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Analysis of Volatile Compounds of *Curcuma longa* (Turmeric) and Investigation of the Antioxidant Activity of Rhizome Extracts

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Abstract

Fragrances originating from plants are widely believed to have therapeutic properties. The volatile compounds originating from *Curcuma longa* (turmeric) plant cultivated in a medicinal plant garden located in southern Tokyo were investigated using thermal desorption-gas chromatography-mass spectrometry. Sampling from rhizomes of *C. longa* was performed at three different development stages, i.e., July (when young rhizomes emerge), September (flowers bloom), and November (ready for harvest). Using a polydimethylsiloxane (PDMS)-coated bar as an adsorption device for volatile compounds, 1,8-cineol, α-terpinolene, β-caryophyllene, and ar-curcumene were the predominant constituents in most cases. Additional volatile compounds such as *α*-terpinene, *p*-cymene, and (*E*)-β-farnesene were identified when PDMS/carboxene/divinylbenzene-coated fiber was used. ar-Turmerone was found from ripened rhizomes (in September and November). The leaves of *C. longa* yielded the same compounds as the rhizomes as well as compound characteristic of leaves such as 3-hexen-1-ol. The volatile compounds obtained from *C. longa* roots were the same as those from the rhizomes.

The antioxidant activity of both water and methanol extracts of *C. longa* rhizomes collected from the medicinal plant garden was confirmed using electron spin-resonance spin-trapping method with potent scavenging activity against superoxide anion radical ($O_2^{\cdot -}$). Extracts from ripe rhizomes ready for harvest exhibited greater antioxidant activity than those obtained from young rhizomes.

Keywords: *Curcuma longa*; Volatile compound; Thermal desorption-gas chromatography-mass spectrometry; Antioxidant activity; Electron spin-resonance spectrometry

Introduction

Curcuma longa is a plant in the family Zingiberaceae, which is native to India. Rhizomes of *C. longa*, with cork layers surrounded by roots located underground, are harvested, washed, peeled or unpeeled, warmed in a vessel placed in hot water, dried, and then cut into small pieces to prepare granules, powder, tablets, and drinks. It is commonly known as turmeric or as ukon in Japan. The main rhizome is nearly ovoid and the lateral rhizome is cylindrical. The cut surfaces of rhizomes are yellow-brown to red-brown in color [1]. Turmeric has been used as a yellow dye, a cooking spice, especially in curry, a health drink to prevent hangovers, and for medicinal purposes, e.g., to treat stomachache and as a blood purifier, carminative, appetite stimulant, and cholagogue [2].

The medicinal plant garden of Hoshi University (southern Tokyo) is home to many medicinal plants, and contains *C. longa* plants. The leaves are green sheaths, and the subterranean parts comprise rhizomes and roots. Seed rhizomes, which were harvested the previous November and then stored in their soil and root balls in a warm room over the winter, are planted in the ground in the garden in May (spring). Green leaves gradually grow aboveground from the new annual planting, and young, slender, white rhizomes emerge from underground in July (summer). The white turmeric flowers bloom in September. With the coming of winter, the aboveground leaves wither, and underground rhizomes turn yellow-brown to red-brown in color and generally grow thick enough for harvest. They are harvested in late November before the first frost of the year.

In our continuous studies of volatile compounds originating from fresh plants, volatile compounds from living clove buds cultivated in the garden have been investigated [3]. An odor is composed of several volatile compounds which are assumed to differ under the influence of several conditions such as the cultivar, time of harvest and treatment, and surrounding environment [4]. Volatile compounds from *C. longa* are assumed to differ with each development stage. With the gradual development of the rhizomes, their odor also changes. Our investigations evaluate the seasonal changes in the volatile compounds produced by rhizomes and compare them with the compounds originating from the roots and leaves.

In the present study, two types of absorption device for volatile compounds originating from *C. longa* plants were examined: a solid-phase micro extraction (SPME) fiber and a bar coated with polydimethylsiloxane (PDMS). The volatile compounds absorbed onto the devices were immediately analyzed using thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) procedures. The effectiveness of PDMS/carboxene (CAR)-coated and PDMS/CAR/divinylbenzene (DVB)-coated fibers in detecting volatile compounds was compared with that of PDMS-coated devices. Although volatile compounds of essential oils obtained by hydrodistillation of *C. longa* plants were analyzed previously [2,5], to the best of our knowledge this is the first investigation focusing on the fragrances produced by fresh plants.

