 7/24/03, <biz.thestar.com.my/news/story.asp?file=/2003/1/8/business/nusking24b&sec=business>

4. R. Tisserand, T. Balacs, *Essential Oil Safety*. Harcourt Publishers Limited, Edinburgh, 2000.

5. Native Confers of North America, *Nearctica*, <www.nearctica.com/trees/conifer/juniper/Jscop.htm>. 6/27/2003.

6. W. Weber, *Rocky Mountain Flora*. Colorado Assoc University Press, Boulder, 1976.

7. Juniper Berry essential Oils, *The Kevala Centre for Holistic Heath*, <www.Kevala.co.uk/aromatherapy/juniper.cfm> 6/27/2003.

8. Plants for a Future: *Salvia apiana, Plants for a Future,* <www.scs.leeds.ac.uk/cgibin/pfaf/arr_html?Salvia+apiana&CAN=LATIND> 7/15/03.

9. White Sage, *Earth Observatory: NASA*, <earthobservatory.nasa.gov/Laboratory/Biome/seedsage.html> 7/15/03.

10. J.E. Bowers, *Shrubs and Trees of the Southwest Deserts.* Southwest Parks and Monuments Association, Tucson, 1993.

11. W.W. Dunmire, G.D. Tierney, *Wild Plants of the Pueblo Province*. New Mexico Press, Santa Fe, 1991.

12. R.P. Adams, Geographic variation of essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia, in *Biochemical Systematics and Ecolog,* **29**, pp. 609-619, 2000.

13. J. Mastelic, M. Milos, D. Kustrak, A. Radonic, Essential Oil and Gylcosidicallly bounds volatile Compounds for the Needles of Common Juniper, in *Croatica Chemica Acta*, **73**, pp. 585-593, 2000.

14. R.P. Adams, The leaf essential oils and chemotaxonomy of *Juniperus* sect. *Juniperus*, in *Biochemical Systematics and Ecology*, **26**, pp. 637-645, 1998.

15. C. Bicchi, P. Rubiolo, H. Marschall, P. Weyerstahl, R. Laurent. Constituents of Artemisia roxburghiana Besser essential oil, in *Flavor and Fragrance Journal,* **13**, pp.40-46, 1998.

16. R. Karousou, D. Vokou, S. Kokkini, Variation of Salvia fruticosa essential oils on the island of Crete (Greece), in *Botanica Acta*, **111**, pp. 250-254, 1998.

17. M. Skoula, I.E. Hilali, A.M. Makris, Evaluation of the genetic diversity of *Salvia fruticosa* Mill. Clones using RAPD markers and comparison with the essential oil profiles, in *Biochemical Systematics and Ecology*, **27**, pp. 559-568, 1999.

18. M.Z. Haznedaroglu, N.U. Karabay, U. Zeybek, Antibacterial activity of *Salvia tomentosa* essential oil, in *Fitoterapia*, **72**, pp. 829-831, 2001.

19. Chemical Ecology of Browsing, Tasmanian School of Pharmacy, http://www.healthsci.utas.edu.au/pharmacy/ChemicalEcologyofBrowsing.htm, 8/6/2003.

20. Camphor Revisited: Focus of Toxicity (RE9422), in *American Academy of Pediatrics*, **94**, pp. 127-128, 1994.

21. Opinion of the Scientific Committee on Food on Thujone, European Commission of Health & Consumer Protection Directorate-General, 2 Dec. 2002, 6 Feb. 2003.

INSTRUMENTATION: ThermoFinnigan SSQ, gas chromatograph/quadrapole mass spectrometer

GC Column: J&W Scientific, DB-5ms column, 60m x 0.25 mm, 1 µm df

GC Parameters: Method Name: "nonylphenol"

Temperatures	Oven Init.	Oven Ramp	Oven Final	Injector	Transfer line
	50°C for 5.0 min	25°C/min	300°C for 10.0 min	280°C	280°C

Injection Volume: 0.2 µL, splitless
Carrier Gas: Helium at 37 psi constant pressure

MS Parameters: Method Name: "acqscan"

Full scan: 33 to 350 amu
Filament Delay: 6.0 minutes
Source Temp: 150°C
Manifold Temp: 70°C

Figure 1: Gas Chromatography/Mass Spectrometry analysis conditions.

