

## Sesquiterpene hydrocarbons of the essential oil of *Actinolema macrolema* Boiss

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**Abstract:** The essential oils of *Actinolema macrolema* Boiss. (Apiaceae) were obtained by hydrodistillation in the first 3 (sample A) and the following 3 h (sample B) from crushed fruits and dried leaves (sample C), which were subsequently analyzed by GC and GC-MS. Overall, 64 components were characterized, representing 93% of the leaf oil. Thirty components were characterized from the fruit oil representing 95% of the first fraction and 90% of the second fraction consecutively. Guaia-5,7(11)-diene, selina-3,7(11)-diene, and juniper camphor were isolated from the oils by column chromatography and their structures were elucidated by GC-MS, <sup>1</sup>H NMR, and <sup>13</sup>C NMR. The occurrence of guaia-5,7(11)-diene in nature is reported for the first time. Guaia-5,7(11)-diene (37% and 30%), germacrene-B (25% and 21%), and selina-3,7(11)-diene (both 12%) were found as major components in the oil of sample A and the following sample B, respectively. In sample C, 1-octadecanol (24%) and hexadecanoic acid (19%) were identified as the major components. Additionally, antimicrobial activities of the fruit oils were determined using broth microdilution. Sample A exhibited relatively good inhibition of *Staphylococcus epidermidis* (MIC 62.5 µg/mL). The 2 fruit essential oils showed inhibitory (MIC 125 µg/mL) effects equal to those of the standard antifungal agent used against *C. albicans*.

**Key words:** *Actinolema macrolema*, Apiaceae, essential oil, isolation, guaia-5,7(11)-diene, GC-MS, biological activity

### 1. Introduction

The family Apiaceae, comprising about 300 genera and 3000 species worldwide, is widespread and common in Turkey. The genus *Actinolema* Fenzl is represented in the *Flora of Turkey* by 2 species, namely *Actinolema macrolema* Boiss. and *A. eryngioides* Fenzl.<sup>1</sup>

A literature survey showed that there have only been a few phytochemical investigations of *Actinolema* species, and these revealed the presence of fatty acids and proteins.<sup>2,3</sup> The extraction of seed oils of *A. macrolema* and *A. eryngioides* and their oil yields were reported as 34.6% and 40.4%, respectively. Furthermore, the fatty acid composition of the seed oils was determined by GC. The major fatty acids of both species were found to be palmitic acid (6.6% and 3.8%), stearic acid (1.7% and 0.6%), petroselinic acid (43.7% and 59.6%), oleic acid (26.9% and 21.5%), and octadecadienoic acid (18.9% and 13.8%), respectively.<sup>2</sup> In another previous work, the fatty acid yield of *A. macrolema* fruits was reported as 48.4%.<sup>3</sup> Protein contents of *A. macrolema* and *A. eryngioides* fruits were determined as 17.5% and 16.8%, respectively.<sup>2</sup>

The present work reports on the chemical composition of *A. macrolema* leaf and fruit essential oils analyzed by both gas chromatography (GC) and gas chromatography–mass spectroscopy (GC-MS) systems,

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simultaneously. GC-MS analyses showed an unknown compound (**1**) as the major constituent; it was isolated by column chromatography along with 2 other known compounds (**2** and **3**) elucidated by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR studies.

The fruit essential oils were also investigated for their antibacterial activity against a panel of gram-positive and gram-negative human pathogenic bacteria, as well for their antifungal activity against the yeast *Candida albicans* using a broth microdilution assay.

To the best of our knowledge, this is the first report on evaluation of the essential oil chemistry and antimicrobial activity of *A. macrolema*.

