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**Prabodh Satyal**  
 Department of Chemistry,  
 University of Alabama in  
 Huntsville, Huntsville, USA

**William N Setzer**  
 Department of Chemistry,  
 University of Alabama in  
 Huntsville, Huntsville, USA

## Chemical composition and enantiomeric distribution of monoterpenoids of the essential oil of *Abies spectabilis* from Nepal

Prabodh Satyal and William N Setzer

### Abstract

Two subspecies of *Abies spectabilis*, *A. spectabilis* subsp. *densa* and *A. spectabilis* subsp. *langtangensis*, were collected from Dhankuta (eastern Himalaya) and Jumla (western Himalaya) of Nepal, respectively. The leaf (needle) essential oils were obtained by hydrodistillation and analyzed by gas chromatography – mass spectrometry as well as chiral gas chromatography. The major components in the essential oil of *A. s. densa* were limonene (26.9%, 99% (–)-enantiomer),  $\alpha$ -pinene (22.2%, 90% (–)-enantiomer), bornyl acetate (10.3%, 100% (–)-enantiomer),  $\beta$ -phellandrene (9.7%, 99% (–)-enantiomer), camphene (7.1%, 100% (+)-enantiomer), and  $\beta$ -pinene (6.5%, 96% (–)-enantiomer). *A. s. langtangensis* was composed largely of  $\alpha$ -pinene (33.4%, 75% (+)-enantiomer), bornyl acetate (10.9%, 100% (–)-enantiomer), selin-11-en-4 $\beta$ -ol (9.4%), and  $\alpha$ -bisabolol (4.0%).

**Keywords:** essential oil, chemical composition, enantiomeric distribution, chiral gas chromatography, *Abies spectabilis*.

### 1. Introduction

The genus *Abies* (Pinaceae) is comprised of about 46 northern temperate species<sup>[1]</sup>. *Abies spectabilis* (D. Don) Spach is a high altitude conifer of the Himalayas from Afghanistan to Nepal<sup>[2]</sup>, including Kashmir<sup>[3]</sup> and Tibet<sup>[4]</sup>. In Nepal, the tree ranges in elevation from about 2500 m to 4000 m<sup>[5-7]</sup>. The leaves (needles) are used to treat bronchitis and whooping cough<sup>[8, 9]</sup>. Previous phytochemical investigations of *A. spectabilis* have focused on solvent extracts of the aerial parts<sup>[10]</sup> and the bark<sup>[11]</sup>. From the aerial parts, three triterpenoids, 23 diterpenoids, a sesquiterpenoid, 13 flavonoids, 12 lignans, and 20 miscellaneous compounds were identified<sup>[10]</sup>, while the bark extract showed several condensed tannins<sup>[11]</sup>. The essential oil compositions of several species of *Abies* have been investigated, including *A. alba*<sup>[12, 13]</sup>, *A. balsamea*<sup>[14]</sup>, *A. cilicica*<sup>[15]</sup>, *A. koreana*<sup>[16, 17]</sup>, *A. nephrolepis*<sup>[18]</sup>, *A. pindrow*<sup>[19]</sup>, and *A. sachalinensis*<sup>[20]</sup>. In this work, we present the chemical compositions of two subspecies of *A. spectabilis*, *A. spectabilis* subsp. *densa* (Griff.) Silba, from eastern Nepal, and *A. spectabilis* subsp. *langtangensis* (Silba) Silba, from western Nepal. To our knowledge, this work represents the first report of the chemical compositions of *A. spectabilis* essential oils, including enantiomeric distribution of monoterpenoid constituents.

### 2. Materials and Methods

#### 2.1 Plant Material

*A. spectabilis* subsp. *langtangensis* from Jumla, Nepal (29° 16' 28.99" N, 82° 11' 1.79" E, elevation 2500 m asl), was identified by Nawal Shrestha, and collected on July 2, 2016. The fresh needles (100 g) were hydrodistilled for 4 h using a Clevenger-type apparatus and collected to give 200 mg colorless essential oil after drying with Na<sub>2</sub>SO<sub>4</sub>. *A. spectabilis* subsp. *densa* from Dhankuta Nepal (26° 59' 0.44" N, 87° 19' 17.37" E, elevation 2700 m asl) was identified by Saugat Shrestha, and collected on September 1, 2016. The fresh needles (100 g) were hydrodistilled for 4 h using a Clevenger-type apparatus and collected to yield 280 mg pale yellow essential oil after drying with Na<sub>2</sub>SO<sub>4</sub>.

