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Full Length Research Paper

Chemical composition and antinociceptive activity of California sagebrush (*Artemisia californica)*

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***Artemisia californica*, California sagebrush, has been reported to have pain relieving activity and is a traditional medicine of the Chumash Indians of California. Pain relieving activity of a traditional sagebrush preparation was examined in patients suffering from arthritis and other pain. The preparation was examined by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography-mass spectrometry (HPLC-MS) to identify the compounds present. A traditional tincture of sagebrush was produced and used on 42 patients with moderate to severe pain. All patients reported pain relief within 10 to 20 min. Sagebrush was examined by GC-MS and HPLC-MS and was found to contain monoterpenoids, lipids, flavonoids and sesquiterpenes. The major monoterpenoid found is eucalyptol. Of the monoterpenoids, camphor and eucalyptol have reported pain relieving activity. They interact with transient receptor potential cation channel vanilloid 3 (TRPV3), transient receptor potential ankyrin-repeat 1 (TRPA1) and transient receptor potential melastatin 8 (TRPM8) receptors to produce pain relief that lasts for several hours.**

Key words: California sagebrush, *Artemisia californica*, Asteraceae, pain relief, anti-inflammatory, arthritis, transient receptor potential cation channels, transient receptor potential melastatin 8 (TRPM8), transient receptor potential ankyrin-repeat 1 (TRPA1), transient receptor potential cation channel vanilloid 3 (TRPV3).

INTRODUCTION

Artemisia californica Less. also known as California sagebrush (*khapshikh* in Chumash), is a species of the genus *Artemisia*, and belongs to the Asteraceae family. It grows in chaparral in the foothills near the coast from San Francisco to Baja California. *Artemisia* plants are very important medicinal plants throughout the world. The Costanoan Indians of California use the leaves for tooth aches and to poultice wounds (Garcia and Adams, 2012). The Chumash Indians use a decoction of the leaves and stems externally for colds, asthma and arthritis (Garcia and Adams, 2012). In fact, a tincture of *A. californica* has been recommended for use by arthritic patients. Several patients suffering from moderate or severe pain were treated with a sagebrush tincture; case reports were presented.

The chemistry of *A. californica* has not been described previously, except for a report of finding a sesquiterpene named artecalin (Geissman et al., 1969) and a recent review article written to complement the present work

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Table 1. Characteristics of the six different fractions collected from the *A. californica* extract.

Fraction number	Color	Volume (ml)
1	Translucent	10
2	Dark green	15
3	Light green	15
4	Yellow	50
5	Brown-yellow band	5
6	Light yellow	10

(Adams, 2012). The goal of the present work was to find out if antinociceptive compounds are present in *A. californica* that may help explain the use of the tincture for pain control. Tinctures of the plant were examined by gas chromatography-mass spectrometry (GC-MS), high performance liquid chromatography-mass spectrometry (HPLC-MS) and other techniques to characterize the compounds present.

MATERIALS AND METHODS

General experimental procedures

Isolated compounds were characterized by spectroscopic methods. Nuclear magnetic resonance (NMR) spectra (^{1}H and ^{13}C NMR) were recorded at room temperature on a Varian Mercury Plus instrument at 400 MHz. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS). GC-MS employed a Thermo-Fisher FOCUS-DSQ II gas chromatograph with a mass-selective detector. The column temperature was: 40°C for 10 min, which increased 2°C per min to a final temperature of 250°C for 5 min. HPLC-MS analysis involved a Thermo Finnigan LCQ DECA with a reverse phase column. The solvent system consisted of 10% MeOH in water that increased at 2% per min to 100% MeOH. Column chromatography was performed with Silicagel 60 columns (EBM, Germany) that were developed with the following solvents, AcOEt-Ether (90:10), AcOEt-MeOH (50:50) and pure MeOH and it enabled the fractionation of the *A. californica* extract into six different fractions. The six fractions collected had the characteristics presented in Table 1. Thin layer chromatography (TLC) was performed with Silicagel 60 plates (EBM, Germany). The mobile phase consisted of AcOEt-Ether (90:10). The plant extract gave the following spots on TLC plates, Rf = 0, 0.06, 0.68, 0.83, and 0.95.

Plants

A. californica is a perennial shrub that grows to 1.5 to 2.5 m high and branches from the base. The branches are about 1 m long, flexible and canescent. The leaves are thread like, light green, about 5 cm long and may be 2 to 4 pinnately lobed. Flower heads are less than 5 mm wide, yellow or white, contain 6 to 10 pistillate flowers and 15 to 30 disk flowers. The branches of *A. californica* used in this study were collected near Pasadena, California. White sage (*Salvia apiana*) leaves were also collected. All plant materials were collected in early May, 2010. Voucher specimens of *A. californica* are available at the Rancho Santa Ana Botanic Garden, Claremont.

Extraction and tincture preparation

The alcoholic sagebrush tincture was prepared following the methods of traditional Chumash healers (Garcia and Adams, 2012). Eighty-six grams of *A. californica* branches, one leaf of *S. apiana*, one avocado seed (*Persea americana*) and 500 ml of 70% isopropanol were introduced into an amber-colored glass bottle. A total of 10 L of tincture were produced. This tincture was used to treat patients.

A sagebrush extract, for chemical analysis, was prepared with 290 ml of 99.8% isopropanol as well as 29.0 g of *A. californica* leaves and stems. This *A. californica* extract was used to identify, characterize and isolate the compounds present.

Patient treatment

Patients were recruited from a senior citizen center and from the community. Each patient reported pain of moderate to extreme intensity using an Osteoarthritis Research Society (OARSI) pain scale (Hawker et al., 2008) modified for sites in addition to the hips and knees. Patient data is presented in the results subsequently. Each patient was allowed to apply sagebrush tincture topically to the painful site with a cotton ball. Patients were then questioned at 10 min intervals and were asked to rate their pain as nonexistent, mild, moderate, severe or extreme. Results were recorded at the time of each interview.

