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Analysis of Volatile Compounds and Enantiomeric Separation of Chiral Compounds of Dried *Sancho* (*Zanthoxylum schinifolium* Siebold & Zucc)

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Abstract The volatile compounds of dried *sancho* (*Zanthoxylum schinifolium*), an aromatic plant were extracted by simultaneous distillation and extraction (SDE) method and identified by gas chromatograph-mass spectrometry (GC-MS). Selected chiral constituents of *sancho* oil were characterized by enantiodifferentiation using multidimensional gas chromatograph (MDGC)-MS. A total of 57 compounds were identified and quantified, and the major compounds were identified estragole, nonanoic acid, octanoic acid, β-phellandrenene, and limonene. Among them, estragol (63.9%) was found as the predominantly abundant component of *sancho*. α-Pinene and nerolidol, and β-pinene and linalool were determined to be enantiomerically pure (100%) for their (*S*)-form and (*R*)-form, respectively. The enantiomeric composition of limonene in *sancho* revealed 83.9% purity for the (*S*)-enantiomer, whereas (*E*)- and (*Z*)-rose oxides showed mixtures of both enantiomers. The enantiomeric excess (%) for citronellal was 22.6% with the (*R*)-enantiomer as major enantiomer. The enantiomeric composition of these compounds can be used as parameter for authenticity control of *sancho*.

Keywords: *Zanthoxylum schinifolium*, essential oil, enantiomeric composition, multidimensional gas chromatograph

Introduction

Sancho (*Zanthoxylum schinifolium*) Siebold & Zucc (in Korean) is a deciduous shrub belonging to the *sancho* species of the Rutaceae family, and is distributed in China, Korea, and Japan. Containing pungent compounds (sanshools), essential oil, and flavonoids, it has been widely used in Korea not only as a condiment, but also as a medicinal material for the treatment of vomiting, diarrhea, abdominal pain, ascariasis, and dermal diseases for a long time (1,2).

Recently, consumption of aromatic medicinal plants such as *sancho* has been increasing due to the increasing interest in personal health. Among the constitutes of these plants, the essential oil is well known to have biological activities and considerable commercial value either as a raw material for precious fragrance compositions or as a mass product for flavoring food, beverages, cosmetics, and detergents (3-6). A large proportion of essential oil constituents, mainly terpenoid compounds, are chiral (7), and the sensory properties and biological activities differ between enantiomers (8). Moreover, the chiral components that occur in nature are enantiomeric mixtures and the enantiomeric proportion of chiral components is often specific to the natural source or origin (9). Chirality evaluations have already been proven to be a reliable indicator for adulteration (10,11) and the enantiomeric purity of linalool and linalyl acetate has been adopted by European Pharmacopoeia Commission in monograph No. 1338 Lavender Oil of European Pharmacopoeia (12).

Essential oils from various species of *sancho* have been widely investigated, however mostly from different species in China, Japan, Vietnam, India, etc (13). In Korea, some of the research into the essential oils of *sancho* was carried out on the fresh leaves and fruits, but no adequate research has been conducted on the essential oils of commercial dried products. Specially, the chiral compounds in the essential oil from *sancho* have not been analyzed for their enantiomeric composition. In the present investigation, we analyzed the volatile compounds of *sancho* and evaluated the characteristics of its essential oils. In addition, to provide useful data for authenticity evaluation of *sancho*, enantiomeric compositions were determined by multidimensional gas chromatograph-mass spectrometry (MDGC-MS).

Materials and Methods

Materials Dried *sancho* (*Zanthoxylum schinifolium* Siebold & Zucc) fruits were purchased from a dispensary of Korea Medicine Herbal, Gwangju, Korea, in August 2005. This sample was stored at −18°C until required for the experiments.

Chemicals All the reagents used in the experiments were purchased from Sigma-Aldrich (Steinheim, Germany) and Fisher Scientific (Schwerte, Germany). The organic solvents used for the extraction and chromatography were redistilled before use and Milli-Q water was generated with

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a water purification system (S.A. 67120; Millipore Co., Molshem, France). Authentic references were commercially available from Fluka (Deisenhofen, Germany) and Sigma-Aldrich.

Extraction of volatile compounds One-hundred g samples were taken and homogenized in a blender (MR 350CA; Braun, Alcobendas, Spain) and mixed with 1 L distilled water. While maintaining the pH value at 6.5, the resultant slurry was used for the quantitative analysis with 1 μL of *n*-butyl benzene added as an internal standard. The sample were subjected for 2 hr with 200 mL *n*-pentane/diethyl ether (1:1, v/v) to simultaneous distillation and extraction (SDE, Likens & Nickerson type) apparatus as modified by Nikerson and Likens (14) and Schultz *et al.* (15) under atmospheric pressure. The extracts were dehydrated for 12 hr over anhydrous sodium sulfate, and carefully concentrated to approximately 1.5 mL using a Vigreux column (45°C) and subsequently subjected to GC-MS analysis.

