

Aroma Chemicals Isolated and Identified from Leaves of *Aloe arborescens* Mill. Var. *natalensis* Berger

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Extracts from leaves of aloe (*Aloe arborescens* Mill. var. *natalensis* Berger) were obtained using two methods: steam distillation under reduced pressure followed by dichloromethane extraction (DRP) and simultaneous purging and extraction (SPE). A total of 123 aroma chemicals were identified in the extracts obtained by both methods using gas chromatography and gas chromatography/mass spectrometry. There were 42 alcohols, 23 terpenoids, 21 aldehydes, 9 esters, 8 ketones, 6 acids, 5 phenols, and 9 miscellaneous compounds. The major aroma constituents of this extract by DRP were (*Z*)-3-hexenol (29.89%), (*Z*)-3-hexenal (18.86%), (*E*)-hexenal (7.31%), 4-methyl-3-pentenol (5.66%), and butanol (4.29%). The major aroma constituents of this extract by SPE were (*E*)-2-hexenal (45.46%), (*Z*)-3-hexenal (32.12%), hexanal (9.14%), (*Z*)-3-hexenol (1.60%), and 3-pentanone (1.41%). Terpenoids were also found as one of the major constituents. The fresh green note of aloe leaves is due to the presence of these C₆ alcohols and aldehydes as well as terpenoids.

Keywords: *Aloe leaves; aroma chemicals; gas chromatography*

INTRODUCTION

There are >300 different species of aloe that grow in the dry regions of the United States, Africa, Asia, and Europe. Aloe has long been used as a folk medicine in many countries including Mexico, China, and Japan. The plant was first mentioned by Celsius (BC 25–50 AD) in Greek pharmacology. Studies on the medicinal characteristics of aloe vera gel began at the beginning of this century. There has been much scientific evidence of the healing properties of aloe leaves in the past three decades. For example, the positive influence of an aqueous extract from aloe leaves on the healing of full-thickness wounds in diabetic rats has been reported (Chithra et al., 1998). The organic solvent extract of aloe leaves has also been reported to possess antiinflammatory activity in the experimental rat (Vazquez et al., 1996).

Analysis of the pharmaceutically active components in aloe leaves has recently begun. Some phenolic compounds have been isolated and identified as an active component of aloe leaf extracts. Several phenolic compounds and their derivatives such as aloesin and hydroxyaloin were separated and identified by high-performance liquid chromatography (HPLC) (Zonta et al., 1995). 1,1-Diphenylethane derivative, which occurs in the drug, was found in Cape aloe (Speranza et al., 1994). Later, the same researcher found two additional phenol derivatives (5-methylchromones) and hypothesized their occurrence in the drug (Speranza et al., 1997). Terminal younger *Aloe hereroensis* leaves contained significantly higher concentrations of the secondary phenolic compound homonataloin than all the lower

and older leaves (Chauser-Volfson and Guterman, 1997). The presence of phenol derivatives in aloe is interesting because some of them are known to possess potent antioxidative activities (Namiki, 1990).

Even though aloe leaf extract has been used widely for manufacturing food products and beverages because of its aroma and taste (Zonta et al., 1995), there are only a few studies on the analysis of aroma chemicals in an aloe leaf extract (Kameoka et al., 1981). In the present study, aroma chemicals isolated from aloe leaves were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

EXPERIMENTAL PROCEDURES

Materials. Aloe leaves (*Aloe arborescens* Mill. var. *natalensis* Berger) were bought from a local market. Authentic compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI), Tokyo Kasei Organic Chemicals (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Fluka Chemical Co. (Ronkonkoma, NY) or obtained from Takata Koryo Co., Ltd. (Osaka, Japan) as a gift. Chemicals not commercially available were synthesized according to published methods.

Isolation of Aroma Chemicals by Steam Distillation under Reduced Pressure (DRP). Aloe leaves (400 g) were cut into small pieces (1 cm) by a knife and homogenized in a blender with 1400 mL of distilled water and 210 mL of saturated sodium chloride solution and then placed in a 3 L round-bottom flask. The solution was steam distilled at 32 °C for 30 min under reduced pressure (30 mmHg). The distillate (900 mL) was extracted with 90 mL of dichloromethane using a liquid–liquid continuous extractor for 4 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by distillation with a Vigreux column. The distillation was stopped when the volume of extract was reduced to 1 mL, and then the solvent was further removed under a purified nitrogen stream until the weight was reduced to 100 mg. The sample was stored at 5 °C until analysis.