INSTRUMENTATION: Hewlett Packard 5890A gas chromatograph/flame ionization detector

GC Column: J&W Scientific, DB-5ms column, 30m x .25 mm, 1 µm df

GC Parameters: Method Name: "GC Gen"

Temperatures	Oven Init.	Oven Ramp	Oven Final	Injector	Transfer line
	50°C for 2.0 min	20°C/min	280°C for 2.0 min	245°C	250°C

Injection Volume: 0.2 µL, 200:1 split
Carrier Gas: Helium at 15 psi constant pressure

Figure 2: **Gas Chromatography/Flame Ionization Detection analysis conditions.**

RT	Constituent	Identification	Per cent (%)
11.56	tricyclene	Ref, Lib	trace
11.97	α-thujene	Ref, Lib	0.76
12.14	α-pinene	GC/MS,GC/FID, Ref Lib	3.50
12.42	camphene	Ref, Lib	1.25
12.54	sabinene	Ref, Lib	49.91
12.59	myrcene	GC/MS, GC/FID	trace
12.69	β-pinene	Ref, Lib	0.23
12.94	α-phellandrene	Ref, Lib	trace
13.03	α-terpinene	GC/MS,GC/FID, Ref, Lib	9.95
13.1	limonene/ β-phellandrene	GC/MS,GC/FID, Ref, Lib	4.77
13.2	1,8 cineole	GC/MS,GC/FID, Ref, Lib	2.93
13.59	linalool	GC/MS,GC/FID, Ref, Lib	0.68
13.8	thujone	GC/FID, Ref	0.77
13.97	β-ocimene	Ref	trace
14.02	cis-sabinene hydrate	Ref, Lib	0.33
14.29	eucarvone	Ref, Lib	0.25
14.35	camphor	GC/MS,GC/FID, Ref, Lib	3.55
14.49	4-terpineol	GC/MS,GC/FID, Ref, Lib	6.79
14.58	α-terpineol	Ref, Lib	0.55
14.78	unknown acetate		0.88
14.91	geranyl acetate	Ref	0.38
15.14	4-terpinyl acetate	Ref	0.46
15.25	bornyl acetate	Ref, Lib	0.45
15.33	unknown acetate		0.45
16.01	β-cubebene	Ref	trace
16.68	germacrene D	Ref	0.33
16.79	α-muurolene	Ref, Lib	0.24
16.96	α-amorphene	Ref, Lib	0.41
17.15	elemol	Ref	1.41
17.68	bisbolene	Ref	0.76
17.89	δ-salinene	Ref	0.44
18.01	?-cadinene	Ref	trace

GC/FID = comparison with the GC/FID of standard
GC/MS = comparison with the GC/MS of standard
Ref = comparison with GC and MS literature values
Lib=Computer Library Identification

Table 1: Chemical constituents in the essential oil of *Juniperus scopulorum* by order of their retention times.