GC-MS For quantitative GC-MS analysis a HP Agilent 6890 Series gas chromatograph with split injection (220; 1:20) directly coupled to an HP Agilent 5973 Network mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) was used. The electron energy for the electron impact (EI)-mass spectra and temperature of ion source were 70 eV and 220°C. The mass spectrometer scanned from 38 to 450 *m/z*. The volatile compounds were separated on a DB-Wax fused silica capillary column (30×0.25 mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA, USA). The temperature program was as follows: 3 min isothermal at 50°C, then raised at 4°C/min to 220°C. Identification was performed by comparison of linear retention indices and mass spectrometric data of sample constituents with that of authentic reference compounds. Mass spectra of volatile compounds were identified with the aid of our own mass spectral data of authentic reference compounds and those contained within the WILEY275, and NIST98 libraries and mass spectral data books (16,17) as well as by the comparison of retention indices to reference data (18,19). The quantitative analysis was carried out with the help of peak area percent of internal standard (*n*-butyl benzene) and not considered detector responses of each chemical.

MDGC-MS A double-oven MDGC system (oven I, Fisons GC 8160; oven II, Fisons GC 8130) coupled to a Fisons MD 800 mass spectrometer (Fisons Instruments, Mainz, Germany) was used. The chiral target compounds were transferred from the precolumn to the main column via a moving column stream switching (MCSS) system at defined cut time. The preseparation was performed using a DB-Wax column (30×0.25 mm i.d., 0.25-μm film thickness, J&W Scientific) and cut times were determined with a flame-ionization detector (FID) as monitor detector. As transfer lines between the GC ovens I and II as well as the GC oven II and the mass spectrometer, deactivated fused silica capillaries (0.5 m×0.25 mm i.d.), each kept at 200°C were used. Further enantiomeric separation was performed using 2 different types of modified cyclodextrin phase (Mega, Legnano, Italy). Separation of α- and β-pinene, limonene, (*E*)- and (*Z*)-rose oxide, linalool, and nerolidol enantiomers were performed on a fused-silica column coated with 2,3-diethyl-6-*tert*-butyldimethyl-silyl-β-cyclodextrin in PS 086 (25 m×0.25 mm i.d., 0.15-μm film thickness) and citronellal enantiomers were separated on a fused-silica column coated with 2,3-diacetyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin in OV1701 (25 m×0.25 mm i.d., 0.15-μm film thickness). For cut times and temperature programs see Table 5. The MS conditions were as follows: the carrier gas was helium at 1.6 mL/min; temperature of ion source and connecting parts were 220 and 230°C, respectively. The ionization voltage for EI-mass spectra was 70 eV. The enantiomeric excess were calculated from peak areas obtained from the total ion chromatogram and excess of major enantiomer was expressed as %, i.e., [(major enantiomer−minor enantiomer)/(major enantiomer+minor enantiomer)]×100 (20).

Results and Discussion

Volatile compounds of *sancho* The volatile compounds from *sancho* were extracted using simultaneous distillation and extraction (SDE) and subsequently detected and identified by GC-MS. The GC/MS chromatogram is shown in Fig. 1, and the identified volatile components from GC-MS analysis and their relative peak area percentages are shown in Table 1.

The volatile oils in *sancho* were obtained at a yield of

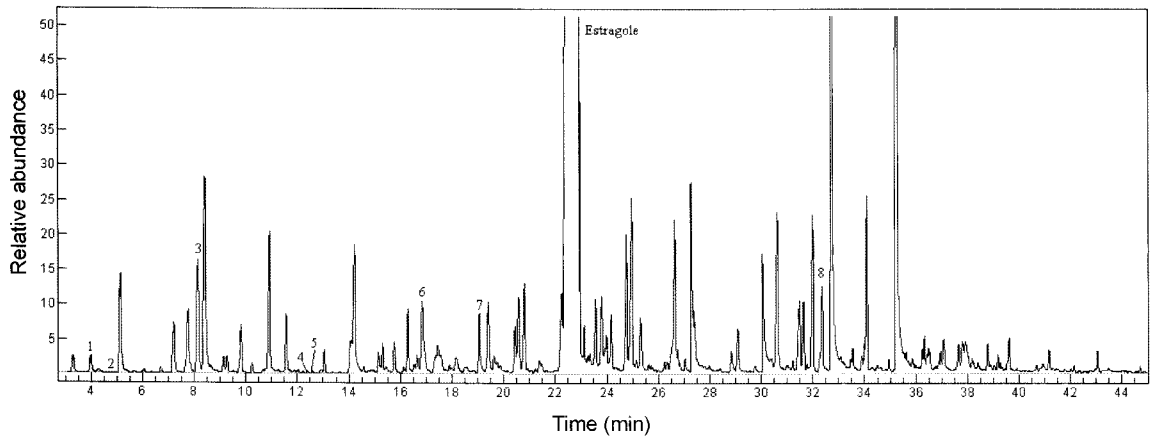


Fig. 1. GC/MS chromatogram of volatile compounds from dried *sancho*.