## 2. Experimental

### 2.1. General

The density of the fruit essential oils was determined by using a Drummond capillary, refractive index by using a Shimadzu Bausch and Lomb Abbe Refractometer, and optical rotation by using a Bellingham and Stanley Digital Polarimeter Model P20.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker BioSpin system at 500 and 125 MHz, respectively. Tetramethylsilane (TMS) at 0.0 ppm was referenced as internal standard in  $\text{CDCl}_3$ . All chemicals, standard substances, solvents, and culture media of high purity (>99%) were purchased from Sigma-Aldrich (Taufkirchen, Germany) or Merck (Darmstadt, Germany) if not otherwise stated.

### 2.2. Plant material

The plant material was collected from Hadim village in Konya Province at 1450 m on 09.07.2005. A voucher specimen is kept at the Herbarium of Anadolu University, Faculty of Pharmacy, Eskişehir, Turkey (ESSE 14422).

### 2.3. Isolation of the essential oils

The essential oils were obtained by hydrodistillation from the fruits and leaves of *A. macrolema* using a Clevenger-type apparatus.<sup>4</sup> Air-dried fruits were crushed using a mortar and immediately hydrodistilled. The essential oils were isolated by hydrodistillation in the first 3 h (A) and the following 3 h (B) from the fruits to provide an essential oil in 2.0% and 0.3% (v/w) yields on a dry weight basis, respectively. Moreover, the air-dried leaves of the plant were hydrodistilled for 3 h to produce a small amount of essential oil, which was trapped in *n*-hexane. Physicochemical properties of the fruit essential oils are given in Table 1.

**Table 1.** Physicochemical properties of the fruit essential oils.

	A	B
Density $d^{25}$	0.8932	0.9108
Specific rotation $[\alpha]_D^{25}$	-20	-10
Refractive index $[n]_D^{25}$	1.515	1.515

A: Fruit oil in the first 3 h

B: Fruit oil in the last 3 h

### 2.4. Gas chromatography–mass spectrometry (GC-MS) analysis

An Agilent 5975 GC-MSD system was used. An Innowax FSC column (60 m  $\times$  0.25 mm, 0.25- $\mu\text{m}$  film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then

programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted to 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from  $m/z$  35 to 450.

## 2.5. Gas chromatography analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 °C. To obtain the same elution order as GC-MS, simultaneous autoinjection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analyses are shown in Tables 2 and 3.

**Table 2.** The composition of the leaf essential oil of *A. macrolema* (sample C).

RRI	Compound	%
1032	$\alpha$ -Pinene	1.8
1244	2-Pentyl furan	0.1
1294	1,2,4-Trimethyl benzene	0.1
1296	Octanal	tr
1300	Tridecane	0.1
1355	1,2,3-Trimethyl benzene	tr
1400	Nonanal	1.4
1497	$\alpha$ -Copaene	0.1
1506	Decanal	0.1
1528	$\alpha$ -Bourbonene	tr
1535	$\beta$ -Bourbonene	0.2
1549	1-Pentadecene	0.1
1562	Octanol	0.1
1589	$\beta$ -Ylangene	0.1
1597	$\beta$ -Copaene	0.1
1612	$\beta$ -Caryophyllene	0.1
1655	( <i>E</i> )-2-Decenal	0.1
1683	<i>trans</i> -Verbenol	0.1
1704	$\gamma$ -Muurolene	0.3
1726	Germacrene-D	2.8
1740	$\alpha$ -Muurolene	0.1
1747	Guaia -5,7(11)-diene	0.5
1755	Bicyclgermacrene	tr
1766	1-Decanol	0.1
1773	$\delta$ -Cadinene	0.2
1776	$\gamma$ -Cadinene	0.2
1796	Selina-3,7(11)-diene	0.2
1827	( <i>E, E</i> )-2,4-Decadienal	0.1
1854	Germacrene-B	0.7
1868	( <i>E</i> )-Geranyl acetone	0.9
1900	Nonadecane	0.2
1941	$\alpha$ -Calacorene	0.2
1945	1,5-Epoxysalvial-4(14)-ene	0.2
1958	( <i>E</i> )- $\beta$ -Ionone	0.2
1973	1-Dodecanol	0.2
2019	2,3,6-Trimethyl benzaldehyde	1.0
2037	Salvial-4(14)en-1-one	0.7