#### 2.2 Gas Chromatography – Mass Spectrometry

The essential oils of *A. spectabilis* chemotypes were analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40–400 amu, scan rate = 3.0 scans/sec, and GC-MS solution software.

**Correspondence:**  
**William N Setzer**  
 Department of Chemistry,  
 University of Alabama in  
 Huntsville, Huntsville, USA

The GC column was a ZB-5 fused silica capillary column with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25  $\mu\text{m}$ . The carrier gas was helium with a column head pressure of 80 psi and flow rate of 1.37 mL/min. Injector temperature was 250  $^{\circ}\text{C}$  and the ion source temperature was 200  $^{\circ}\text{C}$ . The GC oven temperature program was programmed for 50  $^{\circ}\text{C}$  initial temperature, temperature increased at a rate of 2  $^{\circ}\text{C}/\text{min}$  to 260  $^{\circ}\text{C}$ . A 5% w/v solution of the sample in  $\text{CH}_2\text{Cl}_2$  was prepared and 0.1  $\mu\text{L}$  was injected with a splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [21], and stored in our in-house MS library.

### 2.3 Chiral Gas Chromatography

Chiral analysis of the essential oils was performed on a Shimadzu GCMS-QP2010S operated in the EI mode (electron energy = 70 eV), scan range = 40–400 amu, scan rate = 3.0 scans/sec. GC was equipped with a Restek B-Dex 325 capillary column (30 m  $\times$  0.25 mm ID  $\times$  0.25  $\mu\text{m}$  film). Oven temperature was started at 50  $^{\circ}\text{C}$ , and then gradually raised to 120  $^{\circ}\text{C}$  at 1.5  $^{\circ}\text{C}/\text{min}$ . The oven was then raised to 200  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$  and held for 5 min. Helium was the carrier gas and the flow rate was maintained at 1.8 mL/min. Samples were diluted 3% w/v with  $\text{CH}_2\text{Cl}_2$  and then a 0.1  $\mu\text{L}$  sample was injected in a split mode with a split ratio of 1:45.

### 3. Results and Discussion

The essential oil compositions of the two subspecies of *A. spectabilis* are compiled in Table 1. A total of 108 compounds were identified in *A. spectabilis* subsp. *densa*, accounting for 99.8% of the total composition, while 75 compounds were identified in *A. spectabilis* subsp. *langtangensis*, 98.6% of the total composition. The major components in *A. s. densa* were limonene (26.9%),  $\alpha$ -pinene (22.2%), bornyl acetate (10.3%),  $\beta$ -phellandrene (9.7%), camphene (7.1%), and  $\beta$ -pinene (6.5%). The essential oil of *A. s. langtangensis* was dominated by  $\alpha$ -pinene (33.4%), bornyl acetate (10.9%), selin-11-en-4 $\beta$ -ol (9.4%) and  $\alpha$ -bisabolol (4.0%). The needle oil of another Himalayan *Abies* species, *A. pindrow*, was shown to be rich in limonene (21.0%), camphene (19.9%),  $\alpha$ -pinene (16.8%),  $\beta$ -pinene (6.5%), and myrcene (6.7%) [19]. Padalia *et al.* have summarized the compositions of several *Abies* essential oils and found that the oils are generally composed of large concentrations of monoterpenoids,  $\alpha$ -pinene,  $\beta$ -pinene,  $\delta$ -3-carene, limonene,  $\beta$ -phellandrene, myrcene, camphene, and bornyl acetate [19]. Thus, for example, essential oils of *Abies sachalinensis* and *A. mayriana* from Finland were both dominated by bornyl acetate (27.9% and 26.0%, respectively), camphene (18.3% and 16.6%, respectively),  $\alpha$ -pinene (12.2% and 12.5%, respectively),  $\beta$ -phellandrene (7.5% and 8.9%, respectively),  $\beta$ -pinene (7.0% and 7.3%, respectively), and limonene (6.5% and 6.4%, respectively) [22].

**Table 1:** Chemical compositions of the needle essential oils of *Abies spectabilis* subsp. *densa* and *Abies spectabilis* subsp. *langtangensis* collected in Nepal.