Table 1. Volatile components identified from dried *sancho*[1)]

RI	Compound name	MF	Mw	mg/kg	Area%	ID	No.
1018	α-Pinene	$C_{10}H_{16}$	136	5.1	0.23	RI, MS, STD	1
1077	Hexanal	$C_6H_{12}O$	100	28.8	1.33	RI, MS	
1101	β-Pinene	$C_{10}H_{16}$	136	0.3	0.01	RI, MS, STD	2
1116	Sabinene	$C_{10}H_{16}$	136	1.2	0.05	RI, MS	
1129	2-Butyl furan	$C_8H_{12}O$	124	0.2	0.01	RI, MS	
1142	Butanol	$C_4H_{10}O$	74	1.3	0.06	RI, MS	
1160	β-Myrcene	$C_{10}H_{16}$	136	14.2	0.66	RI, MS	
1179	Heptanal	$C_7H_{14}O$	114	18.9	0.87	RI, MS	
1191	Limonene	$C_{10}H_{16}$	136	32.3	1.50	RI, MS, STD	3
1199	β-Phellandrene	$C_{10}H_{16}$	136	56.3	2.61	RI, MS	
1226	5-Pentyl furan	$C_9H_{14}O$	138	3.1	0.14	RI, MS	
1231	(*Z*)-β-Ocimene	$C_{10}H_{16}$	136	3.7	0.17	RI, MS	
1249	Pentanol	$C_5H_{12}O$	88	10.8	0.50	RI, MS	
1263	γ-Cymene	$C_{10}H_{16}$	136	1.9	0.09	RI, MS	
1275	α-Terpinolene	$C_{10}H_{16}$	136	0.6	0.03	RI, MS	
1278	2-Octanone	$C_8H_{16}O$	128	1.0	0.05	RI, MS	
1282	Octanal	$C_8H_{16}O$	128	33.4	1.55	RI, MS	
1302	Butylbenzene	$C_{10}H_{14}$	134	-	-	-	
1346	(*Z*)-Rose oxide	$C_{10}H_{18}O$	154	1.1	0.05	RI, MS, STD	4
1351	Hexansol	$C_6H_{14}O$	102	3.5	0.16	RI, MS	
1361	(*E*)-Rose oxide	$C_{10}H_{18}O$	154	0.3	0.01	RI, MS, STD	5
1381	2-Nonanone	$C_9H_{18}O$	142	8.0	0.37	RI, MS	
1385	Nonanal	$C_9H_{18}O$	142	29.8	1.38	RI, MS	
1435	(*Z*)-Linalool oxide	$C_{10}H_{18}O_2$	170	5.5	0.26	RI, MS	
1452	Heptanol	$C_7H_{16}O$	116	10.3	0.48	RI, MS	
1463	(*E*)-Linalool oxide	$C_{10}H_{18}O_2$	170	2.8	0.13	RI, MS	
1469	Citronellal	$C_{10}H_{18}O$	154	19.1	0.89	RI, MS, STD	6
1480	α-Copaene	$C_{15}H_{24}$	204	0.7	0.03	RI, MS	
1484	Methyl nonanoate	$C_{10}H_{20}O_2$	172	6.2	0.29	RI, MS	
1488	Decanal	$C_{10}H_{20}O$	156	7.0	0.32	RI, MS	
1515	2-Nonanol	$C_9H_{20}O$	144	0.3	0.01	RI, MS	
1521	(*E*)-2-Nonenal	$C_9H_{16}O$	140	1.8	0.08	RI, MS	
1541	Linalool	$C_{10}H_{18}O$	154	9.9	0.46	RI, MS	7
1552	Octanol	$C_8H_{18}O$	130	12.7	0.59	RI, MS	
1578	β-Elemene	$C_{15}H_{24}$	204	6.9	0.32	RI, MS	
1582	(*E*)-β-Caryophyllene	$C_{15}H_{24}$	204	13.9	0.64	RI, MS	
1589	2-Undecanone	$C_{11}H_{22}O$	170	16.2	0.75	RI, MS	
1633	(*E*)-2-Decenal	$C_{10}H_{18}O$	154	16.3	0.75	RI, MS	
1661	Estragole	$C_{10}H_{12}O$	148	1378.7	63.91	RI, MS, STD	
1663	α-Humulene	$C_{15}H_{24}$	204	18.7	0.87	RI, MS	
1735	(*E*)-2-Undecenal	$C_{11}H_{20}O$	168	21.3	0.99	RI, MS	
1740	δ-Cadinene	$C_{15}H_{24}$	204	3.9	0.18	RI, MS	
1744	Geranyl acetate	$C_{12}H_{20}O_2$	196	24.0	1.11	RI, MS	
1750	β-Citronellol	$C_{10}H_{20}O$	156	0.7	0.03	RI, MS	
1790	(*E,E*)-2,4-Decadienal	$C_{10}H_{16}O$	152	1.2	0.06	RI, MS	
1835	Hexanoic acid	$C_6H_{12}O_2$	116	19.6	0.91	RI, MS	
1889	γ-Octalactone	$C_8H_{14}O_2$	142	3.6	0.17	RI, MS	
1946	Heptanoic acid	$C_7H_{14}O_2$	130	9.3	0.43	RI, MS	
1958	Caryophyllene oxide	$C_{15}H_{24}O$	220	28.8	1.33	RI, MS	
2013	Humulene oxide	$C_{15}H_{24}O$	220	27.9	1.29	RI, MS	
2026	Nerolidol	$C_{15}H_{26}O$	222	16.5	0.76	RI, MS, STD	8
2045	Octanoic acid	$C_8H_{16}O_2$	144	84.2	3.90	RI, MS, STD	
2101	Spathulenol	$C_{15}H_{24}O$	220	26.5	1.23	RI, MS	