**Table 2.** Continued.

RRI	Compound	%
2053	Germacrene-D 1,10-epoxide	0.5
2077	1-Tridecanol	0.1
2100	Heneicosane	0.2
2130	Salviadienol	0.5
2131	Hexahydrofarnesyl acetone	3.2
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	0.2
2179	1-Tetradecanol	1.0
RRI	Compound	%
2200	Docosane	0.1
2240	1-Methyl ethyl hexadecanoate	0.4
2174	Pentadecanol	0.2
2300	Tricosane	1.9
2369	Eudesma-4(15),7-dien-1 $\beta$ -ol	1.3
2380	Hexyl cinnamic aldehyde	0.5
2384	1-Hexadecanol	5.0
2384	Farnesyl acetone	1.2
2400	Tetracosane	1.4
2500	Pentacosane	8.0
2503	Dodecanoic acid	tr
2600	Hexacosane	0.5
2607	1-Octadecanol	23.6
2622	Phytol	1.7
2670	Tetradecanoic acid	1.0
2700	Heptacosane	5.8
2794	Eicosanol	1.3
2800	Octacosane	tr
2900	Nonacosane	1.1
2931	Hexadecanoic acid	19.0
	Monoterpene hydrocarbons	1.8
	Oxygenated monoterpenes	0.1
	Sesquiterpene hydrocarbons	5.8
	Oxygenated sesquiterpene	3.2
	Diterpenes	1.7
	Alkanes and alkenes	19.6
	Fatty acid + esters	20.4
	Alcohols	31.6
	Others	5.9
	Total	93.1

RRI: Relative retention indices calculated against *n*-alkanes

%; calculated from FID data

tr: Trace (<0.1%)

## 2.6. Identification of components

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial<sup>5,6</sup> (Wiley GC-MS Library, Adams Library, MassFinder 3 Library) and

in-house “Başer Library of Essential Oil Constituents”<sup>7</sup> built up by genuine compounds and components of known oils, as well as MS literature data,<sup>8–10</sup> was used for the identification.

**Table 3.** The composition (area percent  $\pm$  SD) of the fruit essential oils of *A. macrolema*.#

RRI	Compound	A	B
1032	$\alpha$ -Pinene	tr	tr
1093	Hexanal	tr	tr
1400	Nonanal	tr	tr
1452	1-Octen-3-ol	tr	tr
1479	$\delta$ -Elemene	0.10 $\pm$ 0.00	0.06 $\pm$ 0.10
1493	$\alpha$ -Ylangene	tr	tr
1568	<i>cis</i> -Eudesma-6,11-diene	0.20 $\pm$ 0.00	0.20 $\pm$ 0.10
1586	Cascarilladiene	0.10 $\pm$ 0.00	0.1 $\pm$ 0.00
1597	$\beta$ -Copaene	tr	tr
1600	$\beta$ -Elemene	0.07 $\pm$ 0.06	tr
1612	$\beta$ -Caryophyllene	tr	tr
1650	$\gamma$ -Elemene	5.13 $\pm$ 1.50	6.77 $\pm$ 0.68
1677	Sibirene	1 $\pm$ 0.00	1 $\pm$ 0
1707	$\delta$ -Selinene	0.10 $\pm$ 0.00	0.1 $\pm$ 0
1718	4,6-Guaiadiene	10.33 $\pm$ 0.58	8.17 $\pm$ 0.76
1742	$\beta$ -Selinene	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
1747	Guaia -5,7(11)-diene ( <b>1</b> )	36.60 $\pm$ 0.72	30.30 $\pm$ 0.53
1751	Isoeremofilen	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
1796	Selina-3,7(11)-diene ( <b>2</b> )	11.50 $\pm$ 0.5	11.67 $\pm$ 0.58
1854	Germacrene-B	24.67 $\pm$ 1.53	20.67 $\pm$ 0.58
2096	Elemol	tr	0.1 $\pm$ 0
RRI	Compound	A	B
2100	Guaiyl acetate	1.00 $\pm$ 0.00	1.20 $\pm$ 0.20
2144	Rosifoliol	tr	0.10 $\pm$ 0.00
2185	$\gamma$ -Eudesmol	tr	0.10 $\pm$ 0.00
2187	T-Cadinol	1.00 $\pm$ 0.00	3.33 $\pm$ 0.58
2202	1(10),5-Germacradiene-4 $\alpha$ -ol	0.37 $\pm$ 0.06	1.00 $\pm$ 0.00
2200	$\alpha$ -Guaiol	0.07 $\pm$ 0.06	0.17 $\pm$ 0.06
2250	$\alpha$ -Eudesmol	0.23 $\pm$ 0.05	1.00 $\pm$ 0.00
2257	$\beta$ -Eudesmol	0.10 $\pm$ 0.00	0.63 $\pm$ 0.32
2320	Juniper camphor ( <b>3</b> )	0.27 $\pm$ 0.06	1.06 $\pm$ 0.11
	Monoterpene hydrocarbons	tr	tr
	Oxygenated monoterpenes	tr	tr
	Sesquiterpene hydrocarbons	91.8	81.04
	Oxygenated sesquiterpene	3.04	8.69
	Others	tr	tr
	Total	94.84	89.73