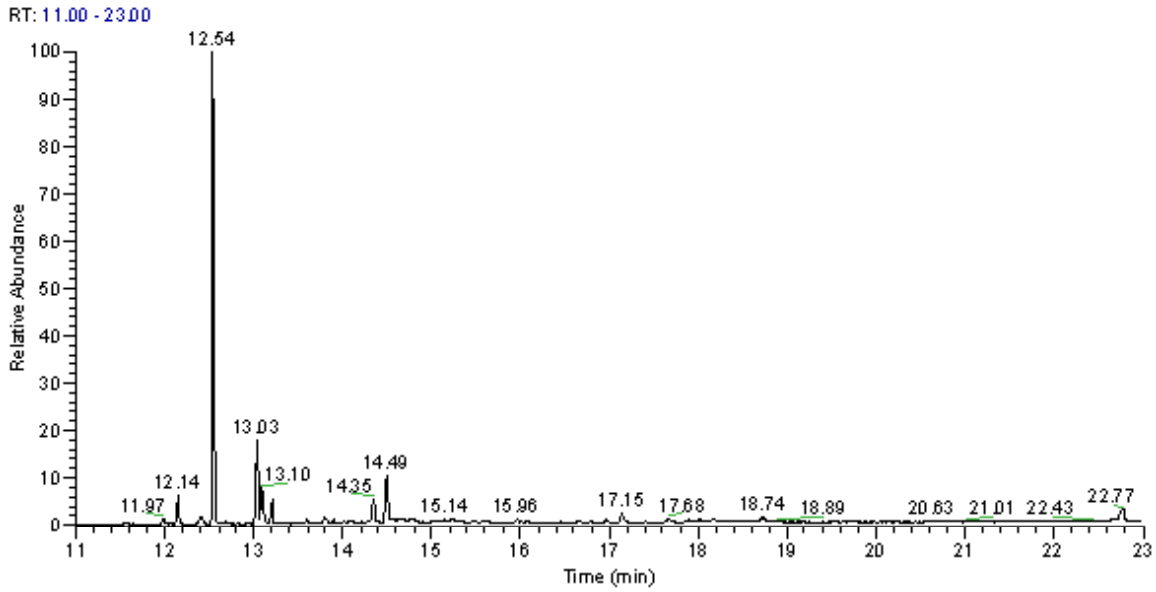

Figure 3: Total ion scan of *Juniperus scopulorum* in acetonitrile.

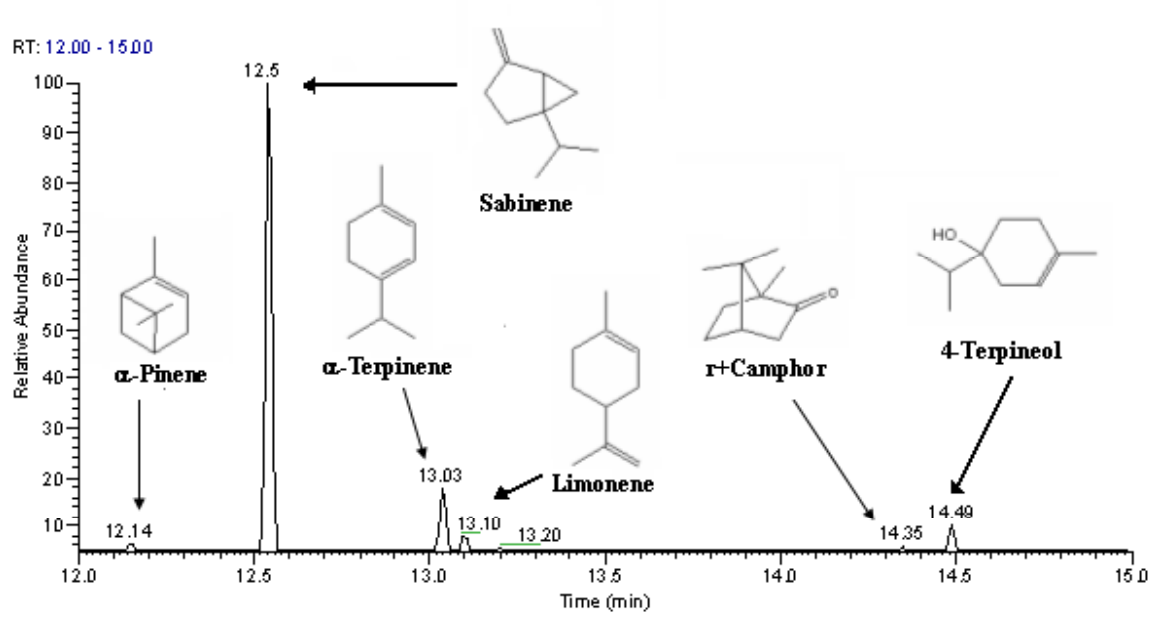

Figure 4: *Juniperus scopulorum* in acetonitrile with a 5% baseline threshold.