Headspace Isolation by Simultaneous Purging and Extraction (SPE). Aloe leaves (370 g) were cut into small

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Table 1. Aroma Chemicals Identified in Aloe Leaf Extracts

compound	<i>I</i> ^b	GC peak area % ^a		compound	<i>I</i> ^b	GC peak area % ^a		
		DRP ^c	SPE ^d			DRP ^c	SPE ^d	
Alcohols				Ketones				
2-methyl-1-propanol	1091	0.11		acetone	820	0.26	<i>e</i>	
3-pentanol	1109	<i>e</i>		2-butanone	923	0.86		
2-pentanol	1119	0.12		3-pentanone	975		1.41	
butanol	1143	4.29	0.89	butane-2,3-dione	977	0.13		
1-penten-3-ol	1158	<i>e</i>	0.67	1-penten-3-one	1017		1.25	
4-methyl-2-pentanol	1168	<i>e</i>		1-methoxypropan-2-one	1100	<i>e</i>		
(<i>E</i>)-2-penten-4-ol	1170	<i>e</i>		acetoine	1280	1.88		
2-methyl-1-butanol + isoamyl alcohol	1207	4.41	0.28	2-hydroxy-2-methyl-4-pentanone	1358	<i>e</i>		
3-methyl-3-buten-1-ol	1248	0.08		Acids				
pentanol	1250	0.40	0.07	3-methylbutyric acid	1674	0.07		
cyclopentanol	1303	<i>e</i>		hexanoic acid	1849	0.59		
(<i>Z</i>)-3-pentenol	1307	<i>e</i>		octanoic acid	2065	0.53		
(<i>E</i>)-2-pentenol	1313	0.06		decanoic acid	2278	0.73		
4-methyl-1-pentanol	1316	0.65		4-hydroxycinnamic acid	2382	0.10		
(<i>Z</i>)-2-pentenol	1321	1.02	0.12	dodecanoic acid	2477	0.30		
hexanol	1354	1.89	0.25	Phenols				
(<i>E</i>)-3-hexenol	1365	0.10		guaiacol	1851	<i>e</i>		
(<i>Z</i>)-3-hexenol	1386	29.89	1.60	phenol	2000	0.15		
4-methyl-3-pentenol	1390	5.66		<i>p</i> -cresol	2084	0.17		
ethylene glycol monobutyl ether	1402	0.26		4-vinylguaiacol	2188	0.32		
(<i>Z</i>)-2-hexenol	1403	0.13	0.32	3- <i>tert</i> -butyl-4-hydroxyanisol	2520	<i>e</i>		
(<i>E</i>)-2-hexenol	1407	2.90		Esters				
(<i>E</i>)-4-hexenol	1413	<i>e</i>		ethyl acetate	908	<i>e</i>		
1-octen-3-ol	1452		<i>e</i>	ethyl butanoate	1033		<i>e</i>	
6-methyl-5-hepten-2-ol	1468	<i>e</i>		2-methylbutyl acetate	1119		0.06	
2-ethyl-1-hexanol	1490		<i>e</i>	(<i>Z</i>)-3-hexenyl acetate	1314		<i>e</i>	
2-methylthioethanol	1526	<i>e</i>		diethyl fumarate	1647	<i>e</i>		
octanol	1560		<i>e</i>	butane-1,4-diol diacetate	1768	<i>e</i>		
2,4-hexadienol	1570	0.07		α -methylbenzyl acetate	1810	0.08		
2,4-hexadienol isomer ^f	1588	<i>e</i>		diethyl phthalate	2358	<i>e</i>		
furfuryl alcohol	1659	<i>e</i>		dibutyl phthalate	1680	0.73		
3,7-dimethyl-1-octanol	1664	<i>e</i>		Terpenoids				
<i>trans-p</i> -mentha-2,8-dien-1-ol	1670	<i>e</i>		<i>d</i> -limonene	1197	<i>e</i>	1.02	
3-hydroxymethyl furan	1679	0.19		γ -terpinene	1240		<i>e</i>	
(<i>E,E</i>)-2,4-heptadien-1-ol	1682	<i>e</i>		<i>p</i> -cymene	1264		<i>e</i>	
(<i>E,Z</i>)-3,5-octadien-1-ol	1736	<i>e</i>		terpinolene	1280		<i>e</i>	
(<i>E,E</i>)-3,5-octadien-1-ol	1742	<i>e</i>		rose oxide	1350	0.22	0.07	
(<i>Z,Z</i>)-3,6-nonadien-1-ol	1759	<i>e</i>		rose oxide isomer ^f	1364	0.06	<i>e</i>	
α,α -dimethyl benzyl alcohol	1759	<i>e</i>		decanal	1491	0.10	<i>e</i>	
benzyl alcohol	1878	3.40		linalool oxide <i>cis</i> -furanoid	1444	<i>e</i>	<i>e</i>	
phenylethyl alcohol	1907	0.26		nerol oxide	1467	0.09	<i>e</i>	
4-methylbenzyl alcohol	1967	<i>e</i>		linalool oxide <i>trans</i> -furanoid	1471	<i>e</i>		
Aldehydes				2-hydroxy-5-ethyltetrahydrofuran	1557	<i>e</i>		
acetaldehyde	690		0.32	β -cyclocitral	1613		<i>e</i>	
2-methyl-(<i>Z</i>)-2-butenal	1012	0.07	<i>e</i>	<i>l</i> -menthol	1642	<i>e</i>		
4-methylpentanal	1039	<i>e</i>	0.26	neral	1676	0.18	<i>e</i>	
2-methyl-(<i>E</i>)-2-butenal	1069	<i>e</i>		α -terpineol	1705	0.06	<i>e</i>	
hexanal	1080	0.16	9.14	geranial	1727	<i>e</i>	<i>e</i>	
(<i>Z</i>)-2-pentenal	1105	0.06	0.26	isopiperitenol	1752	<i>e</i>		
(<i>E</i>)-2-pentenal	1124		0.94	citronellol	1768	<i>e</i>		
(<i>E</i>)-3-hexenal	1134	0.0	1.37	nerol	1801	2.91		
(<i>Z</i>)-3-hexenal	1139	18.88	32.12	<i>p</i> -cymen-8-ol	1849	0.08		
2-methyl-2-pentenal	1155	0.73	0.05	isopulegol	1879	0.29		
(<i>Z</i>)-2-hexenal	1200	0.15	0.98	eugenol	2161	<i>e</i>		
(<i>E</i>)-2-hexenal	1215	7.31	45.46	thymol	2166	<i>e</i>		
octanal	1284		<i>e</i>	Miscellaneous Compounds				
(<i>Z</i>)-2-heptenal	1318		0.06	2-ethylfuran	947		0.20	
6-methyloctanal	1357	0.28		toluene	1036	<i>e</i>	0.18	
nonanal	1389		<i>e</i>	2-pentylfuran	1228		<i>e</i>	
(<i>E</i>)-2-octenal	1423		<i>e</i>	1,2,4-trimethylbenzene	1276		<i>e</i>	
(<i>E,Z</i>)-2,4-heptadienal	1459	0.10		α -methylstyrene	1325	<i>e</i>		
(<i>E,E</i>)-2,4-heptadienal	1487	0.06	<i>e</i>	pentadecane	1500	<i>e</i>	<i>e</i>	
benzaldehyde	1514	<i>e</i>	<i>e</i>	γ -butyrolactone	1620	<i>e</i>		
(<i>E,Z</i>)-2,6-nonadienal	1580		<i>e</i>	γ -hexalactone	1696	<i>e</i>		
				nonadecane	1900		<i>e</i>	