RESULTS

Phytochemical characterizations by GC-MS

In total, 19 compounds were found by GC-MS and were characterized with a GC-MS database. Compounds found by GC-MS data were identified by comparison of mass total ion count (TIC) fragmentation spectra with authentic samples reported in the NIST/EPA/NIH Mass Spectral Library (Version 2.0 d) build 26 April, 2005 using the NIST Mass Spectral Search Program (Stein et al., 2005). Out of the 19 identified, 15 turned out to be known monoterpenes-camphene, menthadiene, β-pinene, eucalyptol, isopropenylmethylcyclohexanol, trimethylheptadienol, isopropylmethylbicyclohexanol (also called 4-methyl-1-propan-2-yl-bicyclo[3.1.0]hexan-5-ol), 3-thujanone (also called α-thujone), β-thujone, chrysanthenone, camphor, borneol, carene, menthenol and menthadienol (Figure 1). The remaining 4 compounds were characterized as known lipids and diterpenoids, retinol acetate, dimethylmethylene cyclohexenyl diene methylbutylester acetic acid, methylhexadecanol and tetratetracontane. The retention times (RTs), characteristic fragments and base peaks of each of these 19 chemical compounds are presented in Table 2. Four of them, all monoterpenoids, appeared to be the main compounds present in the *A. californica* tincture since they corresponded to the peaks with the highest relative abundances (> 8% of the total), eucalyptol (24%), camphor (18%), carene (14%), and menthadienol (9%).

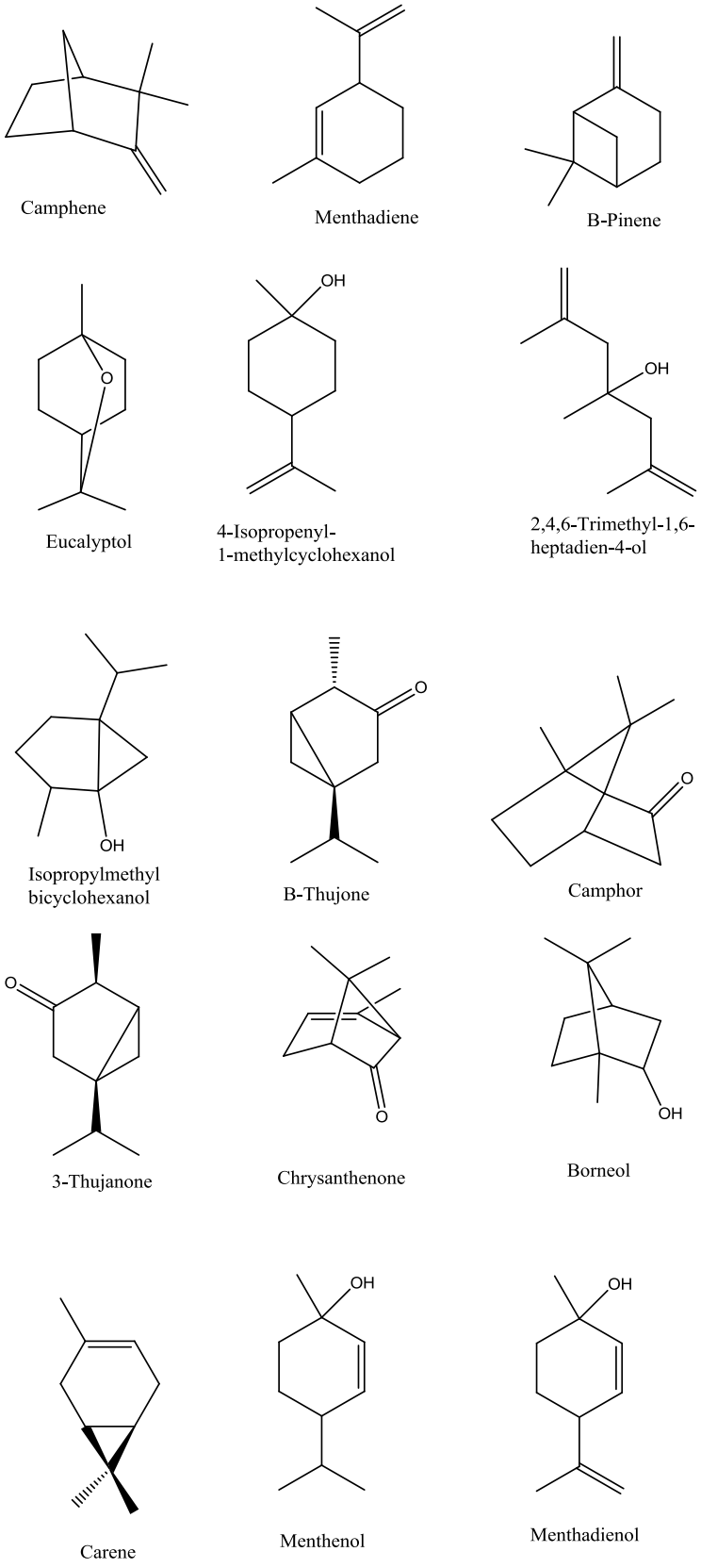

Figure 1. Monoterpenoids found in *A. californica*.

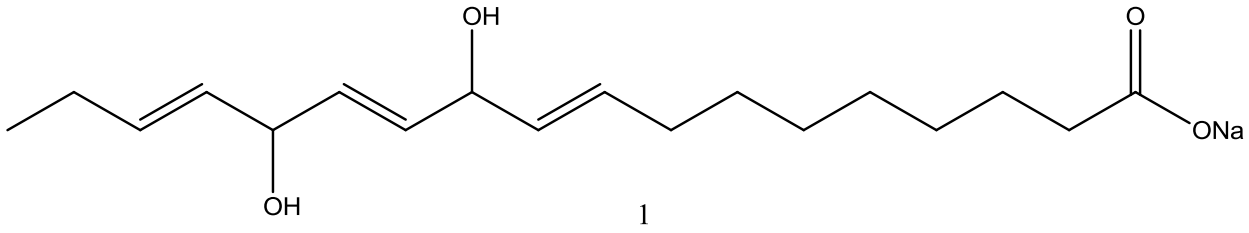

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Figure 2. Dihydroxylinolenic acid found in *A. californica*.