Table 1. Continued

RI	Compound name	MF	Mw	mg/kg	Area%	ID	No.
2152	Nonanoic acid	$C_9H_{18}O_2$	158	100.8	4.67	RI, MS, STD	
2425	Methyl oleate	$C_{19}H_{36}O_2$	296	3.7	0.17	RI, MS, STD	
2473	Methyl linoleate	$C_{19}H_{34}O_2$	294	1.3	0.06	RI, MS, STD	
2500	Ethyl linoleate	$C_{20}H_{36}O_2$	308	0.9	0.04	RI, MS	
2502	Methyl linolenate	$C_{19}H_{32}O_2$	292	0.7	0.03	RI, MS, STD	

[1)]RI, retention index; MF, molecular formula; Mw, molecular weight; MS, mass spectrum; STD, standard mass spectrum.

Table 2. Relative content of functional groups in identified volatile components from dried *sancho*

Functional group	No.	mg/kg	Relative area%
Acids	4	213.9	9.9
Alcohols	10	91.8	4.3
Aldehydes	10	178.1	8.2
Esters	6	36.8	1.7
Ether	1	1,378.7	63.9
Ketones	4	28.8	1.4
Hydrocarbons	14	159.6	7.4
Miscellaneous	8	69.6	3.2
Total	57	2,157.3	100

0.2% and a total of 57 compounds were identified and quantified in the oil, comprising 14 hydrocarbons, 10 alcohols, 10 aldehydes, 6 esters, 4 acids, 4 ketones, 1 ether, and 8 miscellaneous, see Table 2.

Ether (estragole, 63.9%) constituted one of the main functional groups in the volatile compounds of *sancho*. Estragole (4-methoxyallylbenzene) is the structurally related to *p*-allylalkoxybenzene which occurs naturally in a variety of traditional foods and spices such as tarragon, basil, fennel, star anise, and anise (21). It has antibacterial and antifungal activities and it is particularly known to inhibit the growth of *Escherichia coli*, *Streptomyces aureus*, *Bacillus subtilis*, and *Vibrio parahaemolyticus* (22-24). Estragole is the major component of *sancho* fruit and a minor component of the leaves. However, it is progressed to the leaves according to the stage of fruit ripening (25,26).

The following aliphatic acids (9.9%) constituted the second functional group in the sample: hexanoic, heptanoic, octanoic, and nonanoic acid. Octanoic and nonanoic acid were the main acids, and contained 3.9 and 5.0%, respectively. These results were in agreement with the report by Lee *et al.* (27), presumably because the sample is a seed of *sancho*.

Aldehydes constituted 8.2% of the volatile compounds, with octanal (1.60%), nonanal (1.4%), and hexanal (1.3%) as the main constituents. Citronellal was also detected and most of the aldehydes were aliphatic aldehydes.

The main hydrocarbons, consisting of mono- and sesquiterpenes totaling 7.4%, were β-phellandrene (2.6%) and limonene (1.5%). Also detected in considerable amounts were β-myrcene, α-humulene, and β-caryophyllene. Although other mono- and sesquiterpenes were detected only at levels lower than 0.5%, the total amount of these compounds was considerable.

Alcohols constituted 4.3% of the volatile compounds, spathulenol, nerolidol, linalool, and β-citronellol, which are terpene alcohols and butanol, pentanol, hexanol, heptanol, octanol, and 2-nonanol which can occur naturally after hydrolysis or enzymatic reduction reactions were detected. Ketones (1.4%) such as 2-octa/nona/undecanone and esters (1.7%) such as geranyl acetate, methyl- and ethyl of fatty acids were the minor compounds in the volatile compounds of *sancho*. In addition, furans (2-butyl and 5-pentyl furan) and oxides (rose, linalool, caryophyllene, and humulene oxide) were detected.