^a Solvent peak excluded. ^b On DB-Wax. ^c Chemicals obtained by steam distillation under reduced pressure. ^d Chemicals obtained by simultaneous purging and extraction. ^e GC peak area % <0.01. ^f Correct isomer not identified.

pieces (1 cm) by a knife and then placed in a 1 L round-bottom flask with 210 mL of saturated sodium chloride solution. The flask was connected to an SPE apparatus previously prepared (Umamo and Shibamoto, 1987). The flask was kept at 40 °C. Headspace aroma chemicals were purged into 200 mL of

distilled water, which was extracted with 30 mL of dichloromethane simultaneously for 2 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by distillation with a Vigreux column. The distillation was stopped when the volume of extract was reduced to 1 mL, and

then the solvent was further removed under a purified nitrogen stream until the weight was reduced to 100 mg. The sample was stored at 5 °C until analysis.

Instrumental Analyses of Components. All samples were analyzed with Kovats GC retention index *I* (Kovats, 1965) and GC/MS. The GC retention index and MS fragmentation pattern of each component were compared with those of the authentic compound for qualitative analysis. A Hewlett-Packard (HP) 5890 Series II GC equipped with a 60 m × 0.25 mm ($d_f = 0.25 \mu\text{m}$) DB-Wax bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) and a flame ionization detector (FID) were used for routine quantitative analysis. The oven temperature was held at 40 °C for 2 min and then programmed to 200 °C at 2 °C/min. Detector and injector temperatures were 250 °C. Linear velocity of helium carrier gas flow rate was 30 cm/s at split ratio of 30:1.