Phytochemical characterizations by low resolution HPLC-MS

The first fraction from silica gel column chromatography was found to contain a fatty acid that appeared to be dihydroxylinolenic acid (1) (Figure 2). Purity was demonstrated by HPLC-MS as subsequently shown. The ^{1}H NMR signals of the compound were observed at δ 1.5 to 1.2 as a multiplet characteristic of alkyl protons, 2.1 for the proton on C17, 2.2 for the proton on C2, 3.6 for the proton on C14, 3.9 for the proton on C11, 5.0 to 5.6 as a multiplet characteristic of protons for C9, OH, C10, C12, C13, C15 and C16. Positive ion HPLC-MS showed an ion of *m/z* value of 610.3 (base peak) that corresponds to a dimer of dihydroxylinolenic acid (1). Other column fractions were impure and were not analyzed by NMR.

HPLC-MS enabled the identification of several sesquiterpenes and flavonoids, all of which are known chemical compounds often found in the *Artemisia* genus. Some compounds were retrieved in both the positive and negative ion modes whereas some others were either found in the negative or the positive ion mode. For instance, quercetin hexose (Yin et al., 2008) was found in both modes (RT = 18.01 min) and they demonstrated the characteristic fragment of quercetin itself (MW = 302). In the positive ion mode, there were also the two following fragments, 655.35 which corresponds to the addition of a molecule of ketohexose ($C_7H_9O_6$, MW = 190) (Tan et al., 2008) to quercetin hexose and, 433.67 that is the loss of CH_4O from quercetin hexose. However, in the negative ion mode there was only one additional fragment, 615.25, characteristic of the addition of a pentose (MW = 150) onto quercetin hexose.

A few other compounds were detected in both positive and negative ion modes tamarixetin glycoside (Avula et al., 2009), jaceosidin (Kazuno et al., 2005; Yin et al., 2008), 6-methoxytricin (ACD/Labs Mass Spectrometry Database, National Institute of Standards mass spectrometry database), and chrysosplenetin (Wollenweber et al., 1991). Tamarixetin glycoside showed several different fragments in the two modes. In the positive ion mode (RT = 21.35 min), a 259.05 ion that could be attributed to the loss of $C_4H_8O_2$ (MW = 88) was found (ACD/Labs Mass Spectrometry Database). Isomers of tamarixetin glycoside were however found twice in the negative ion mode at two successive RTs of 20.97 and 21.35 min. Many higher molecular weight fragments than in the positive mode were found in the isomer at RT = 20.97 min in the negative ion mode such as 1332.62, which could be called tamarixetin quadra hexose, dipentose (MW = 1336); 647.62 that could be tamarixetin hexose, pentose diglycoside followed by 579.42 and 445.67 ions that are probably tamarixetin diglycoside fragments.

Jaceosidin (RT = 23.38 min) had a fragment at 286.37, which is the loss of $C_3H_5O_2$ in negative ion mode. However, a fragment at 168.21 for the neutral loss of a flavone glycoside fragment (MW = 162, Kazuno et al., 2005) was found in positive ion mode. 6-Methoxytricin was identified in the two modes. In positive ion mode, 331.46 was found, which is the result of the loss of two methyls ($2\times CH_3 = 30$) and, 229.23 that corresponds to the loss of $C_5H_9O_2$ (ACD/Labs Mass Spectrometry Database, MW = 101).

Chrysosplenetin in the negative ion mode, led to a 343.65 ion, which is a characteristic of the loss of a molecule of CH_2O. In positive ion mode, a molecule of H_2O was lost to produce a 299.55 ion. Several chemicals were detected only in negative ion mode. Isoorientin (RT = 15.10 min) and tanapartholide A (RT = 15.70 min) (Wen et al, 2010) that both led to respective ions at 293.29 and 243.13 by loss of H_2O_2. Secogorgonolide (Ortet et al., 2008) at a RT of 15.99 min, gave an ion at 192.98, which is equivalent to loss of C_5H_9O (ACD/Labs Mass Spectrometry Database). Finally, methoxyflavone hexose (MW = 462) was found at a RT of 22.47 min. Several ions were found including 1120.24 methoxy-flavone trihexose, dipentose; 1064.53 methoxyflavone hexose, quadra pentose; 1049.18 methoxyflavone glycoside fragment; 763.87 methoxy-flavone hexose, dipentose; 749.36 which may be a multiple pentose fragment; and 299.36, which is the loss of a flavone glycoside fragment (MW = 162) (Kazuno et al., 2005).

Finally, a compound that could be luteolin gentiobioside or quercetin glucoside rhamnoside was eluted at RT = 6.73 min in the positive ion mode (Sakushima et al., 1988; Kazuno et al., 2005). Usaramine was found at RT = 14.93 min (ACD/Labs Mass Spectrometry Database). Leucodin was only retrieved in the positive mode and had

Table 2. Retention times, characteristic ions and base peaks of the nineteen *A. californica* monoterpenes and lipids found by GC/MS.