The percentage composition of the essential oil provides probably the most important parameter to characterize the respective plant (28). To allow a comparison, the essential oil groups of volatile organic compounds identified from Table 1 are summarized in Table 3. Thirty compounds of the volatile constituents from *sancho* were terpenoids, which constituted 80% of the total volatile contents.

Oxygenated monoterpenes (68.0%) iders 2916 (2001)s estersrs represented the larger fraction of *sancho* oil and mostly consisted of estragole (63.9%) and geranyl acetate (1.1%). Cho *et al.* (26) reported that estragole was detected at above 70% and that geranyl acetate not detected. However, Lee *et al.* (27) and Lee and Chung (29) detected geranyl acetate at 11.1 and 23.9%, respectively, and did not detect estragol. These results prove that estragol is progressed to the leaves according to the stage of fruit ripening (25,26), and that the *sancho* fruit used by Lee *et al.*(27) and Lee and Chung (29) maybe have been much more ripened than the sample used by Cho *et al.* (26) and this study. Monoterpene hydrocarbons represented 5.4% of

Table 3. Relative concentration by terpenoid groups in dried *sancho*

Functional group		mg/kg	%Total
Monoterpenes (C_{10})	Monoterpenes	115.5	5.4
	Oxygenated monoterpenes	1,466.6	68.0
Sesquiterpenes (C_{15})	Sesquiterpenes	44.0	2.0
	Oxygenated sesquiterpenes	99.7	4.6
Total		1,725.8	80.0

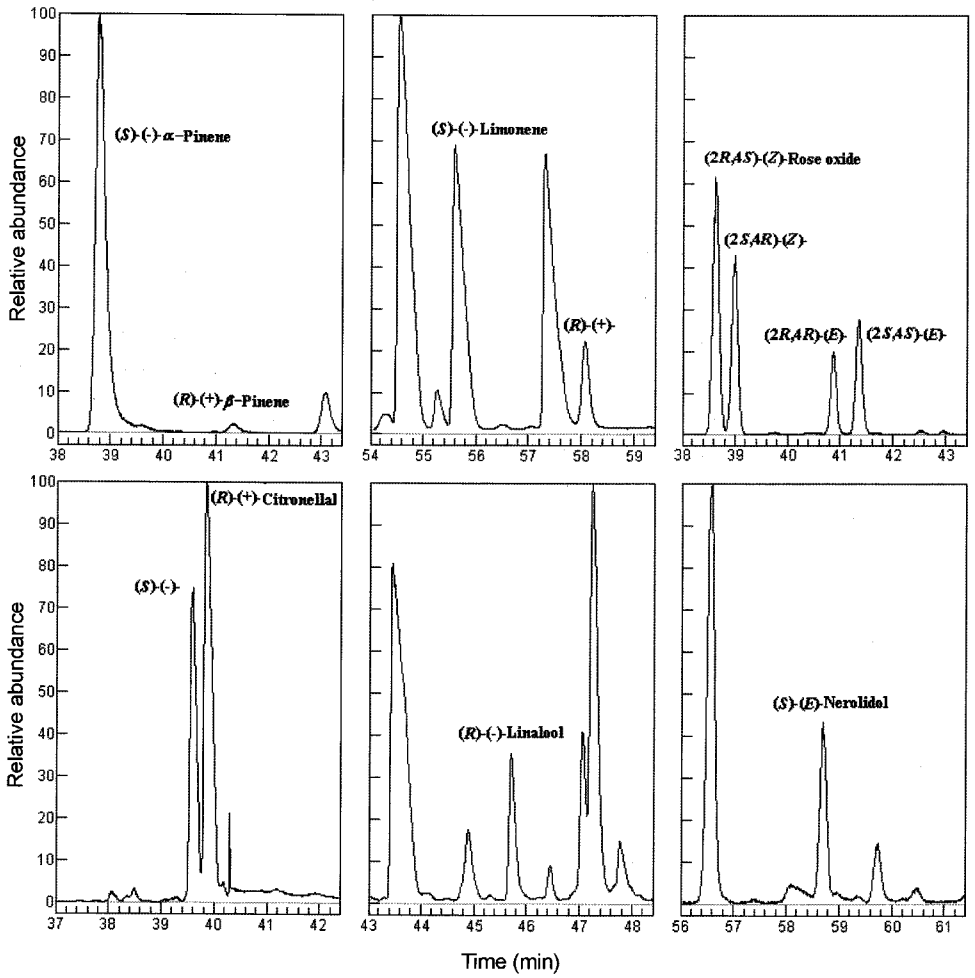


Fig. 2. MDGC/MS chromatograms of selected chiral compounds in dried *sancho*.

the volatile composition in *sancho* of which β-phellandrene and limonene were the main compounds. The composition of sesquiterpenes was 6.7%, consisting of 2.0% hydrocarbons and 4.6% oxygenated compounds. These mono- and sesquiterpenes stimulate a wide spectrum of aromas, most of which are perceived as being very pleasant (30).