RT	Constituent	Identification	Percent (%)
12.03	α-thujene	Ref, Lib	0.69
12.22	α-pinene	GC/MS,GC/FID, Ref, Lib	10.14
12.46	camphene	Ref, Lib	0.82
12.59	myrcene	GC/MS,GC/FID, Ref, Lib	2.42
12.76	β-pinene	Ref, Lib	10.68
13	3-carene	Ref, Lib	3.19
13.18	para-cymene	GC/MS,GC/FID, Ref, Lib	3.10
13.32	1,8 cineole	GC/MS,GC/FID, Ref, Lib	60.65
13.64	linalool	GC/MS,GC/FID, Ref, Lib	0.22
14.43	camphor	GC/MS,GC/FID, Ref, Lib	2.66
14.57	4-terpineol	GC/MS,GC/FID, Ref, Lib	0.47
16.53	β-carophyllene	Ref	1.39
17.05	α-amorphene	Ref	0.33
			94.59%

GC/FID = comparison with the GC/FID of standard
GC/MS = comparison with the GC/MS of standard
Ref = comparison with GC and MS literature values
Lib=Computer Library Identification

Table 2: Chemical constituents in the essential oil of *Salvia apiana* by order of their retention times.

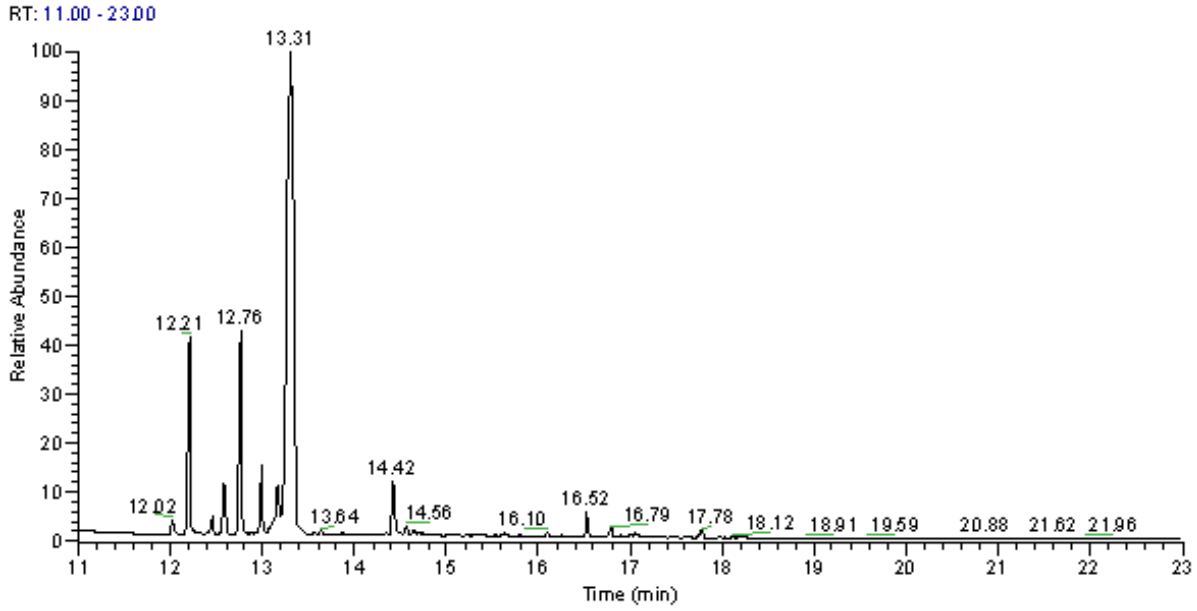

Figure 5: Total ion scan of *Salvia apiana* in hexane.