Next, our attention was focused on the antioxidant activities of extracts from *C. longa* rhizomes cultivated in the garden. The extracts of medicinal plants become a great source of antioxidant property [6,7]. It was reported the rhizomes and leaves of *C. longa* yield essential oils possessing antibacterial, antifungal, anti-inflammatory, antihepatotoxic, antiarthritic, antioxidant and insecticidal activities [2,8,9].

To measure antioxidant activity of *C. longa* rhizomes cultivated in the medicinal plant garden electron spin-resonance (ESR) spin-trapping method was performed. Superoxide anion radical ($O_2^{\cdot -}$), which is one of the strongest harmful reactive oxygen species (ROS), is trapped using a spin-trapping agent such as 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) to form a spin adduct such as DMPO-OO^- so that the antioxidant potency can be measured. When a decrease in the ESR signal intensity of DMPO-OO^- was observed with the addition of a sample extract to the reaction system, inhibition of the formation of DMPO-OO^- due to competitive reaction with the antioxidant with potent scavenging activity against $O_2^{\cdot -}$ was seen, reflecting the antioxidant activity of the sample extract.

Materials and Methodology

Plant material

C. longa rhizomes, roots, and leaves were collected from plants cultivated in the medicinal plant garden of Hoshi University. Each sample was cleaned and washed with water and then cut into pieces of approximately 2 mm × 2 mm with scalpel prior to analyses.

Chemicals

For TD-GC-MS measurements, standard reagents of β-caryophyllene and 3-hexen-1-ol were purchased from Wako Pure Chemical Industries (Osaka, Japan); p-cymene, (*E*)-β-farnesene, β-myrcene, α-phellandrene, α-terpinene, and α-terpinolene were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); and 1,8-cineol was from Sigma-Aldrich (St. Louis, MO, USA). For the determination of retention indices using a homologous series of C_9-C_{33} *n*-alkanes, Naginata criteria sample Mix II in dichloromethane was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan).

For ESR measurement, DMPO was purchased from Labotec (Tokyo, Japan), and hypoxanthine (HPX) and xanthine oxidase (XOD) from bovine milk were from Sigma-Aldrich. SOD and sodium dihydrogen phosphate were obtained from Wako Pure Chemical Industries. Methanol (MeOH) and sodium hydroxide were from Kanto Chemical Co., Inc. (Tokyo, Japan). All aqueous solutions were prepared using water filtered through Autopure WD501UV from Yamato (Tokyo, Japan).

Instrumentation

TD-GC-MS analysis was performed with a TD unit (TDU) equipped with a CIS4 programmed temperature vaporization inlet (Gerstel, Mülheim an der Ruhr, Germany), installed on a 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and a JMS-700 mass spectrometer (JEOL, Tokyo, Japan) equipped with a DB-5 column (30-m × 0.25-mm internal diameter) coated with a 0.25-μm film consisting of 5% phenyl and 95% dimethyl polysiloxane (Agilent Technologies).

ESR spectra were recorded on a JES-RE1X ESR spectrometer (JEOL). The measurement conditions were: magnetic field, 336 ± 5 mT; microwave power, 9 mW; modulation width, 0.063 mT; sweep time, 30 s; and time constant, 0.03 s. In ESR measurements, the signal intensity was normalized as the relative height against the standard signal intensity of the manganese oxide marker. Each experiment was conducted in duplicate or triplicate.

TD-GC-MS analyses

Absorption of volatile compounds: For absorption of volatile compounds, two types of absorption device, comprising solid-phase micro extraction (SPME) fibers coated with 100-μm-thick film of three materials, i.e., PDMS, PDMS/CAR, and PDMS/CAR/DVB (Supelco, Bellefone, PA, USA), and 10-mm-long bar coated with a layer of PDMS (Gerstel) 0.5 mm thick [10,11] were used. The harvested plant material (ca. 1 g per sample) was cut into pieces of approximately 2 mm × 2 mm and then transferred immediately to a 40-mL vial equipped with a clean pinhole septum (Thermo Fischer Scientific, Waltham, MA, USA). Each device was fixed in the headspace of the vial in which the plant material was placed, and headspace sorptive extraction was carried out at room temperature. After standing for 60 min, the absorption device was removed and transferred to a glass thermal desorption liner.