RRI: Relative retention indices calculated against *n*-alkanes

A: Fruit oil in the first 3 h

B: Fruit oil in the last 3 h

%: calculated from FID data

tr: Trace (<0.1%)

#: The analyses were carried out in triplicate

## 2.7. Isolation of guaia-5,7(11)-diene (1)

The compound was isolated from the hydrodistilled essential oil of *A. macrolema* by column chromatography. Silica gel 60 G (ca. 3.5 g, Merck 7734) was used as the packing material and was added to a column (10 cm × 1 cm) using *n*-hexane. The hydrodistilled essential oil (148 mg) was applied to the column and *n*-hexane was used as the initial eluent to yield compound **1** (2.4 mg) as a colorless oily material. EIMS  $m/z$ : 204 [ $M^+$ ] (204.3511 cal. for  $C_{15}H_{24}$ ) (87%), 189 [ $M-CH_3$ ]<sup>+</sup> (54), 175 (10), 161 [ $M-C_3H_7$ ]<sup>+</sup> (100), 149 (30), 133 (36), 119 (12), 105 (15), 91 (11), 79 (22), 63 (13), 55 (15), 41 (20); <sup>1</sup>H NMR (500 MHz,  $CDCl_3$ ):  $\delta$  6.17 (1H, s, H-6), 2.51 (1H, m, H-4), 1.89 (2H, m, H-8), 1.75 (6H, s, 2 ×  $CH_3$ , H-12 and H-13), 1.12 (3H, d,  $J$  = 7.92 Hz, H-14), 0.93 (3H, d,  $J$  = 6.66 Hz, H-15); <sup>13</sup>C NMR (125 MHz,  $CDCl_3$ ):  $\delta$  151.99 (C-5), 122.18 (C-6), 131.35 (C-7), 125.71 (C-11), 49.02 (C-1), 40.27 (C-4), 37.44 (C-3), 36.79 (C-9), 34.19 (C-10), 32.00 (C-2), 29.06 (C-8), 20.80 (C-15), 20.66 (C-14), 20.23 (C-12 and C-13).