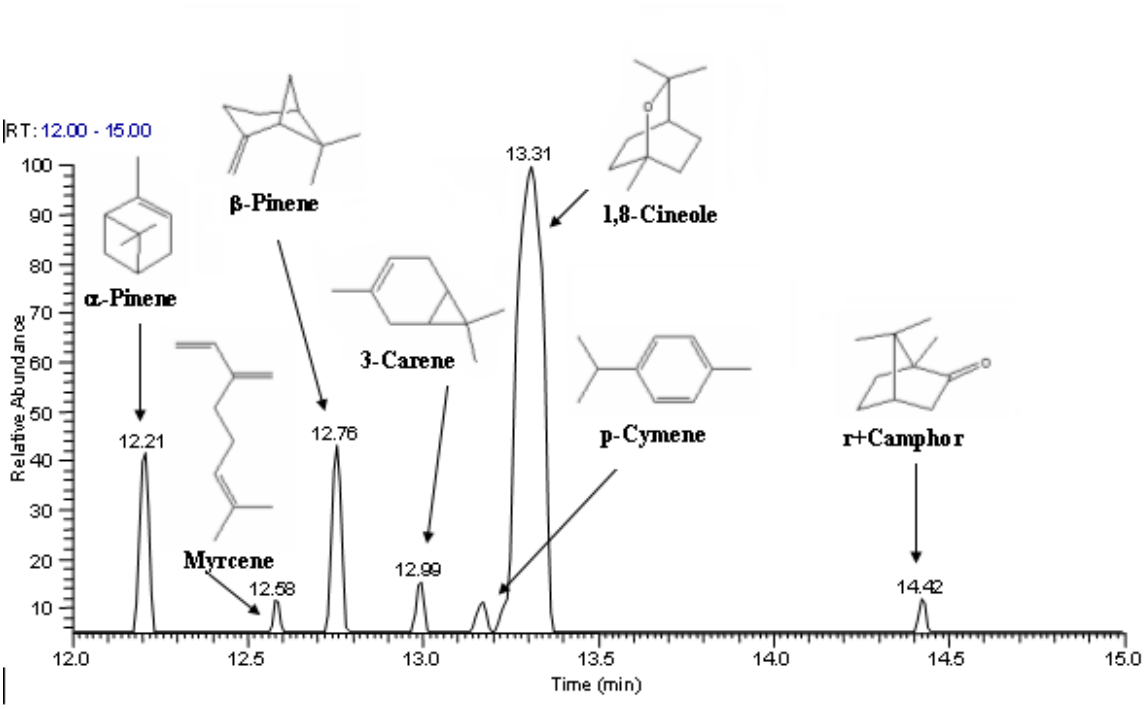

Figure 6: *Salvia apiana* in hexane with a 5% baseline threshold

RT	Constituent	Identification	Percent
11.59	tricyclene	Ref, Lib	2.59
11.68	2-acetylfuran	Ref, Lib	0.31
12.21	α-pinene	GC/MS, GC/FID, Ref, Lib	1.47
12.28	C_8H_{16}	Ref	0.15
12.48	camphene	Ref, Lib	16.88
12.6	sabinene	Ref, Lib	1.10
12.76	β-pinene	Ref, Lib	1.03
13.12	para-cymene	GC/MS, GC/FID, Ref, Lib	2.06
13.28	1,8 cineole	GC/MS, GC/FID, Ref, Lib	13.23
13.9	thujone	GC/MS, GC/FID, Ref	6.36
13.99	ocimene	Ref	2.46
14.46	camphor	GC/MS, GC/FID, Ref, Lib	28.63
14.62	borneol	Ref, Lib	4.97
14.9	geranyl acetate	Ref	0.53
15.32	borneol acetate	Ref, Lib	0.77
15.96	para-cymene acetate	Ref, Lib	0.21
16.11	α-copaene	Ref, Lib	0.54
16.49	β-caryophellene	Ref, Lib	0.21
16.78	β-gurjunene	Ref, Lib	0.28
17.02	δ-cadiene	Ref, Lib	0.52

GC/FID = comparison with the GC/FID of standard
GC/MS = comparison with the GC/MS of standard
Ref = comparison with GC and MS literature values
Lib=Computer Library Identification