RI <sup>a</sup>	Compound	<i>A. s. densa</i>		<i>A. s. langtangensis</i>	
		% <sup>b</sup>	ED <sup>c</sup> (+)/(-)	% <sup>b</sup>	ED <sup>c</sup> (+)/(-)
762	Toluene	tr <sup>d</sup>		0.1	
880	Santene	0.1		---	
923	Tricyclene	0.6		0.3	
925	$\alpha$ -Thujene	0.1	76/24	tr	70/30
933	$\alpha$ -Pinene	22.2	10/90	33.4	75/25
946	$\alpha$ -Fenchene	tr		---	
949	Camphene	7.1	100/0	3.0	75/25
953	Thuja-2,4(10)-diene	tr		0.1	
971	Sabinene	tr		---	
971	3,7,7-Trimethyl-1,3,5-cycloheptatriene	---		0.1	
978	$\beta$ -Pinene	6.5	4/96	3.0	6/94
989	Myrcene	1.5		2.0	
1004	<i>p</i> -Mentha-1(7),8-diene	tr		---	
1006	$\alpha$ -Phellandrene	0.5			
1009	$\delta$ -3-Carene	3.1	98/2	3.3	100/0
1016	$\alpha$ -Terpinene	0.2		---	
1024	<i>p</i> -Cymene	0.2		0.9	
1029	Limonene	26.9	1/99	3.3	20/80
1030	$\beta$ -Phellandrene	9.7	1/99	tr	
1034	( <i>Z</i> )- $\beta$ -Ocimene	tr		---	
1044	( <i>E</i> )- $\beta$ -Ocimene	0.1		---	
1057	$\gamma$ -Terpinene	0.2		---	
1071	Pinol	tr		---	
1080	<i>p</i> -Mentha-2,4(8)-diene	tr		---	
1084	Terpinolene	0.8		---	
1089	2-Nonanone	0.1		---	
1096	6-Camphenone	tr		---	
1098	Perillene	---		0.2	
1099	Linalool	tr		---	
1101	2-Nonanol	tr		---	
1118	<i>endo</i> -Fenchol	tr		0.1	
1123	<i>cis-p</i> -Menth-2-en-1-ol	tr		---	
1126	$\alpha$ -Campholenal	tr		0.2	
1140	<i>trans</i> -Pinocarveol	tr		0.6	
1141	<i>trans-p</i> -Menth-2-en-1-ol	tr		---	

1146	Camphor	tr		---	
1148	<i>trans</i> -Verbenol	tr		0.8	
1150	$\alpha$ -Phellandren-8-ol	---		0.1	
1159	4-Decanone	tr		---	
1162	<i>p</i> -Mentha-1,5-dien-8-ol	---		0.3	
1171	Borneol	0.2	98/2	0.6	40/60
1175	<i>cis</i> -Pinocamphone	tr		---	
1179	Terpinen-4-ol	0.2	70/30	---	
1180	<i>m</i> -Cymen-8-ol	---		1.0	
1184	<i>p</i> -Methylacetophenone	---		tr	
1185	<i>p</i> -Cymen-8-ol	tr		0.9	
1186	Cryptone	tr		0.3	
1194	$\alpha$ -Terpineol	0.6	30/70	1.5	71/29
1205	Decanal	tr		---	
1207	Verbenone	---		0.8	67/33
1208	<i>trans</i> -Piperitol	tr		---	
1210	4-Methyleisophorone	---		0.2	
1218	<i>trans</i> -Carveol	---		0.3	
1225	Citronellol	0.4		---	
1229	Thymol methyl ether	0.5		0.3	
1232	2-Nonyl acetate	tr		---	
1242	Cumin aldehyde	---		0.1	
1244	Carvone	---		0.1	
1246	Car-3-en-2-one	---		0.1	
1252	Piperitone	0.1	0/100	---	
1271	(2 <i>E</i> )-Decen-1-ol	tr		---	
1274	Cyclooctyl acetate	tr		---	
1276	Phellandral	0.1		---	
1279	Isopulegyl acetate	tr		---	
1284	Bornyl acetate	10.3	0/100	10.9	0/100
1292	2-Undecanone	0.2		0.2	
1294	Methyl myrtenate	tr		---	
1345	$\alpha$ -Cubebene	tr		---	
1349	Citronellyl acetate	0.2		0.3	
1368	$\alpha$ -Ylangene	tr		---	
1376	$\alpha$ -Copaene	0.1		0.1	
1377	Geranyl acetate	tr		0.1	
1379	<i>trans-p</i> -Menth-6-en-2,8-diol	---		0.2	
1383	$\alpha$ -Bourbonene	1.8		0.9	
1385	$\beta$ -Bourbonene	0.1		---	
1388	$\beta$ -Elemene	0.1		---	
1393	1,5-di- <i>epi</i> - $\alpha$ -Bourbonene	tr		---	
1395	Ethyl decanoate	---		0.3	
1401	Isocycloseychellene	0.1		---	
1404	$\alpha$ -Funebrene	tr		---	
1408	Dodecanal + Longifolene	0.1		---	
1410	Acora-3,7(14)-diene + Longifolene + Dodecanal	---		0.1	
1419	( <i>E</i> )-Caryophyllene	0.9		0.3	
1430	$\beta$ -Copaene	0.2		tr	
1433	$\alpha$ - <i>trans</i> -Bergamotene	---		0.1	
1434	Aromadendrene	tr		---	
1436	$\gamma$ -Elemene	tr		---	
1443	<i>cis</i> -Muurolo-3,5-diene	0.1		---	
1447	<i>trans</i> -Muurolo-3,5-diene	tr		---	
1454	$\alpha$ -Humulene	0.3		0.3	
1460	<i>cis</i> -Muurolo-4(14),5-diene	tr		---	
1466	Methyl 3,6-dodecadienoate	tr		---	
1468	Undecenyl acetate	0.1		0.2	
1470	<i>cis</i> -Cadina-1(6),4-diene	tr		---	
1475	<i>trans</i> -Cadina-1(6),4-diene	0.2		0.8	
1477	$\alpha$ -Amorphene	tr		---	
1479	Germacrene D	0.1		---	
1482	$\beta$ -Chamigrene	0.3		1.2	
1485	$\delta$ -Selinene	tr		---	
1489	$\beta$ -Selinene	0.1		0.4	
1490	<i>trans</i> -Muurolo-4(14),5-diene	0.1		---	
1492	$\gamma$ -Amorphene	---		0.1	
1494	$\beta$ -Alaskene	---		0.3	
1495	$\alpha$ -Selinene	0.3		---	
1496	<i>trans</i> - $\beta$ -Guaiene	---		1.3	
1498	$\alpha$ -Muuroloene	0.2		0.2	
1501	Aciphyllyene	0.3		---	