Retention Time (min)	Fragment 1 (Relative Abundance)	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Base Peak	Compound
7.01	93.05 (100)	28.03 (63)	-	-	-	93.05	Camphene
8.21	93.05 (100)	28.04 (83)	-	-	-	93.05	Menthadiene
9.5	93.03 (92)	28.03 (100)	-	-	-	28.03	β- Pinene
12.82	81.00 (52)	71.00 (52)	43.04 (100)	-	-	43.04	Unknown
12.96	43.02 (100)	-	-	-	-	43.02	Eucalyptol
15.41	82.99 (100)	-	-	-	-	82.99	Isopropenylmethylcyclohexanol
16.87	85.02 (100)	-	-	-	-	85.02	Trimethylheptadienol
17.46	93.06 (100)	70.99 (72)	43.07 (82)	28.03 (56)	-	93.06	Isopropylmethylbicyclohexanol
17.71	110.10 (82)	95.05 (76)	81.06 (100)	69.06 (74)	67.04 (92)	81.06	Thujanone
18.48	110.10 (92)	95.02 (100)	81.05 (98)	69.08 (78)	67.05 (90)	95.02	Thujone
19.08	107.05 (100)	91.00 (78)	79.05 (52)	-	-	107.05	Chrysanthenone
20.24	95.02 (100)	81.06 (56)	41.06 (52)	-	-	95.02	Camphor
21.74	95.06 (100)			-	-	95.06	Borneol
22.97	93.05 (92)	91.02 (74)	79.05 (100)	77.02 (58)	42.99 (94)	79.05	Carene
23.84	136.12 (64)	121.07 (56)	93.06 (100)	59.01 (98)	43.05 (60)	93.06	Menthenol
30.27	119.04 (69)	43.04 (100)		-	-	43.04	Menthadienol
77.04	-	-		-	-	-	Retinol, acetate
86.91	173.09 (72)	43.05 (100)	28.03 (56)	-	-	43.05	Dimethylmethylene cyclohexenyl diene methylbutylester acetic acid
96.53	97.10 (78)	83.08 (55)	69.07 (68)	57.03 (76)	55.05 (54)	43.09	Methylhexadecanol
103.34	71.07 (90)	57.05 (100)	43.09 (71)	-	-	57.05	Tetratetracontane

two characteristic fragments, a 243.30 ion that can be named dehydroleucodine (MW = 244) and 215.51 that is the result of loss of O_2 (Glasl et al., 2002; Ando et al., 1994). Near the RT of leucodin, pestalodiopsolide A was found to loose H_2O_2 to give a fragment at 261.15 (Magnani et al., 2003; Huang et al, 2009). A few minutes later, echinolactone B eluted and was characterized by its fragment at 243.39 that results from the loss of H_2O (Suzuki et al., 2005). Marmin (ACD/Labs Mass Spectrometry Database), chromonar (ACD/Labs Mass Spectrometry Database), and xanthohumol disaccharide were identified through the positive ion mode and had only molecular ions without other fragments. Apigeninidin glucoside (Swinny et al., 2000) was found and had a fragment that came from the loss of either C_5H_{11} or $C_3H_3O_2$ (MW = 71). Rupestine (MW = 245) (Su et al., 2008) had a characteristic ion at 227.51 that can be attributed to the loss of a molecule of H_2O.

The characteristic ions, base peaks and RTs of all the compounds retrieved in *A. californica* are presented in Tables 3 and 4. Ultraviolet (UV) spectral characteristic peaks for each compound are reported in Table 5.

Out of all the known flavonoids, sesquiterpenes and alkaloids found in *A. californica* tincture, four of the flavonoids, appeared to be the primary non-monoterpenoid compounds present in the extract. These compounds correspond to the peaks with the highest relative abundances on the total ion current, jaceosidin, 6-methoxytricin, chrysosplenetin, and quercetin hexose.

CASE REPORTS

Knee pain was reported by several patients. A 57 year old Oriental woman with a broken left patella reported extreme pain. Her pain was not diminished by 2 tablets of hydrocodone (7.5 mg)/acetaminophen (750 mg). She reported her pain as moderate within 20 min of one topical application of *A. californica* tincture. She also noted that the swelling of her knee diminished somewhat. She applied another topical application 3 h later, when the pain returned. By 10 min, she reported that her pain was gone. The next day, she began to use naproxen as needed. Other knee pain patients included, a 72 year old Caucasian woman with arthritis in the right knee that caused moderate pain and a slight limp. Within 10 min of topical application of the tincture, she reported that her pain was mild. A 60 year old Caucasian woman had

Table 3. Retention times, characteristic ions and base peaks of the *A. californica* compounds found by low resolution HPLC-MS (+ ion mode) in the different fractions.

Retention time (min)	Fragment 1 (relative abundance)	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Fragment 7	Fragment 8	Base peak
6.73	610.09 (100) Luteolin gentiobioside, Quercetin glucoside rhamnoside (MW=610)	536.15 (60)	520.07 (45)	503.29 (20)	403.21 (15)	355.11 (25)	299.17 (55)	194.18 (45)	610.09
14.93	351.32 Usaramine	346.2	261.33	243.37	215.4	-	-	-	261.33
15.75	565.24 (30)	296.27 (85)	247.32 (100) Leucodin (MW= 246)	243.30 (40) Dehydroleucodine (MW= 244)	215.51 (12) M-O2 (MW= 32)	183.47 (6)	-	-	247.32
15.96	296.15 (100) Pestalodio-psolide A (MW= 295)	261.15 (66) M-H2O2	243.25 (30)	215.37 (13)	199.22 (5)	-	-	-	296.15
18.01	655.35 (100) M+190 Ketohexose (C7H9O6)	608.19 (25)	523.19 (30)	499.35 (45)	465.35 (89) Quercetin hexose (MW= 465)	433.67 (39) M-CH4O	325.27 (20)	303.58 (25) Quercetin (MW= 302)	528.69
18.65	261.42 (100) Echinolactone B (MW=260)	243.39 (8) M-H2O	169.10 (4)	-	-	-	-	-	261.42
20.58	423.49 (90)	418.40 (100) Apigeninidin glucoside (MW= 417)	347.47 (25) M-C5H11 M-C3H3O2 (MW= 71)	277.25 (40)	261.32 (66)	243.45 (38)	237.08 (23)	-	418.4
21.35	400.30 (70)	347.44 (100)	317.44 (20) Tamarixetin (glycoside) (MW= 316)	259.05 (10) M-C4H8O2 (MW= 88)	177.33 (10)	-	-	-	347.44
22.65	1314.16	597.97	332.3 Marmin	302.24	243.39	167.05	-	-	332.3
22.82	362 (20)	331.51 (20)	303.27 (25)	245.40 (100) Rupestine (MW= 245)	227.51 (2) M-H2O	-	-	-	245.4
23.38	361.44	331.48 (100) Jaceosidin (MW= 330)	316.49 (20)	301.68 (7)	229.24	168.21 (1) M-162	-	-	331.48
23.71	361.40 6-methoxy-tricin (MW= 360)	331.46 (15) M-30 (2× CH3)	303.42	229.23 (1) 331-C5H9O2 (MW= 101)	-	-	-	-	361.4
23.73	361.74 Chromonar	303.52	257.34	169.19	-	-	-	-	361.74

Table 3. Contd.