Measurements: The absorption device was thermally desorbed by programming the TDU from 40°C (held for 0.2 min) to 250°C (held for 5 min) at the rate of 720°C/min in splitless mode. Desorbed compounds were focused at –50°C on the CIS4 inlet liner, and then by programming the temperature from –50°C (held for 0.5 min) to 260°C (held for 5 min) at the rate of 720°C/min. The trapped compounds were then injected onto the analytical column with a split ratio of 10:1. Helium was used as the carrier gas at a constant flow of 1.5 mL/min, with an ion-chamber temperature of 250°C. The column-oven temperature was held at 40°C for 3 min, increased to 250°C at the rate of 5°C/min, and then held at the final temperature. The mass spectrometer was operated at a filament current of 300 μA and accelerating voltage of 10 kV with electron ionization mode of 70 eV.

ESR spin-trapping analyses

Superoxide anion radical-scavenging activity (SOSA) was investigated following the method in previous reports [12-15]. $O_2^{\cdot -}$ was generated from the HPX–XOD reaction. The SOSA of the extracts was compared with that of superoxide dismutase (SOD) as a standard.

Sample preparation: Small cut pieces of *C. longa* rhizomes from plants cultivated in the medicinal plant garden, totaling 0.1 g each, were extracted with either 1 mL of sodium phosphate buffer–water solution (PB; pH 7.8) or with 1 mL of MeOH, with shaking for 1 h using a PIC-100S shaking incubator (AS ONE, Osaka, Japan), and supernatants were obtained by centrifugation (Kubota model 5922, Kubota Corp., Tokyo, Japan) at 9160 g for 5 min at 4°C. One supernatant was further filtered through a 0.45-μm HLC-Disk filter (Kanto Chemical Co. Inc.) to prepare a filtration sample referred to as the "filtrated PB extract" and the other without filtration was prepared as the "supernatant of PB extract" sample. Samples were stored at -78°C until further analysis.

Measurement: First, ESR measurement of SOD as a standard was carried out. Next, sample extracts were analyzed. For the water extract, 15 μL of DMPO, 50 μL of 5 mM HPX dissolved in PB (water at pH 7.8), 35 μL of PB, and 50 μL of PB extract from a sample (or 50 μL of

PB solution of SOD) were placed in a test tube and mixed. Fifty microliters of 1.2 U/mL XOD in PB was added to the combined solution and mixed using a TM-251 test tube mixer (Iwaki, Tokyo, Japan). Two hundred microliters of the mixture was placed in a flat glass cell, and recording of the ESR spectrum started 60 s after the addition of XOD.

Results and Discussion

Volatile compounds originating from freshly harvested rhizomes, leaves, and roots using PDMS-coated bars

Volatile compounds originating from freshly harvested *C. longa* rhizomes cultivated in the medicinal plant garden were analyzed using TD-GC-MS. Sampling was performed at three different development stages of the rhizomes, i.e., in July, September, and November, using PDMS-coated bars as an adsorption device to investigate differences in volatile compounds. Odor compositions detected at different development stages are shown in Table 1. 1,8-Cineol, α-terpinolene, β-caryophyllene, and ar-curcumene were the predominant constituents in most cases. ar-Turmerone possessing fungicidal, mosquitocidal, anticonvulsant, and anticancer activities [8,16-18] was found from ripened rhizomes (in September and November). Changes in volatile compounds with development stage were observed. Old rhizomes that had been harvested the previous November and then stored over the winter yielded additional compounds such as β-myrcene, β-elemene, and β-bisabolene, which were not found in rhizomes freshly harvested in November (data not shown). Fresh leaves harvested in July yielded both the same compounds as identified from rhizomes as well as compound characteristic of leaves, i.e., 3-hexen-1-ol. When volatile compounds from the roots were also investigated, the same compounds as from the rhizomes were identified.