Enantiomeric composition of chiral compounds of *sancho* oil In order to provide information to support authenticity control, the enantiomeric composition of chiral compounds which are α- and β-pinene, limonene, (*E*)- and (*Z*)-rose oxides, citronellal, linalool, and nerolidol selected among flavor of dried *sancho* were determined by MDGC-MS. Rose oxide and nerolidol were detected on *m/z* 139 and 161, respectively, because of co-elution of other compounds (Fig. 2). The characteristics of these compounds are shown in Table 4, and their enantiomeric compositions are summarized in Table 5 in which the MDGC-MS conditions are also outlined.

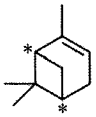
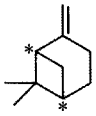
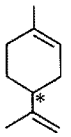
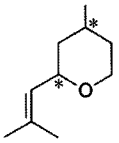
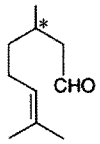
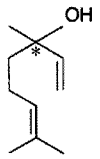
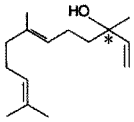
α- And β-pinene were detected enantiomerically pure in the (*S*)-form and (*R*)-form, respectively. In a study on the enantioselective analysis of essential oil compounds (31), the enantiomeric purity of α-pinene in thyme, tea tree, and eucalyptus oil was 89-93, 86-91, and 93-99% in the (*R*)-form, respectively. In addition, it has been reported that β-pinene showed mixtures of both enantiomers in these oils (31), and also in the case of *Mentha piperita* plants (32). Consequently, the enantiomeric compositions of α- and β-pinene in *sancho* oil were characteristic in comparison with those of other plants.

The enantiomeric composition of limonene in *sancho* was 83.9% for (*S*)-enantiomer. In fruit such as lemon, grapefruit, and orange, their enantiomeric ratio was enantiomerically pure up to 98% for (*R*)-enantiomer (33, 34), confirming that the enantiomeric ratio of limonene was characteristic in *sancho*. Whereas the (-)-*S*-enantiomer has a turpentine (mint) like odor, the (+)-*R*-enantiomer is associated with an orange-type smell (35).

Although (*E*)- and (*Z*)-rose oxides are the minor compounds present only in trace amounts, their enantiomers were clearly separated at the selected ion monitoring (SIM) mode. In geranium oil, (2*S*,4*R*)-(*Z*)- and (2*R*,4*R*)-(*E*)-rose oxides are predominant (36) while (2*S*,4*SR*)-(*Z*)-rose oxide occurs more profusely in nature (37). However, they showed mixtures of both enantiomers in *sancho*, and the enantiomeric excess percentage of (*E*)- and (*Z*)-rose oxides were 19.4 and 18.2% with the (*R*)- and (*S*)-enantiomer as the main enantiomer, respectively.

The enantiomeric excess percentage of citronellal was 22.6% with the (*R*)-enantiomer as the major enantiomer. Citronellal (3,7-dimethyl-6-octanal), a monoterpene which occurs in the L or D form, bears distinct odor characteristics

Table 4. Chiral compounds identified in dried *sancho*

No.	Compounds	Structure	Enantiomer	Odor description[1)]	Threshold[1)]
1	α-Pinene		(1*R*,5*R*)-(+)- (1*S*,5*S*)-(-)-	Harsh, terpene-like, minty Harsh, terpene-like, coniferous	2,100 3,300 (in air)
2	β-Pinene		(1*R*,5*R*)-(+)- (1*S*,5*S*)-(-)-	(Musty, green, sweet, pine, resin, turpentine, woody)	140[2)]
3	Limonene		(*R*)-(+)- (*S*)-(-)-	Fresh citrus, orange-like Harsh, turpentine-like, lemon note	200 500
4	Rose oxide		cis-(2*R*,4*S*)-(+)- cis-(2*S*,4*R*)-(-)- trans-(2*R*,4*R*)-(-)- trans-(2*S*,4*S*)-(+)-	Floral, green, clean, sharp, metallic, light, rose, green Herbal, green, floral, hay, earthy, heavy Floral, green, herbal (minty), fruity Herbal, green, floral, fruity, rose, citrus (bitter peel)	50 0.5 160 80
5	Citronellal		(*R*)-(+)- (*S*)-(-)-	Powerful, fresh, herbaceous-citrus Powerful, fresh, bright clean, herbaceous-citrus	25 25
6	Linalool		(*R*)-(-)- (*S*)-(+)-	Floral, woody lavender note Sweet, floral; odor reminiscent of petitgrain and lavender	0.8 7.4
7	(*E*)- Nerolidol		(*R*)-(+)- (*S*)-(-)-	Pleasant, woody, warm, musty Slightly sweet, mild, soft, flowery	120[2)]

[1)]Leffingwell & Associates, USA, [2)]Scent and Fragrances, Springer-Verlag (Ref. no. 35); *Chiral center.