25.69	375.45 (100) Chrysosplenetin (MW= 374)	317.48 (10)	299.55 (2) M-H2O	274.34 (2)	201.08 (1)	194.38 (1)	-	-	375.45
40.77	1369.86 (9)	998.29 (30)	759.33 (25)	685.35 (100) Xanthohumol disaccharide (MW= 685)	355.26 (20)	299.17 (22)	-	-	685.35

arthritis of the left knee that made sitting or straightening her leg extremely painful. Within 10 min of topical application of the tincture, she reported that her pain was mild. A 63 year old Indian man had a swollen, painful knee from rheumatoid arthritis. He applied the liniment and reported that his pain went from 7 to 3 within 20 min. He continued to use the liniment for 3 weeks with continued success and no adverse reactions. A 27 year old Caucasian woman presented with moderate tendon pain due to having the left leg longer than the right. She applied the tincture and within 10 min reported nonexistent pain and could run 6 miles. A 55 year old Caucasian man complained of severe pain and fatigue after running a marathon. Within 10 min of topical application of the tincture, he reported nonexistent pain. A 73 year old Caucasian woman had a knee replacement that failed and was done again. She started to use *A. californica* tincture every morning and reported pain relief within 15 min that lasted for about 3 h. Her pain was severe before applying the tincture and became mild after the tincture. She used the tincture daily for 2 months during her recovery. Hip pain was found in one patient. A 55 year old Caucasian man presented with severe tail bone pain due to a fall. The pain made sitting and sleeping very difficult. Within 10 min of topical application of the tincture, he reported mild pain and could sit and sleep normally.

Hand pain was very common. In a crafts workshop in a retirement community, the 26 students, Caucasian and Latino, were having trouble completing the crafts due to severe to moderate arthritis pain in their hands. Both men and women participated and were aged between 70 and 80. All used the tincture topically and reported nonexistent or mild pain within 10 min. The treatment allowed them to work on their projects. A 72 year old Latino man presented with moderate arthritis pain in his hands and shoulders that prevented him from performing his job. Within 10 min of topical application of the tincture, he reported nonexistent pain and could perform his job.

Muscle pain was a common complaint. A 45 year old Caucasian man reported severe pain in his right hand from manual labor. Within 10 min of application of the tincture, he reported his pain was gone and his hand motion was no longer limited. A 67 year old Caucasian woman had severe neck pain from lifting a heavy object. She reported that her pain was gone within 10 min of application of the tincture. Three patients, 45 to 63 years old, complained of severe low back pain from lifting heavy objects. They all reported their pain was gone within 10 min of application of the tincture. A 56 year old Caucasian man reported twisting his ankle while running. He was in severe pain and walked with a cane. Ten minutes after applying the tincture, the pain had decreased to moderate. Another application of the tincture decreased the pain to mild within 10 minutes. The patient was able to sleep normally. A third application of the tincture the next morning allowed the man to walk and climb stairs without a cane and without significant pain.

A 78 year old woman with hepatic cancer was treated with high dose morphine that did not control her pain. She said her pain was extreme. The sagebrush tincture was applied to her chest with a cloth. Within 10 min, she reported her pain was moderate.

DISCUSSION

The genus *Artemisia* with between 200 to 400 species belonging to the family Asteraceae, is very important medicinally and is used throughout the world. Various species have previously been described as possessing antimalarial, antifungal, anti-inflammatory, antibacterial and antiviral agents (Garcia and Adams, 2009). These activities have been attributed to flavonoids (Giangaspero et al., 2009; Yin et al., 2008),

Table 4. Retention times, characteristic ions and base peaks of the *A. californica* compounds found by low resolution HPLC-MS (- ion mode) in the different fractions.

Retention time (min)	Fragment 1	Fragment 2	Fragment 3	Fragment 4	Fragment 5	Fragment 6	Fragment 7	Base peak
6.02	1445.81 Unknown	-	-	-	-	-	-	1445.81
15.1	327.25 (100) Isoorientin (MW= 327)	293.29 (35) M-H2O2	277.27 (15)	265.41 (5)	180.41 (2)	-	-	327.25
15.7	277.42 (100) Tanapartholide A (MW=278)	243.13 (6) M-H2O2	175.10 (4)	-	-	-	-	277.42
15.99	401.15 (33)	355.15 (10)	277.12 (100) Seco-orgonolide (MW= 278)	192.98 (5) M- C5H9O	107.94 (5)	-	-	277.12
18.01	927.08 (20)	615.25 (17) M+152 (pentose)	463.31 (100) Quercetin hexose (MW= 464)	301.31 (11) Quercetin (MW= 302)	285.33 (1)	179.07 (1)	-	463.31
20.97	1332.62 Tamarixetin quadrahexose, dipentose (MW= 1336)	647.62 Tamarixetin hexose, pentose diglycoside	579.42 Tamarixetin diglycoside fragment (578)	445.67 Tamarixetin diglycoside fragment	315.37 (100) Tamarixetin glycoside (MW= 316)	277.37 (65)	-	315.37
21.35	345.40 (35)	315.24 (100) Tamarixetin (glycoside) (MW= 316)	299.43 (16)	285.44 (10)	247.63 (5)	160.40 (3)	-	315.24
22.47	1120.24 (35) Methoxyflavone trihexose, dipentose	1064.53 (59) 763+300 Methoxyflavone hexose quadrapentose	1049.18 (100)	763.87 (64) Methoxyflavone hexose dipentose	749.36 (100)	461.49 (69) Methoxyflavone hexose (MW= 462)	299.36 (23) M-162 Flavone glycoside fragment	1049.18
23.38	359.31 (75)	329.31 (100) Jaceosidin (MW= 330)	314.34 (15)	286.37 (4) M-C3H5O2	263.37	-	-	329.35
23.71	359.42 (100) 6-methoxy-tricin (MW= 360)	329.37	299.34 (15)	286.06 (8)	263.37	202.34 (2)	-	359.42
25.69	373.50 (100) Chrysosplenetin (MW= 374)	343.65 (22) M-CH2O	329.44 (11)	300.49 (8)	257.39 (5)	213.38 (2)	-	373.50

Table 5. UV characteristic peaks of the *A. californica* compounds found by Low Resolution HPLC-MS in the different fractions.