Compound[a]	RI[b]	RI		Rhizome			D[e]
		lit[c]	Leaf	July	September	November	
3-hexen-1-ol	*d*		○	-	-	-	MS, Std
β-Myrcene	982	982	-	-	○	-	MS, RI, Std
1,8-cineole	1026	1026	○	○	○	○	MS, RI, Std
α-Terpinolene	1085	1085	○	○	○	○	MS, RI, Std
β-Elemene	1390	1390	○	○	○	-	MS, RI
β-Caryophyllene	1420	1420	○	○	○	○	MS, RI, Std
ar-Curcumene	1482	1482	○	○	○	○	MS, RI
β-Bisabolene	1508	1508	-	-	○	-	MS, RI
ar-Tumerone	1663	1664	-	-	○	○	MS, RI

Table 1: Odor components of *C. longa* rhizome and leaf identified by TD-GC-MS using PDMS-coated bar. [a]Compounds are listed in order of their elution from a DB-5 column. [b]RI on DB-5 column, experimentally determined using homologous series of C_9-C_{33} *n*-alkanes. [c]RI taken from previously analyzed compounds in Aroma office data base [21]. [d]Retention time is outside of retention times of homologous series of C_9-C_{33} *n*-alkanes. [e]Identification methods: MS, by comparing their mass spectra with those in the NIST library; RI, by comparing RIs with those reported in the literature recorded in Aroma Office database; Std, by comparing retention time and mass spectrum with those of available authentic standard. ○- identified;-not identified.

Comparison of adsorption devices

Second, analyses of volatile compounds originating from rhizomes harvested in November were carried out using SPME fiber adsorption devices. The effectiveness of PDMS/CAR-coated and PDMS/CAR/DVB-coated fibers in detecting volatile compounds was compared with that when only PDMS-coated fiber was used. As shown in Table 2, additional volatile compounds were detected when using the CAR- and DVB-coated devices. The PDMS/CAR/DVB-coated fiber allowed the detection of additional compounds such as β-myrcene, α-terpinene, p-cymene, and ar-turmerone from the rhizomes in November, which were not detected when the PDMS-coated fiber was used. Although ar-turmerone was not detected with the PDMS-coated fiber, it was detected with the PDMS-coated bar (Table 1). This was assumed to be due to the differences in the PDMS-coated area on each device. The PDMS-coated bar has a wider area of PDMS film compared with the fiber device.

As more volatile compounds were identified when PDMS/CAR/DVB-coated fiber was used, volatile compounds originating from rhizomes in July were subsequently investigated using this device. β-Myrcene, α-phellandrene, α-terpinene, p-cymene, γ-elemene, (*E*)-β-farnesene, and β-bisabolene were additionally obtained from the rhizomes harvested in July but were not when the PDMS-coated bar was used (data not shown).

Antioxidant activity

The relative percentage of SOSA was plotted against the concentration of the logarithm of sample extract in the reaction system. From the relationship between SOSA and concentration, the median inhibitory dose (ID_{50}) on SOSA was obtained. Blank solutions, i.e., without sample in PB or in MeOH, were used as controls when the relative percentage of SOSA was calculated.

Comparison of SOSA of the supernatant of PB extract and filtrated PB extract

For medicinal purposes, a granulated or powdered form of turmeric is generally dissolved in (hot) water and then drunk. In this study, rhizomes of *C. longa* were extracted with PB, and then the antioxidant activities of the supernatant with (filtrated PB extract) and without filtration (supernatant of PB extract) were evaluated. Figure 1 shows the relationship between the relative inhibitory effects of the formation of DMPO-OO^- and logarithm of various concentrations of PB extract from *C. longa* rhizomes. The relative inhibition of the formation of DMPO-OO^- increased with the increasing concentration of PB extract. The ID_{50} values of the supernatant of PB extract and filtrated PB extract were 6.3 mg/mL and 8.2 mg/mL, respectively. As shown in Figure 1, the supernatant of PB extract tended to exhibit greater scavenging activity against $O_2^{\cdot -}$. Suspension in the PB extract solution was assumed to exert effective antioxidant activity. Thus, extract solutions without filtration were used in the following experiments.

Compound	RI	RI	SPME fiber			ID
		lit	PDMS	PDMS/CAR	PDMS/CAR/DVB	
β-Myrcene	992	992	-	-	○	MS, RI, Std
α-Terpinene	1016	1016	-	-	○	MS, RI, Std
p-Cymene	1024	1024	-	○	○	MS, RI, Std
1,8-Cineole	1031	1031	○	○	○	MS, RI, Std
α-Terpinolene	1091	1091	○	○	○	MS, RI, Std
β-Elemene	1395	1394	○	○	○	MS, RI
β-Caryophyllene	1424	1423	○	○	○	MS, RI, Std
(*E*)-*β*-Farnesene	1458	1458	○	○	○	MS, RI, Std
ar-Curcumene	1485	1485	○	○	○	MS, RI
β-Bisabolene	1512	1512	○	○	○	MS, RI
β-Sesquiphellandrene	1529	1531	○	-	○	MS, RI
ar-Turmerone	1663	1664	-	-	○	MS, RI

Table 2: Influence of material coated on SPME fiber on detected volatile compounds originating from *C. longa* rhizome harvested in November.