Table 5. Enantiomeric distribution of chiral compounds from dried *sancho*

Compound	Cut time (min)	Temperature program		Enantiomeric ratio		ee (%)[1)]
		Oven I	Oven II	R	S	
α-Pinene	3.25-4.25	40-220°C (1°C/min)	40 (20 min)-200°C (1°C/min)	0	100	100
β-Pinene	5.20-6.20	40-220°C (1°C/min)	40 (20 min)-200°C (1°C/min)	100	0	100
Limonene	4.47-5.26	40-220°C (10°C/min)	40 (20 min)-200°C (1°C/min)	16.1	83.9	67.8
(*Z*)-Rose oxide	6.25-7.15	60-220°C (5°C/min)	60 (15 min)-200°C (1°C/min)	59.1	40.9	18.2
(*E*)-Rose oxide	6.25-7.15	60-220°C (5°C/min)	60 (15 min)-200°C (1°C/min)	40.3	59.7	19.4
Citronellal	6.24-6.84	60-220°C (10°C/min)	60 (15 min)-200°C (1°C/min)	61.3	38.7	22.6
Linalool	6.94-7.50	60-220°C (10°C/min)	60 (15 min)-200°C (1°C/min)	100	0	100
Nerolidol	8.35-8.85	100-220°C (10°C/min)	100 (20 min)-200°C (1°C/min)	0	100	100

[1)]Enantiomeric excess.

and also occurs enantiomerically pure (*R*, 91.2%) as a major constituent of citronella oil (38). Its enantiomeric ratio varies slightly from plant to plant.

Linalool and nerolidol were detected enantiomerically pure for (*R*)- and (*S*)-enantiomer, respectively. The high enantiomeric purity of linalool was the same in Japanese pepper (*Zanthoxylum piperitum* D.C.), which is the same species (39). It is known that plants mostly produce only one linalool isomer, so that the enantiomeric excess can be used as an indicator for authenticity. However it cannot be parameter to distinguish between *sancho* and *Z. piperitum*. The enantiomeric composition of nerolidol was not

available in the literature.

Consequently, the findings presented here confirm that the enantiomeric composition of α- and β-pinene, limonene, (*E*)- and (*Z*)-rose oxides, chitronellal, and nerolidol can be used as parameter for the authenticity control of *sancho*.