## 2.8. Isolation of selina-3,7(11)-diene [3,7(11)-eudesmadiene] (2)

The same chromatographical procedure was applied as above for compound **1** to obtain compound **2** (6.4 mg). EIMS  $m/z$ : 204 [ $M^+$ ] (204.3511 cal. for  $C_{15}H_{24}$ ) (64%), 189 [ $M-CH_3$ ]<sup>+</sup> (27), 175 [ $M-C_2H_5$ ]<sup>+</sup> (5), 161 [ $M-C_3H_7$ ]<sup>+</sup> (100), 147 (12), 133 (29), 122 (40), 107 (43), 91 (34), 79 (21), 67 (17), 55 (13), 41 (16); <sup>1</sup>H NMR (500 MHz,  $CDCl_3$ ):  $\delta$  5.36 (1H, brs, H-3), 1.68 (3H, brs, H-15), 1.59 (6H, s, 2 ×  $CH_3$ , H-12 and H-13), 1.28 (2H, t, H-9), 0.90 (3H, s, H-14); <sup>13</sup>C NMR (125 MHz,  $CDCl_3$ ):  $\delta$  135.20 (C-4), 131.55 (C-7), 121.23 (C-11), 119.65 (C-3), 47.09 (C-5), 40.74 (C-1), 37.80 (C-9), 32.34 (C-10), 27.47 (C-8), 25.28 (C-6), 23.03 (C-2), 21.08 (C-14), 20.21 (C-12), 20.10 (C-13), 15.16 (C-15).

## 2.9. Isolation of juniper camphor [7(11)-eudesmen-4-ol], [7(11)-selinen-4-ol] (3)

The essential oil (60 mg) was subjected to column chromatography over silica gel, eluted with *n*-hexane:diethyl ether (8:2) to yield an oily substance **3** (3 mg). EIMS  $m/z$ : 222 [ $M^+$ ] (cal. 222.3663 for  $C_{15}H_{26}O$ ) (51%), 204 (50), 189 (100), 161 (49), 148 (17), 135 (44), 121 (35), 105 (32), 93 (40), 81 (56), 67 (29), 55 (30), 43 (62); <sup>1</sup>H NMR (500 MHz,  $CDCl_3$ ):  $\delta$  2.82 (1H, dd, H-6), 2.50 (1H, dd, H-8), 1.84 (1H, brs, H-8'), 1.67 (1H, H-2), 1.58 (6H, s, 2 ×  $CH_3$ , H-12 and H-13), 1.41 (1H, m, H-9), 1.35 (1H, m, H-1), 1.27 (1H, s, H-5), 1.16 (3H, s, H-15), 1.07 (1H, d, H-9'), 1.03 (1H, m, H-1), 0.98 (3H, s, H-14); <sup>13</sup>C NMR (125 MHz,  $CDCl_3$ ):  $\delta$  131.41 (C-7), 120.98 (C-11), 72.32 (C-4), 55.77 (C-5), 45.26 (C-9), 43.59 (C-3), 41.00 (C-1), 34.83 (C-10), 25.46 (C-8), 24.64 (C-6), 22.06 (C-15), 20.21 (C-2), 20.08 (C-12), 20.08 (C-13), 18.09 (C-14).

## 2.10. Antimicrobial activity

### 2.10.1. Microorganisms

Microorganisms were obtained from ATCC, NRRL, and clinical isolates (Faculty of Medicine, Eskişehir Osmangazi University, Turkey) and were stored in 10% glycerol-containing micro-test tubes (Eppendorf) at  $-86^\circ C$ . The yeast *Candida albicans* was inoculated in Sabouraud Dextrose Agar (SDA), whereas the bacteria were inoculated in Mueller Hinton Agar (MHA) at  $37^\circ C$  overnight for purity check. All microorganisms were then transferred to double strength Mueller Hinton Broth (MHB) for further incubation at  $37^\circ C$  for another 24 h.