Comparison of SOSA of *C. longa* rhizomes harvested in July and November

Figure 2 shows ESR spectra of DMPO-OO^- observed upon the addition of various concentrations of PB extracts from *C. longa* rhizomes harvested in November. When a sample extract from *C. longa* rhizomes was added to the reaction system, a reduction in the signal intensity was observed, and this reduction was dependent upon the increase in the concentration of extract. It was thus confirmed that the extract functioned as a superoxide anion radical scavenger.

Figure 3 shows the relationship between the relative inhibitory effects of the formation of DMPO-OO^- and logarithms of various concentrations of PB extract from *C. longa* rhizomes. The relative inhibition of the formation of DMPO-OO^- increased as the concentration of PB extract increased. PB extracts from *C. longa* rhizomes therefore exhibited effective antioxidant activity. The linearity was expressed as $y=15.364\ln(x)-7.6875$; $R^2=0.9697$ for rhizomes harvested in July (PB extract of July), and $y=19.292\ln(x)-8.3069$; $R^2=0.9184$ for rhizomes harvested in November (PB extract of November). The ID_{50} values of PB extracts were 42.7 mg/mL for PB extract of July and 20.5 mg/mL for PB extract of November. When the antioxidant activity of rhizomes harvested in the previous November and then stored in soil and root balls in a warm room over the winter (PB extract of the previous November) was investigated, the linearity was expressed as $y=28.397\ln(x)-12.49$; $R^2=0.9992$, and the ID_{50} value was 9.0 mg/mL. As shown in Figure 3, the PB extract of November tended to exhibit greater scavenging activity against $O_2^{\cdot -}$ than the PB extract of July. The PB extract of the previous November exhibited the greatest activity among the three extract types.

Subsequently, MeOH extracts from rhizomes were analyzed using the same method, and the results were similar to those obtained in PB extracts. The relative inhibition of the formation of DMPO-OO^- increased with the increasing concentration of MeOH extract, as shown in Figure 4. MeOH extracts from *C. longa* rhizomes therefore exhibited effective antioxidant activity. The linearity was expressed as $y=19.3\ln(x)-43.233$; $R^2=0.9996$ with an ID_{50} value of 125.3 mg/mL for the MeOH extract of July, as $y=18.261\ln(x)+20.216$; $R^2=0.9983$ with an ID_{50} value of 5.1 mg/mL for the MeOH extract of November, and as $y=15.481\ln(x)+33.643$; $R^2=0.99$ with an ID_{50} value of 2.9 mg/mL for the MeOH extract of the previous November. The MeOH extract of the previous November exhibited the greatest activity among the three extracts.

The ESR signal intensity of DMPO-OO^- decreased with the increasing sample concentration, and thus each sample functioned as an ROS scavenger. Extracts from *C. longa* rhizomes cultivated in the medicinal plant garden of Hoshi University therefore exhibited antioxidant activity.

To compare the SOSA of each extract, the SOD units of each were calculated. First, the DMPO-OO^- scavenging activity of SOD as a standard was measured using the ESR spin-trapping method, and ID_{50} values of SOD in PB or MeOH were calculated. Next, the DMPO-OO^- scavenging activity of extracts from the rhizomes were measured using the same method. As shown in Table 3, SOD unit values of PB extracts were 0.39 for the PB extract of July, 0.59 for the PB extract of November, and 1.86 for the PB extract of the previous November. The SOD unit values of MeOH extracts were 0.15 for the MeOH extract of July, 3.71 for the MeOH extract of November, and 6.62 for the MeOH extract of the previous November.