Retention time (min)	Peak 1 (nm)	Peak 2 (nm)	Peak 3 (nm)	Peak 4 (nm)	Peak 5 (nm)	Peak 6 (nm)	Maximum (nm)	Compound
2.01	220	260	286	-	-	-	220	Unknown
6.02	203	-	-	-	-	-	203	Unknown
6.73	200	-	-	-	-	-	200	Luteolin gentiobioside, Quercetin glucoside rhamnoside
14.87	220	-	-	-	-	-	220	Usaramine
14.98	219	-	-	-	-	-	219	Isoorientin
15.67	218	-	-	-	-	-	218	Tanapartholide A
15.69	217	265	-	-	-	-	217	Leucodin
15.75	217	265	378	-	-	-	217	Secogorgonolide
15.78	217	-	-	-	-	-	217	Pestalodiopsolide A
18	216	252	270	313	364	-	216	Quercetin hexose
18.46	217	256	-	-		-	217	Echinolactone B
22.47	205	221	271	294	357	379	221	Methoxyflavone hexose
22.65	205	-	-	-	-	-	205	Marmin
21.09	218	270	330	-	-	-	218	Apigeninidin glucoside
21.2	213	304	335	-	-	-	213	Tamarixetin glycoside
22.69	218	253	341	-	-	-	218	Rupestine
23.21	219	253	272	345	-	-	219	Jaceosidin
23.58	221	257	268	350	-	-	221	Chromonar
23.6	218	255	269	351	-	-	218	6-methoxy-tricin
25.5	217	256	270	350	-	-	217	Chrysosplenetin
27.53	223	-	-	-	-	-	223	unknown
28.31	223	-	-	-	-	-	223	Unknown
40.96	225	274	-	-	-	-	225	Xanthohumol disaccharide

monoterpenes (Reddy et al., 2006) or sesquiterpenes (Ishida, 2005).

A. californica tincture was found to contain monoterpenoids, lipids, flavonoids, sesquiterpenes and alkaloids. A tincture of the plant has been used by Chumash people as a powerful, topical pain reliever and anti-inflammatory agent, especially as a long term medicine to treat arthritis and other chronic pain problems. Patients (42) suffering from moderate to extreme pain were treated with *A. californica* tincture. All reported pain relief within 10 min. The pain relief lasted several hours and was associated with an anti-inflammatory effect in some cases. Fifteen monoterpenes were discovered in *A. californica*: camphene, menthadiene, β- pinene, eucalyptol, isopropenylmethylcyclohexanol, trimethylheptadienol, isopropylmethylbicyclohexanol, thujanone, thujone, chrysanthenone, camphor, borneol, carene, menthenol, and menthadienol (Figure 1). Along with their antinociceptive use, monoterpenoids are also anxiolytic, anthelmintic, antibiotic and anti-inflammatory (Ishida, 2005).

Monoterpenoids with known pain relieving activity in *A. californica* include camphor (Xu et al., 2005; Martinez et al., 2009a and b), eucalyptol (Liapi et al., 2007; Martinez et al., 2009a and b), camphene (Martinez et al., 2009a and b), β-pinene (Liapi et al., 2007; Martinez et al., 2009a and b), borneol (Martinez et al., 2009a and b; Granger et al., 2005) and thujone (Hold et al., 2000). Many of them penetrate the skin including β-pinene (Schmitt et al., 2009), and are topically active.

Monoterpenes express antinociceptive activity by binding to transient receptor potential cation channel vanilloid (TRPV1), TRPV3 and TRP melastatin 8 (TRPM8) receptors. TRPV1 and 3 are critically involved in nociception and thermosensing (Vriens et al., 2009). They are expressed in sensory neurons (Caterina et al. 1997) in the skin, keratinocytes and other organs, and in pain pathways including the dorsal root ganglia, trigeminal neurons, and spinal cord (Vriens et al., 2009). TRPM8 is expressed in the majority of cold-sensitive afferents of the skin and other organs (Basbaum et al., 2009) and therefore responds to cold. Monoterpenoids

activate these TRP channels, causing momentary pain, then deactivate the TRP channels, causing long term pain relief. Most of the pain relieving monoterpenoids found in *A. californica* are agonists for TRPV3 (heat-sensitive) including camphor (Xu et al., 2005; Vriens et al., 2009; Vogt-Eisele et al., 2007), borneol, thujone and eucalyptol (Vogt-Eisele et al., 2007). Camphor also blocks TRP ankyrin-repeat 1 (TRPA1, cold-sensitive) receptor and activates the TRPV1 (heat-sensitive) receptor (Xu et al., 2005). Eucalyptol has been reported to also be a TRPM8 (cold-sensitive) receptor agonist (Basbaum et al., 2009) and to exhibit an antinociceptive activity comparable to that of morphine. A synergism exists between morphine and eucalyptol that produces much greater than expected pain relief (Liapi et al., 2007). Anti-inflammatory properties have been reported for some monoterpenoids including camphene, β-pinene (Ishida, 2005; Lin et al., 2008) as well as for some sesquiterpenoids (Ishida, 2005). The monoterpene, borneol has been shown to present high anti-inflamma- tory activity (Tung et al., 2008), which results from the inhibition of nitric oxide (NO) and prostaglandin E2 (PGE2) production as well as an increase in the expression of inhibitor of NF-κB kinase (IKK), inducible nitric oxide synthase (iNOS), nuclear factor κB (NF-κB), and a decrease in inhibitor of NF-κBα (IκBα) expression in dose-dependent manners (Lin et al., 2008; Tung et al., 2008).