### 2.10.2. Antimicrobial assay

Antimicrobial activity of the fruit essential oils was evaluated using the microdilution broth method.<sup>11,12</sup> The essential oils and the antimicrobial standards were first dissolved in dimethyl sulfoxide (DMSO), which was used as a stock to prepare dilution series from 2 to 0.0019 mg/mL in distilled sterile water. The serial dilutions were then transferred into 96-well microtiter plates in 100- $\mu$ L aliquots, where the last row was filled only with water. Overnight grown microorganism suspensions were first diluted in double strength MHB and standardized to  $10^8$  cfu/mL (using McFarland No: 0.5) under sterile conditions. Then each microorganism suspension was pipetted into each well in an equal volume and incubated at 37 °C for 24 h. Chloramphenicol and ampicillin were used as standard antibacterial agents whereas ketoconazole was used as a standard antifungal agent against *Candida albicans*. Sterile distilled water and medium served as a positive growth control. The first well without turbidity was assigned as the minimal inhibitory concentration (MIC, in  $\mu$ g/mL). Average results of 3 separately performed experiments are given in Table 4.

**Table 4.** Minimal inhibitory concentration (MIC) ( $\mu$ g/mL) values for *A. macrolema* fruit essential oils.

Pathogen	Source	A	B	St1	St2
<i>Bacillus cereus</i> , Gr (+)	NRRL B-3711	500	500	125	-
<i>Enterobacter aerogenes</i> , Gr (-)	NRRL 3567	500	500	31.25	-
<i>Escherichia coli</i> , Gr (-)	NRRL B-3008	500	500	31.25	-
<i>Proteus vulgaris</i> , Gr (-)	NRRL B-123	250	250	15.62	-
<i>Pseudomonas aeruginosa</i> , Gr (-)	ATCC 27853	500	500	125	-
<i>Salmonella typhimurium</i> , Gr (-)	ATCC 13311	500	1000	15.62	-
<i>Staphylococcus aureus</i> (MRSA), Gr (+)	Clin. isolate	250	500	31.25	-
<i>Staphylococcus epidermidis</i> , Gr (+)	ATCC 12228	62.5	250	15.62	-
<i>Candida albicans</i> (yeast)	Clin. isolate	125	125	-	125

A: Fruit oil in the first 3 h

B: Fruit oil in the last 3 h

St1: Chloramphenicol (antibacterial)

St2: Ketoconazole (antifungal)

ATCC: American Type Culture Collection

NRRL: Northern Regional Research Lab. Agricultural Res. Service C.C.

Clin. isolate: Clinical isolate from ESOGU, Eskişehir Osmangazi University, Faculty of Medicine

MRSA: Methicillin resistant *Staphylococcus aureus*

Gr (+): Gram positive

Gr (-): Gram negative

### 3. Results and discussion

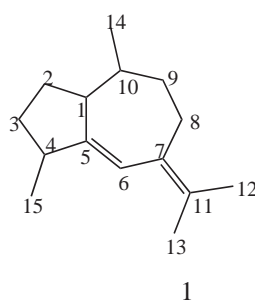
*Actinolema macrolema* Boiss. was collected from its natural habitat in Konya, Turkey. The essential oils were obtained by hydrodistillation in the first 3 h (sample A) and the following 3 h (sample B) from crushed fruits and dried leaves of *A. macrolema*. The fruit essential oils (sample A and sample B) yielded overall 2.0% and 0.3%, whereas the leaf oil was trapped in *n*-hexane. Physicochemical properties of the fruit essential oils are given in Table 1. Due to the low yield of leaf oil (sample C), the density and refractive index of the oil were not determined.