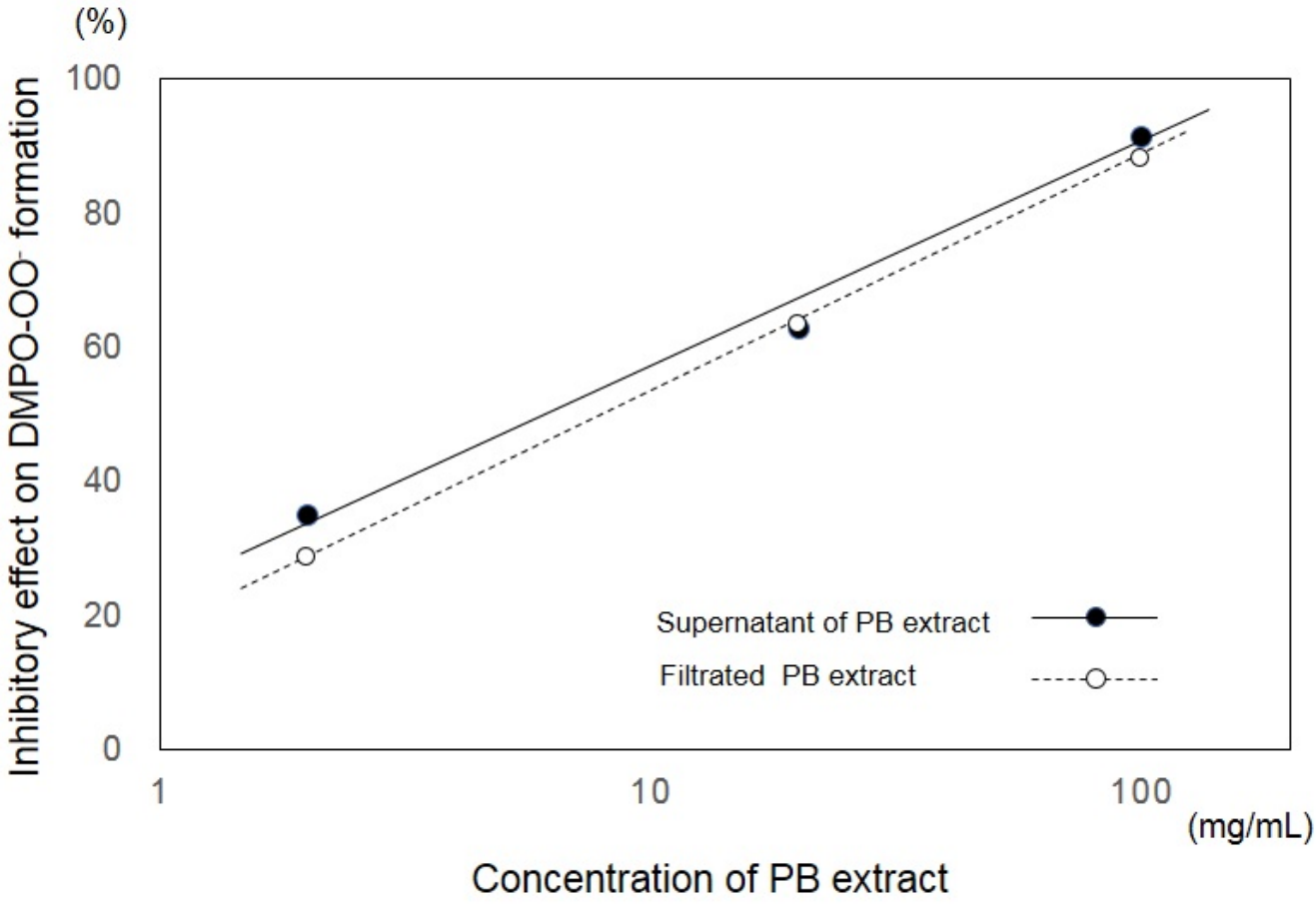

Figure 1: Inhibitory effects of various concentrations of supernatants of PB extracts and filtrated PB extracts from *C. longa* rhizomes on the formation of DMPO-OO^-.