Oral toxicity of monoterpenoids includes seizures reported for camphor (Farhat et al., 2001; Manoguerra et al., 2006), thujone (Farhat et al., 2001; Hall et al., 2004) and camphene (Farhat et al., 2001). However, anti-convulsant properties have been proved for oral β-pinene, eucalyptol (Sayyah et al., 2002) and borneol (Granger et al., 2005). Topical administration of monoterpenoids, in essential oils can cause skin irritation. However, skin penetration of monoterpenoids in quantities sufficient to cause convulsions and other toxicities has not been reported, except in infants.

Several flavonoids are known to be anti-inflammatory and analgesic. For instance, 6-methoxytricin is anti-inflammatory due to its inhibitory activity on the proliferation and activation of T cells (Yin et al., 2008). Quercetin and quercetin glycoside are anti-inflammatory through suppression of synthesis of TNF-α and NO (Sheu et al., 2009) and analgesic through serotonin 5-HT1A receptor activation (Martinez et al., 2009a and b). Jaceosidin is anti-inflammatory and can penetrate the skin to relieve inflammation through an NFkB induction inhibition mechanism (Clavin et al., 2007). These flavonoids undoubtedly add to the analgesic and anti-inflammatory effects of *A. californica* tincture.

Conclusions

A. californica tincture could be a useful pain reliever since it does not interact with cyclooxygenase (COX) like the non-steroidal Anti-inflammatory Drugs (NSAIDs) and therefore should lack their toxicity such as ulcers (Lin et al., 2008). Eucalyptol antinociceptive activity is comparable to that of morphine, except that morphine can cause constipation, respiratory depression, seizures and coma from stimulation of opioid receptors. Monoterpenoids are not known to interact with opioid receptors. This illustrates the great potential of *A. californica* as an antinociceptive and anti-inflammatory medicine for moderate to extreme pain.

REFERENCES

Adams JD (2012). The use of California sagebrush (Artemisia californica) liniment to control pain. Pharmaceuticals 5:1045-1053.

Ando M, Ibayashi K, Minami N, Nakamura T, Isogai K (1994). Studies of the synthesis of sesquiterpenes lactones, 16. The syntheses of 11β, 13-dihydrokauniolide, estafiatin, isodehydrocostuslactone, 2-oxodesoxyligustrin, arborescin, 1, 10-epiarborescin, 11β, 13 dihydroludartin, 8-deoxy-11β, 13-dihydrorupicolin B, 8-deoxyrupicolin B, 3, 4-epiludartin, ludartin, kauniolide, dehydroleucodin, and leucodin. J. Nat. Prod. 57:433-445.

Avula B, Wang YH, Smillie TJ, Mabusela W, Vincent L, Weitz L, Khan IA (2009). Quantitative determination of flavonoids by column high-performance liquid chromatography with mass spectrometry and ultraviolet absorption detection in *Artemisia afra* and comparative studies with various species of Artemisia plants. J. AOAC Int. 92:633-644.

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009). Cellular and molecular mechanisms of pain. Cell 139:267-284.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816-824.

Clavin M, Gorzalczany S, Macho A, Munoz E, Ferraro G, Acevedo C, Martino V (2007). Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. J. Ethnopharmacol. 112:585-589.

Farhat GN, Affara NI, Gali-Muhtasib HU (2001). Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. Toxicon. 39:1601-1605.

Garcia C, Adams J (2012). Healing with Medicinal Plants of the West—Cultural and Scientific Basis for Their Use Third Edition. Abedus Press: La Crescenta.

Geissman TA, Griffin TS, Irwin MA (1969). Sequiterpene lactones of Artemisia. Artecalin from *A. californica* and *A. tripartita ssp rupicola*. Phytochem. 8:1297-1300.

Giangaspero A, Ponti C, Pollastro F, Del Favero G, Della Loggia R, Tubaro A, Appendino G, Sosa S (2009). Topical anti-inflammatory activity of eupatilin, a lipophilic flavonoid from Mountain Wormwood (*Artemisia umbelliformis* Lam.). J. Agric. Food Chem. 57:7726-7730.

Glasl S, Mucaji P, Werner I, Presser A, Jurenitsch J (2002). Sesquiterpenes and flavonoid aglycones from a hungarian taxon of the *Achillea millefolium* group. Z. Naturforsch. 57c:976-982.

Granger RE, Campbell EL, Johnston GA (2005). (+) and (-)-borneol: efficacious positive modulators of GABA action at human recombinant alpha1beta2gamma2L GABA (A) receptors. Biochem. Pharmacol. 69:1101-1111.

Hall AC, Turcotte CM, Betts BA, Yeung WY, Agyeman AS, Burk LA (2004). Modulation of human GABAA and glycine receptor currents by menthol and related monoterpenoids. Eur. J. Pharmacol. 506:9-16.

Hawker GA, Davis AM, French MR, Cibere J, Jordan JM, March L, Saurez-Almazor M, Katz JN, Dieppe P (2008). Development and preliminary psychometric testing of a new OA pain measure- an OARSI/OMERACT initiative. Osteoarthr. Cartilage 16:409-414.

Hold KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE (2000). Alpha-thujone (the active component of absinthe): gamma-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc. Nat. Acad. Sci. USA. 97:4417-4418.

Huang WY, Cai YZ, Surveswaran S, Hyde KD, Corke H, Sun M (2009). Molecular phylogenetic identification of endophytic fungi isolated from three Artemisia species. Fungal Divers. 36:69-88.

Ishida T (2005). Biotransformation of terpenoids by mammals, micro-organisms, and plant-cultured cells. Chem. Biodivers. 2:569-590.

Kazuno S, Yanagida M, Shindo N, Murayama K (2005). Mass spectrometric identification and quantification of glycosyl flavonoids, including dihydrochalcones with neutral loss scan mode. Anal. Biochem. 347:182-192.