All the isolated oils were analyzed by GC and GC-MS, simultaneously. The identified compounds in the leaf and fruit essential oils of *A. macrolema*, along with their relative percentage and their relative retention indices (RRI), are given in Tables 2 and 3, respectively. Overall, 64 components were characterized, representing

93.1% of the leaf oil. 1-Octadecanol (23.6%), hexadecanoic acid (19%), pentacosane (8.0%), heptacosane (5.8%), and 1-hexadecanol (5.0%) were the major constituents of the leaf oil. The leaf oil was characterized by a relatively high content of aliphatic alcohols (31.6%), whereas oxygenated monoterpenes (0.1%) were present in a relatively very low amount. Thirty components were characterized, representing 94.8% of the first fraction and 89.7% of the second fraction. Guaia-5,7(11)-diene (36.6% and 30.3%), germacrene-B (24.7% and 20.7%), selina-3,7(11)-diene (11.5% and 11.7%), 4,6-guaiadiene (10.3% and 8.2%), and  $\gamma$ -elemene (5.1% and 6.8%) were the major constituents of the first and second fractions of the fruit oil. Both fractions were characterized by a high content of sesquiterpene hydrocarbons (91.8% and 81.0%, respectively). As expected, it was observed that oxygenated sesquiterpenes (elemol, guaiyl acetate, rosifoliol,  $\gamma$ -eudesmol, T-cadinol, 1(10),5-germacradiene-4 $\alpha$ -ol,  $\alpha$ -guaiol,  $\alpha$ -eudesmol,  $\beta$ -eudesmol, and juniper camphor) were in relatively low amounts (3.04%) for fraction A, while the same oxygenated sesquiterpenes increased (8.7%) in fraction B. It was also noted that monoterpene hydrocarbons and oxygenated monoterpenes were present in low amounts in both fractions.

The main component (**1**) was purified by column chromatography from sample A. The molecular formula ( $C_{15}H_{24}$ ) was deduced from GC-MS and  $^{13}C$  NMR. DEPT spectra contained 15 carbon signals corresponding to 4 methyls, 4 methylenes, 4 methines, and 3 quaternary carbon atoms. The  $^1H$  NMR spectrum showed a tertiary methyl group at  $\delta$  1.12 and  $\delta$  0.93 (H-14 and H-15). The  $^{13}C$  NMR spectrum indicated quaternary carbon atoms at  $\delta$  151.99,  $\delta$  131.35, and  $\delta$  125.71 ppm (C-5, C-7, C-11).

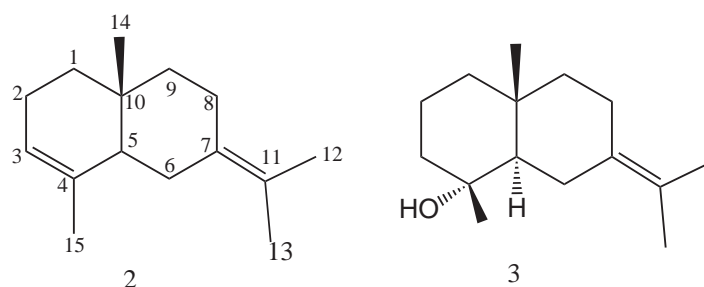
A literature survey showed that this compound was a dehydration product of guaiaen-(5)-ol-(11), which was previously isolated from gurjun balsam (*Dipterocarpus* sp. C.F.Gaertn., Dipterocarpaceae) along with other derivatives by R cker and Hefendehl.<sup>13</sup> Finally, our literature search and comparison with spectral data confirmed the identity of the compound as guaia-5,7(11)-diene (**1**), as shown in Figure 1. However, the information provided in the previous study was tentative, and the authors were uncertain about the absolute confirmation as indicated in the paper.<sup>13,14</sup> In the present study, guaia-5,7(11)-diene (**1**) was shown to be naturally present in both *A. macrolema* essential oils, which was also confirmed by GC-MS analyses for the first time to the best of our knowledge. However, due to the instability and fast decomposition of compound **1**, it was not possible to obtain detailed 2D-NMR data for absolute structure and stereochemical elucidation.



**Figure 1.** Structure of guaia-5,7(11)-diene (**1**).

The structures of selina-3,7(11)-diene (**2**) and juniper camphor (**3**) (see also Figure 2) were confirmed by comparison with the previous reported  $^1H$  and  $^{13}C$  NMR spectral data<sup>15–18</sup> also for the first time in this genus and species, to the best of our knowledge.