Table 3: Chemical constituents in the essential oil of *Artemisia tridentata.*

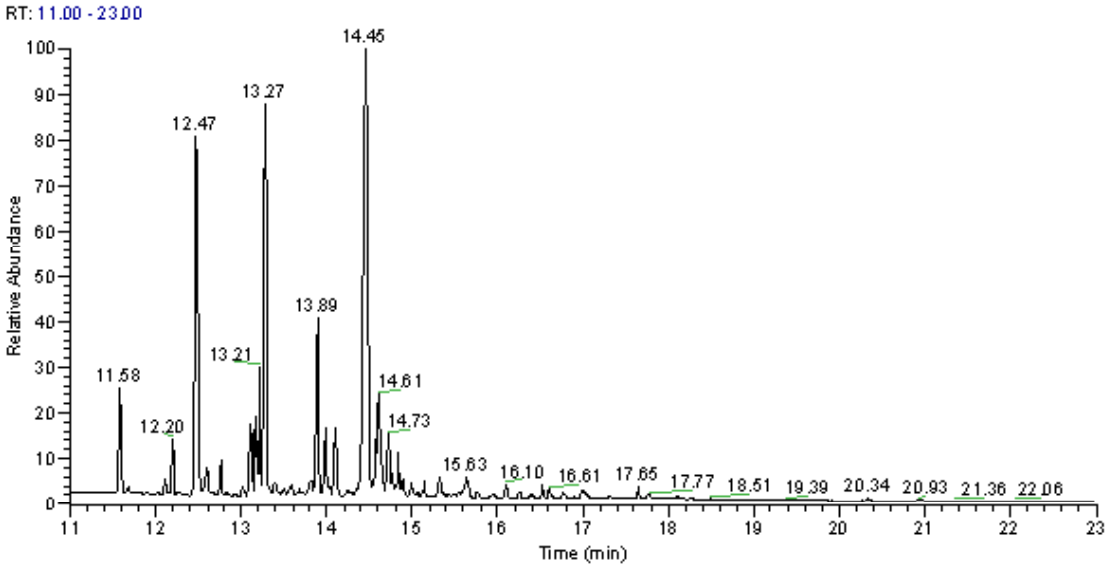

Figure 7: Total ion scan of *Artemisia tridentata* in hexane.

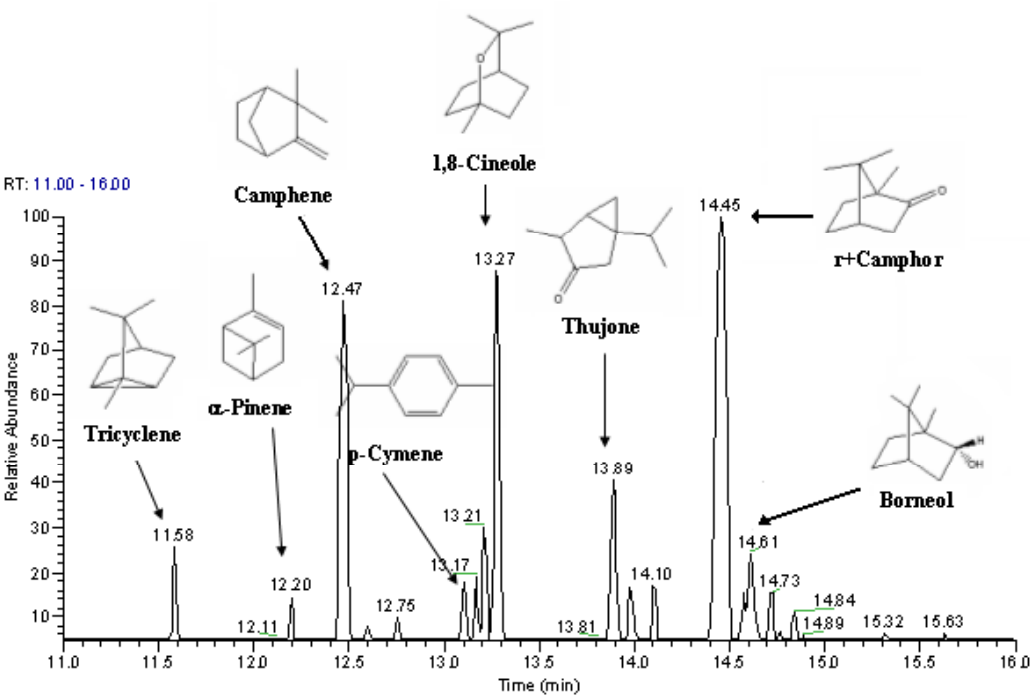

Figure 8: *Artemisia tridentata* in hexane with a 5% baseline threshold

Distribution

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