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References

1. Choi YJ. The Korean Folklore Plants. Academybook, Seoul, Korea. pp. 191-193 (1992)
2. Ko YS, Han HJ. Chemical constituents of Korean *chopi* (*Zanthoxylum piperitum*) and *sancho* (*Zanthoxylum schinifolium*). Korean J. Food Sci. Technol. 28: 19-27 (1996)
3. Janssen AM, Scheffer JJ, Baerheim SA. Antimicrobial activities of essential oils. A 1976-1986 Lature review on possible applications. Pharm. Weekblad 9: 193-197 (1978)
4. Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios 89: 39-46 (1997)
5. Grassmann J, Hippeli S, Dornisch K, Rohnert U, Beuscher N, Elstner EF. Antioxidant properties of essential oils: Possible explanations for their anti-inflammatory effects. Arznei.-Forschung 50: 135-139 (2000)
6. Dang MN, Takacsova M, Nguyen DV, Kristianova K. Antioxidant activity of essential oils from various spices. Nahrung 45: 64-66 (2001)
7. König WA, Fricke C, Saritas Y, Momeni B, Hohenfeld G. Adulteration or natural variability? Enantioselective gas chromatography in purity control of essential oils. J. High Res. Chromatog. 20: 55-61 (1997)
8. Ahuja S. Chiral Separations, Applications, and Technology. American Chemical Society, Washington, DC, USA. p. 201 (1997)
9. Marchelli R, Dossena A, Palla G. The potential of enantioselective analysis as a quality control tool. Trends Food Sci. Tech. 7: 113-119 (1996)
10. Schreier P, Winterhalter P. Progress in Flavour Precursor Studies. Allured Publishing Corporation, Carol Stream, IL, USA. p. 77 (1993)
11. Möllenbeck S, König T, Schreier P, Schwab W, Rajaonarivony J, Ranarivelo L. Chemical composition and analyses of enantiomers of essential oils from Madagasca. Flavour Frag. J. 12: 63-69 (1997)
12. Council of Europe. European Pharmacopoeia. 5th ed. Volume 1. European Directorate for the Quality of Medicines. Strasbourg, France. pp. 1894-1895 (2004)
13. Weyerstahl P, Marschall H, Splittgerber U, Son PT, Giang PM, Kaul VK. Constituents of the essential oil from the fruits of *Zanthoxylum rhetsoides* Drake from Vietnam and from the aerial parts of *Zanthoxylum alatum* Roxb. from India. Flavour Frag. J. 14: 225-229 (1999)
14. Nikerson GB, Likens ST. Gas chromatography evidence for the occurence of hop oil components in beer. J. Chromatogr. 21: 1-5 (1966)
15. Schultz TH, Flath RA, Mon TR, Enggling SB, Teranishi R. Isolation of volatile components from a model system. J. Agr. Food Chem. 25: 446-449 (1977)
16. Sadtler Research Laboratories. The Sadtler standard gas chromatography retention index library. Sadtler, PA, USA (1986)
17. Davies MW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. J. Chromatogr. 503: 1-24 (1990)
18. Stehagen ES, Abbrahansom F, Mclafferty W. The Wiley/NBS Registry of Mass Spectral Data. John Wiley and Sons, New York, NY, USA (1974)
19. Robert PA. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corp., Carol Stream, IL, USA (1995)
20. Schurig V. Terms for the quantitation of a mixture of stereoisomers. Enantiomer 1: 139-143 (1996)
21. Ruff C, Hör K, Weckerle B, König T, Schreier P. Authenticity assessment of estragole and methyl eugenol by on-line gas chromatography-isotope ratio mass spectrometry. J. Agr. Food Chem. 50: 1028-1031 (2002)
22. Bahk JR, Park SH, Kim JO, Kim SW, Lee SY. The effect of estragole identified and extracts form *Agastache rugosa* O. Kuntze on the fungal growth and metabolism. J. Food Hyg. Saf. 12: 63-70 (1997)
23. Wan J, Wilcock A, Coventry MJ. The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. J. Appl. Microbiol. 84: 152-158 (1998)
24. Shin S, Kang CA. Antifungal activity of the essential oil of *Agastache rugosa* Kuntze and its synergism with ketoconazole. Lett. Appl. Microbiol. 36: 111-115 (2003)
25. Chae YA, Cho MG, Song JS. Identification of chemotypes in traditional aromatic plant resources *Z. schinifolium* Siebold et Zucc. and *Z. piperitum* DC. Korean J. Breed. 33: 126-132 (2001)
26. Cho MG, Kim H, Chae YA. Analysis of volatile compounds in leaves and fruits of *Zanthoxylum schinifolium* Siebold et Zucc. & *Zanthoxylum piperitum* DC. Korean J. Med. Crop Sci. 11: 40-45 (2003)
27. Lee JG, Jang HJ, Kwag JJ, Kim OC, Lee GH. Analysis of volatile components of *sancho* (*Zanthoxylum schnifolium*) by solid phase micro extraction. Korean J. Food Nutr. 12: 119-123 (1999)
28. Paula CSG, Manuel FF. Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. J. Agr. Food Chem. 49: 2908-2916 (2001)
29. Lee MS, Chung MS. Analysis of volatile flavor components from *Zanthoxylum schinifolium* and sensory evaluation as natural spice. Korean J. Soc. Food Sci. 16: 216-220 (2000)
30. Belitz H-D, Grosch W. Food Chemistry. Springer-Verlag, Berlin, Germany. pp. 282-290 (1987)
31. Kreck M, Scharrer A, Bilke S, Mosandl A. Enantioselective analysis of monoterpene compounds in essential oils by stir bar sorptive extraction (SBSE)-enantio-MDGC–MS. Flavour Frag. J. 17: 32-40 (2002)
32. Ruiz del Castillo ML, Blanch GP, Herraiz M. Natural variability of the enantiomeric composition of bioactive chiral terpenes in *Mentha piperita*. J. Chromatogr. A 1054: 87-93 (2004)
33. Schurig V, Nowotny H-P. Separation of enantiomers on diluted permethylated γ-cyclodextrin by high-resolution gas chromatography. J. Chromatogr. 441: 155-163 (1988)
34. Fietzek C, Hermle T, Rosenstiel W, Schurig V. Chiral discrimination of limonene by use of β-cyclodextrin-coated quartz-crystal-microbalances (QCMs) and data evaluation by artificial neuronal networks. Fresen. J. Anal. Chem. 371: 58-63 (2001)
35. Ohloff G. Scent and Fragrances. Springer-Verlag, Berlin, Germany. pp. 43-44 (1990)
36. Wüst M, Rexroth A, Beck T, Mosandl A. Structure elucidation of *cis*- and *trans*-rose oxide ketone and its enantioselective analysis in geranium oils. Flavour Frag. J. 12: 381-386 (1997)
37. Dietrich A, Mass B, Karl V, Volker K, Peter DL, Weber B, Mosandl A. Stereoisomeric flavor compounds: Part LV. Stereodifferentiation of some chiral volatiles on heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimet hylsily1)-β-cyclodextrin. J. High Res. Chromatog. 15: 176-179 (1992)
38. Lorenzo D, Dellacassa E, Atti-Serafini L, Santos A, Frizzo C, Paroul CN, Moyna P, Mondello L, Dugo G. Composition and stereoanalysis of *Cymbopogon winterianus* Jowitt oil from Southern Brazil. Flavour Frag. J. 15: 177-181 (2000)
39. Bonnländer B, Cappuccio R, Liverani FS, Winterhalter P. Analysis of enantiomeric linalool ratio in green and roasted coffee. Flavour Frag. J. 21: 637-641 (2006)