**Figure 2.** Structures of selina-3,7(11)-diene (**2**) and juniper camphor (**3**).

Furthermore, fractions A and B were subjected to in vitro antimicrobial activity evaluation using a broth microdilution assay.<sup>12</sup> Common gram-positive and -negative human pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and methicillin-resistant *Staphylococcus aureus* (MRSA) and the yeast *C. albicans* were tested against the fruit oils with antimicrobial standard agents for comparison. The distillation product obtained within the first 3 h (sample A) showed good inhibition on *Staphylococcus epidermidis* (MIC = 62.5  $\mu\text{g/mL}$ ). The 2 fruit essential oils (samples A and B) had the same inhibitory effect on *C. albicans* as the standard antifungal agent ketoconazole (MIC = 125  $\mu\text{g/mL}$ ). Due to low yield of sample C, no antimicrobial activity studies could be performed.

In conclusion, this is the first report on *A. macrolema* essential oils and it yielded a new natural compound, namely guaia-5,7(11)-diene (**1**). Fruit essential oil fractions showed moderate to good inhibitory antimicrobial properties. It is worthwhile to investigate the phytochemistry and the biological activities of this scarcely studied genus.

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### References

1. Davis, P. H. In: *Flora of Turkey and the East Aegean Islands*, Vol. 4; Davis P. H., Ed.; University Press, Edinburgh, 1972.
2. Kleiman, R.; Spencer, G. F. *Journal of American Oil Chemists' Society* **1982**, *59*, 29.
3. Barclay, A. S.; Earle, F. R. *Economic Botany* **1974**, *28*, 178–236.
4. Council of Europe; *European Pharmacopoeia*, Vol. 1; 5th Ed.; Strasbourg, 2005.
5. McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*, J Wiley and Sons, New York, 1989.
6. König, W. A.; Joulain, D.; Hochmuth, D. H. *Terpenoids and Related Constituents of Essential Oils*. Library of MassFinder 3, Hamburg, Germany, 2004.
7. Bařer, K. H. C.; Demirci, F. Chemistry of Essential Oils, In: *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*, Berger, R. G.; Ed. Springer, Berlin, 2007.
8. Jennings, W. G.; Shibamoto, T. *Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC*, Academic Press, New York, 1980.

9. Joulain, D.; König, W. A. *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*. EB-Verlag, Hamburg, 1998.
10. ESO 2000. *The Complete Database of Essential Oils*. Boelens Aroma Chemical Information Service, The Netherlands, 1999.
11. Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn, W. C. *Color Atlas and Textbook of Diagnostic Microbiology*. Lippincott-Raven, Philadelphia, PA, 1997.
12. İscan, G.; Kırmır, N.; Kürkçüođlu, M.; Bařer, K. H. C.; Demirci, F.; *J. Agric. Food Chem.* **2002**, *50*, 394–3946.
13. Růcker, G.; Hefendehl, F. W. *Phytochem.* **1978**, *17*, 809–810.
14. Ferreira, M. J. P.; Oliveira, F. C.; Rodrigues, G. V.; Emerenciano, V. P. *Internet Electronic Journal of Molecular Design* **2004**, *3*, 737–749.
15. Beek, T. A. V.; Kleis, R.; Posthumus, M. A.; Veldhuizen, A. V. *Phytochem.* **1989**, *28*, 1909–1911.
16. Joulain, D.; Kůnig, W. A. Selina-3, 7(11)-dien. In: *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*, E.B. Verlag, Hamburg, 1998.
17. Bakhtiar, A.; Sargent, M. V.; Skelton, B. W.; White, A. H. *Acta Crystallographica C* **2004**, *60*, 503–504.
18. Zhao, Y.; Yue, J.; Lin, Z.; Ding, J.; Sun, H. *Phytochem.* **1997**, *44*, 459–464.