1503	$\beta$ -Dihydroagarofuran	0.1	---	---
1506	Cuparene	tr	---	---
1507	$\beta$ -Bisabolene	---	---	0.8
1512	$\delta$ -Amorphene	0.2	---	0.4
1516	$\delta$ -Cadinene	0.5	---	---
1519	$\alpha$ -Panasinsen	---	---	0.4
1521	Zonarene	tr	---	---
1521	<i>trans</i> -Calamenene	---	---	0.1
1531	<i>trans</i> -Cadine-1,4-diene	tr	---	---
1535	$\alpha$ -Cadinene	tr	---	---
1539	( <i>E</i> )- $\alpha$ -Bisabolene + $\alpha$ -Calcorene	tr	---	---
1554	Isocaryophyllene oxide	tr	---	---
1560	( <i>E</i> )-Nerolidol	0.1	---	1.2
1582	Caryophyllene oxide	tr	---	3.2
1598	<i>cis</i> -Bisabol-11-ol	---	---	tr
1609	Humulene epoxide II	---	---	1.2
1621	Selina-6-en-4-ol	0.1	---	0.2
1626	1- <i>epi</i> -Cubenol	tr	---	---
1637	Caryophylla-4(12),8(13)-dien-5 $\beta$ -ol	---	---	0.1
1640	$\tau$ -Cadinol	tr	---	0.1
1642	$\tau$ -Muurolol	tr	---	0.1
1644	$\alpha$ -Muurolol	tr	---	---
1645	<i>trans</i> -Guai-11-en-10-ol	---	---	0.2
1654	$\alpha$ -Cadinol	tr	---	0.5
1658	$\alpha$ -Bisabolol oxide B	---	---	0.2
1662	Selin-11-en-4 $\beta$ -ol	1.1	---	9.4
1669	14-Hydroxy-9- <i>epi</i> -( <i>E</i> )-caryophyllene	---	---	0.2
1685	<i>epi</i> - $\alpha$ -Bisabolol	---	---	0.2
1687	$\alpha$ -Bisabolol	---	---	4.0
2100	Heneicosane	tr	---	---
2131	Nezukol	---	---	0.1
	Total Identified	99.8	---	98.6

<sup>a</sup> RI = Retention Index based on a series of *n*-alkanes on a ZB-5 capillary column.

<sup>b</sup> The percentages of each component are reported as raw percentages based on total ion current without standardization.