A Varian 3500 GC interfaced to a Finnigan MAT Model 800 ion trap detector was used for MS identification of the GC components. The column and oven conditions were identical to the ones used for the HP GC.

RESULTS AND DISCUSSION

The yields of total aroma chemicals (relative to the fresh aloe leaves used) were 0.0026 and 0.0016% (w/w) from DRP and SPE, respectively. Table 1 shows the compounds identified in extracts obtained from aloe leaves along with their GC peak area percent and Kovats index on a DB-Wax column. A total of 123 components were identified.

The composition of extracts recovered by the two different methods showed significant differences. Among the 101 chemicals identified in the extract obtained by DRP, 42 were alcohols, which composed 59.97% of the total GC peak area. The major aroma constituents of this extract were (*Z*)-3-hexenol (29.89%), (*Z*)-3-hexenal (18.86%), (*E*)-hexenal (7.31%), 4-methyl-3-pentenol (5.66%), and butanol (4.29%). Among 54 chemicals identified in the extract obtained by SPE, 23 were aldehydes, which composed 90.96% of the total GC peak area. The major aroma constituents of this extract were (*E*)-2-hexenal (45.46%), (*Z*)-3-hexenal (32.12%), hexanal (9.14%), (*Z*)-3-hexenol (1.60%), and 3-pentanone (1.41%). (*Z*)-3-Hexenol is a so-called leaf alcohol and possesses a characteristic strong green note. (*Z*)- or (*E*)-3-hexenal is also known to have a green note (Arctander, 1969).

The extracts obtained from aloe leaves possess a strong green note, in particular the ones prepared by SPE. The total GC peak area of (*Z*)- and (*E*)-3-hexenal in the extract from SPE totaled 56.06%; this explains the characteristic strong green aroma of this extract. Fresh aloe leaves have a green note, which might be due to the presence of these C₆ alcohols and aldehydes. Terpenoids, large numbers of which have been found in the essential oils, are also found in the extracts obtained by both DRP and SPE. A total of 23 terpenoids were identified in the present study—19 from DRP and 13 from SPE. Nerol, which has been found in many essential oils and possesses a sweet rosy aroma (Arctander, 1969), was found in the extract from DRP (2.91%) but not in the extract from SPE. On the other hand, *d*-limonene, which is the major monoterpene found in citrus oils and has an orange-like aroma, was found in an extract by SPE in an appreciable level (1.02%) but found in an extract by DRP only at a trace level. The results show that aloe leaves contain many aroma chemicals. In fact, aloe leaves have been used as an aroma ingredient in certain foods and beverages (Zonta et al., 1995) because of their characteristic aroma.

As mentioned above, there are no published studies on the analysis of aroma chemicals in aloe leaves. This may be due to the fact that most analytical efforts have been given to less volatile constituents, such as polyphenols, because of their pharmaceutical properties (Chausser-Volfson and Gutterman, 1997). However, some aroma chemicals have recently been found to possess certain antioxidative activities that are associated with pharmaceutical properties (Singhara et al., 1998). Therefore, the pharmaceutical characteristics of aloe leaves may in part be due to their aroma chemicals.

Two phthalate compounds found in the extracts by DRP must be a contaminant from unknown sources.

It is well-known that the method of isolation of volatile compounds has a great influence on the qualitative and quantitative composition of the extracts (Nitz et al., 1985). In particular, the application of elevated temperatures for distillation causes chemical changes of the original plant components (Fischer et al., 1988). The composition of volatiles in the oil obtained from aloe leaves by steam distillation at 100 °C (Kameoka et al., 1981) was significantly different from the composition of volatiles in the oil obtained in the present study. For example, the oil obtained at the elevated temperature (100 °C) contained a high level of 3-(hydroxymethyl)-furan (31.5%), whereas the oil obtained at the lower temperature in the present study (32 °C) contained a considerably lower level (0.19%). Some compositional changes may be expected during distillation even at a lower temperature. Therefore, headspace sampling has been recommended to obtain aroma chemicals from natural plants (Wyllie et al., 1978). The aroma chemicals recovered by SPE may be most consistent with the ones present in aloe leaves.

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