Liapi C, Anifandis G, Chinou I, Kourounakis AP, Theodosopoulos S, Galanopoulou P (2007). Antinociceptive properties of 1,8-cineole and beta-pinene, from the essential oil of *Eucalyptus camaldulensis* leaves, in rodents. Planta Med. 73:1247-1254.

Lin CT, Chen CJ, Lin TY, Tung JC, Wang SY (2008). Anti-inflammation activity of fruit essential oil from *Cinnamomum insularimontanum* Hayata. Biores. Technol. 99:8783-8787.

Magnani RF, Rodrigues-Fo E, Daolio C, Ferreira AG, de Souza AQL (2003). Three highly oxygenated caryophyllene sesquiterpenes from *Pestalotiopsis sp.*, a fungus isolated from bark of *Pinus taeda*. Zeit. Naturforsch. 58c:319-324.

Manoguerra AS, Erdman AR, Wax PM, Nelson LS, Caravati EM, Cobaugh DJ, Chyka PA, Olson KR, Booze LL, Woolf AD, Keyes DC, Christianson G, Scharman EJ, Troutman WG (2006). American Association of Poison Control Centers. Camphor poisoning: an evidence-based practice guideline for out-of-hospital management. Clin. Tox. Official J. Am. Acad. Clin. Tox. Eur. Assoc. Poison Cent. Clin. Tox. 44:357-370.

Martinez AL, Gonzalez-Trujano ME, Aguirre-Hernandez E, Moreno J, Soto-Hernandez M, Lopez-Munoz FJ (2009a). Antinociceptive activity of *Tilia americana* var. *mexicana* inflorescences and quercetin in the formalin test and in an arthritic pain model in rats. Neuropharmacol. 56:564-571.

Martinez AL, Gonzalez-Trujano ME, Pellicer F, Lopez-Munoz FJ, Navarette A (2009b). Antinociceptive effect and GC/MS analysis of *Rosmarinus officinalis* L. essential oil from its aerial parts. Planta Med. 75:508-511.

Ortet R, Prado S, Mouray E, Thomas OP (2008). Sesquiterpene lactones from the endemic cape Verdean *Artemisia gorgonum*. Phytochem. 69:2961-2965.

Reddy AM, Lee JY, Seo JH, Kim BH, Chung EY, Ryu SY, Kim YS, Lee CK, Min KR, Kim Y (2006). Artemisolide from *Artemisia asiatica*; Nuclear Factor- KB (NF- KB) inhibitor suppressing prostaglandin E2 and nitric oxide production in macrophages. Arch. Pharmaceut. Res. 29:591-597.

Sakushima A, Nishibe S, Takeda T, Ogihara Y (1988). Positive and negative ion mass spectra of flavonoid glycosides by fast atom bombardment. Mass Spectrom. 36:71-80.

Sayyah M, Valizadeh J, Kamalinejad M (2002). Anticonvulsivant activity of the leaf essential oil of *Laurus nobilis* against pentylenetetrazole- and maximal electroshock-induced seizures. Phytomed. 9:212-216.

Schmitt S, Schaefer UF, Doebler L, Reichling J (2009). Cooperative interaction of monoterpenes and phenylpropanoids on the *in vitro* human skin permeation of complex composed essential oils. Planta Med. 75:1381-1385.

Sheu MJ, Chou PY, Cheng HC, Wu CH, Huang GJ, Wang BS, Chen JS, Chien YC, Huang MH (2009). Analgesic and anti-inflammatory activities of a water extract of *Trachelospermum jasminoides* (Apocynaceae). J. Ethnopharmacol. 126:332-338.

Stein S, Mirokhin Y, Tchekhovskov D, Mallard G (2005). The NIST Mass Spectral Search Program. National Institute of Standards and Technology: Gaithersburg.

Su Z, Wu H, Yang Y, Aisa HA, Slukhan U, Aripova S (2008). Preparative isolation of guaipyridine sesquiterpene alkaloid from *Artemisia rupestris* L. flowers using high-speed counter-current chromatography. J. Sep. Sci. 31:2161-2166.

Suzuki S, Murayama T, Shiono Y (2005). Illudalane sesquiterpenoids, echinolactones A and B, from a mycelial culture of *Echinodontium japonicum*. Phytochem. 66:2329-2333.

Swinny EE, Bloor SJ, Wong H (2000). ^{1}H and ^{13}C assignments for the 3-deoxyanthocyanins, luteolinidin-5-glucoside and apigeninidin-5-glucoside. Magnet. Res. Chem. 38:1031-1033.

Tan XJ, Li Q, Chen XH, Wang ZW, Shi ZY, Bi KS, Jia Y (2008). Simultaneous determination of 13 compounds in Herba *Artemisiae Scopariae* (Yin chen) from different harvest seasons by HPLC-DAD. J. Pharmaceut. Biomed. Anal. 47:847-853.

Tung YT, Chua MT, Wang SY, Chang ST (2008). Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. Biores. Technol. 99: 3908-3913.

Vogt-Eisele AK, Weber K, Sherkheli MA, Vielhaber G, Panten J, Gisselmann G, Hatt H (2007). Monoterpenoid agonists of TRPV3. Br. J. Pharmacol. 151:530-540.

Vriens J, Appendino G, Nilius B (2009). Pharmacology of vanilloid transient receptor potential cation channels. Mol. Pharmacol. 75:1262-1279.

Wen J, Shi H, Xu Z, Chang H, Jia C, Zan K, Jiang Y, Tu P (2010). Dimeric guaianolides and sesquiterpenoids from *Artemisia anomala*. J. Nat. Prod. 73:67-70.

Wollenweber E, Rustaiyan A (1991). Exudate flavonoids in three persian *Asteraceae* species. Biochem. Systemat. Ecol. 19: 673-675.

Xu H, Blair NT, Clapham DE (2005). Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. J. Neurosci. 25: 8924-8937.

Yin Y, Gong FY, Wu XX, Sun Y, Li YH, Chen T, Xu Q (2008). Anti-inflammatory and immunosuppressive effect of flavones isolated from *Artemisia vestita*. J. Ethnopharmacol. 120:1-6.