<sup>c</sup> ED = Enantiomeric Distribution determined using a Restek B-Dex 325 capillary column.

<sup>d</sup> tr = "trace" (< 0.05%).

Interestingly, (–)- $\alpha$ -pinene dominated *A. spectabilis* subsp. *densa*, while (+)- $\alpha$ -pinene was the predominant stereoisomer in *A. s. langtangensis*. A similar variation was observed in the needle essential oils of *Abies sibirica*, depending on geographical origin [23]. Both *A. sachalinensis* and *A. mayriana* had preponderance of (–)- $\alpha$ -pinene over the (+)-enantiomer (64% and 67%, respectively [22]). Although there was variation in the (+)- $\alpha$ -pinene / (–)- $\alpha$ -pinene ratio in *Pinus sylvestris* depending on the plant tissue, the needle extracts generally showed a predominance of (+)- $\alpha$ -pinene [24,25]. Similarly, (+)- $\alpha$ -pinene predominated in the headspace of *P. strobus* [26]. Valterová and co-workers have analyzed the resins of several *Pinus* species growing in Cuba and these workers found (+)- $\alpha$ -pinene to predominate in *P. tropicalis* and *P. cubensis*, but (–)- $\alpha$ -pinene was the major enantiomer in *P. caribaea* [27]. (–)- $\alpha$ -Pinene predominated in *Picea abies* volatiles [28]. (–)- $\beta$ -Pinene dominated (94-96%) the leaf oils of both subspecies of *A. spectabilis*. This has been observed on other members of the Pinaceae, including *Abies* [22, 23], *Pinus* [24-27], and *Picea* [28].

(–)-Limonene was the predominant enantiomer in the leaf oils of both *A. spectabilis* subspecies, 99% in *A. s. densa* and 80% in *A. s. langtangensis*. (–)-Limonene also predominated in *A. sachalinensis*, *A. mayriana* [22], *A. alba*, and *A. sibirica* leaf oils [23]. In *Pinus* spp., the (+)-limonene / (–)-limonene ratio has shown variability, depending on species [23, 27], geographical origin [23], tissue [24], or individual [25]. The (–)-enantiomer did predominate in *Picea abies* needles [28]. The major enantiomer of  $\beta$ -phellandrene in *A. s. densa* was the (–)-enantiomer (99%). It was not possible to determine the distribution of  $\beta$ -phellandrene in *A. s. langtangensis* because

the concentration was too low. (–)- $\beta$ -Phellandrene also dominated the volatiles of *Abies* spp. [22], *Pinus* spp. [24, 26, 27], and *Picea abies* [28].

In contrast to the volatiles from the needles of *Abies alba*, *A. sibirica* [23], *A. sachalinensis*, *A. mayriana* [22], *Pinus sylvestris* [24, 25], *P. montana* [23], and *Picea abies* [28], which were dominated by (–)-camphene, (+)-camphene dominated the essential oils of both subspecies of *A. spectabilis*; exclusively (+)-camphene in *A. s. densa*. (+)- $\delta$ -3-Carene has predominated the enantiomeric distribution in *Pinus* [24, 25], *Picea* [28], as well as *Abies spectabilis* as revealed in this study. (+)- $\delta$ -3-Carene dominated both subspecies (98% and 100%).

Although (+)-borneol dominated *A. s. densa* essential oil (98%), the (+)-enantiomer only accounted for 40% of the distribution in *A. s. langtangensis*. A similar reversal in enantiomeric distribution was observed with  $\alpha$ -terpineol; the (–)-enantiomer predominated in *A. s. densa* (70%), but the (+)-enantiomer predominated in *A. s. langtangensis* (71%). However, the (–)-enantiomer of bornyl acetate was the exclusive stereoisomer in both subspecies. (+)- $\alpha$ -Thujene was the predominant enantiomer in both subspecies of *A. spectabilis*. (+)-Verbenone was the predominant stereoisomer in *A. s. langtangensis* (67%), but verbenone was not detected at all in *A. s. densa*.

#### 4. Conclusions

The essential oil compositions of two subspecies of *Abies spectabilis*, *A. spectabilis* ssp. *densa* from eastern Himalayan Nepal, and *A. spectabilis* ssp. *langtangensis*, from western Himalayan Nepal, have been determined for the first time.

Monoterpenoids dominated the essential oils of both subspecies. In addition, the enantiomeric distribution of the monoterpenoids in the essential oils was determined and showed differences between the two subspecies.

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