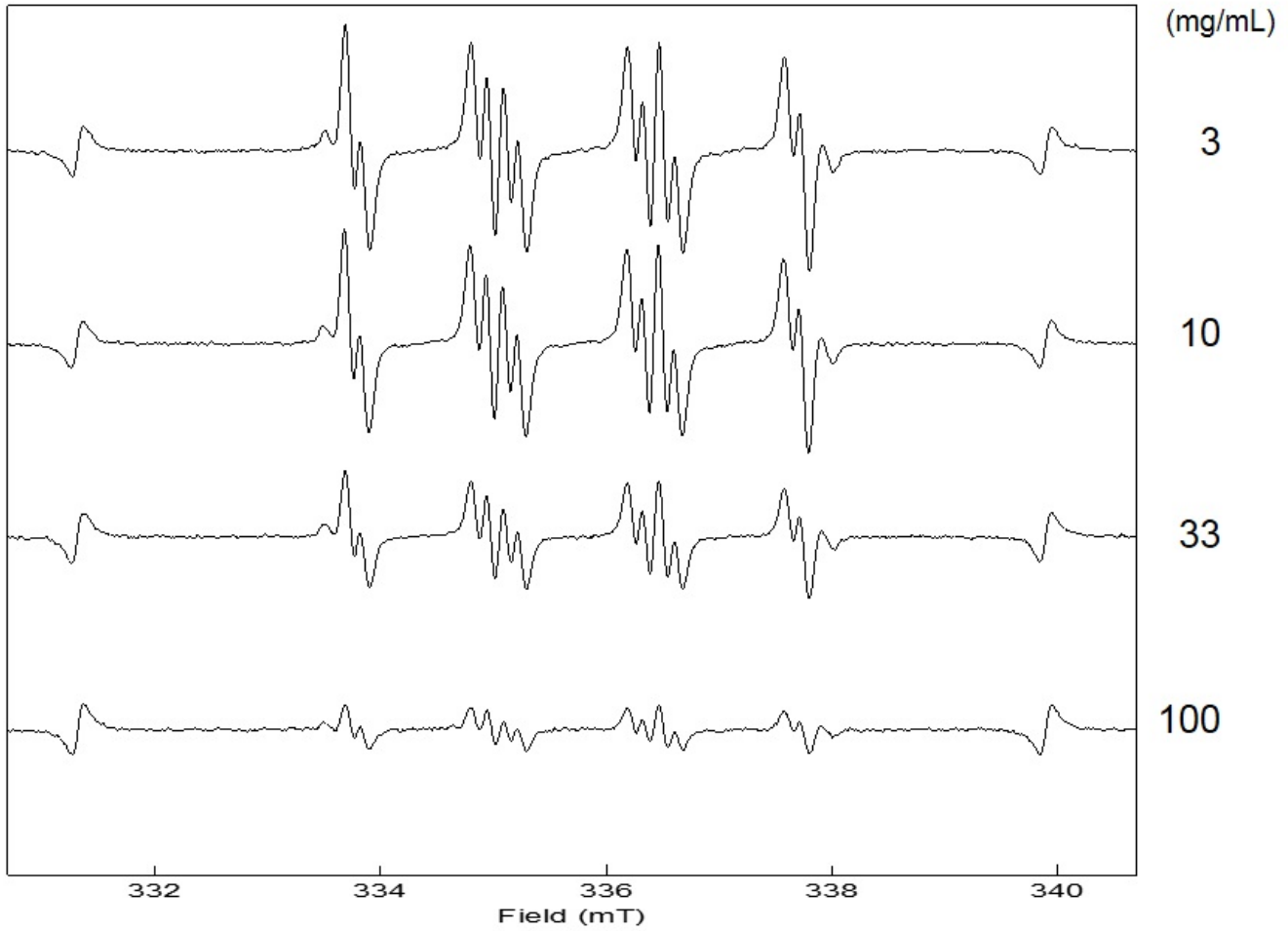

Figure 2: ESR spectra of DMPO-OO^- observed upon the addition of various concentrations of PB extracts from *C. longa* rhizomes harvested in November.

The SOD unit values of both PB and MeOH extracts of November were greater than those of July. Rhizomes ready for harvest in November were therefore assumed to contain higher levels of antioxidant compounds than those in July. In addition, the SOD unit values of extracts from rhizomes harvested the previous November and then stored over the winter indicated greater activity compared with those of rhizomes harvested in the current November. During storage over the winter, the rhizomes harvested the previous November were assumed to accumulate greater activity.

MeOH extracts of November tended to exhibit stronger scavenging activity against $O_2^{\cdot -}$. The SOD unit values of MeOH extracts from the current and previous Novembers were about 6-fold and 4-fold higher than that of the PB extracts, respectively. Phenolic compounds extracted with MeOH such as tetrahydrocurcuminoids produced by *C. longa* play an important role in antioxidant activity [19,20].

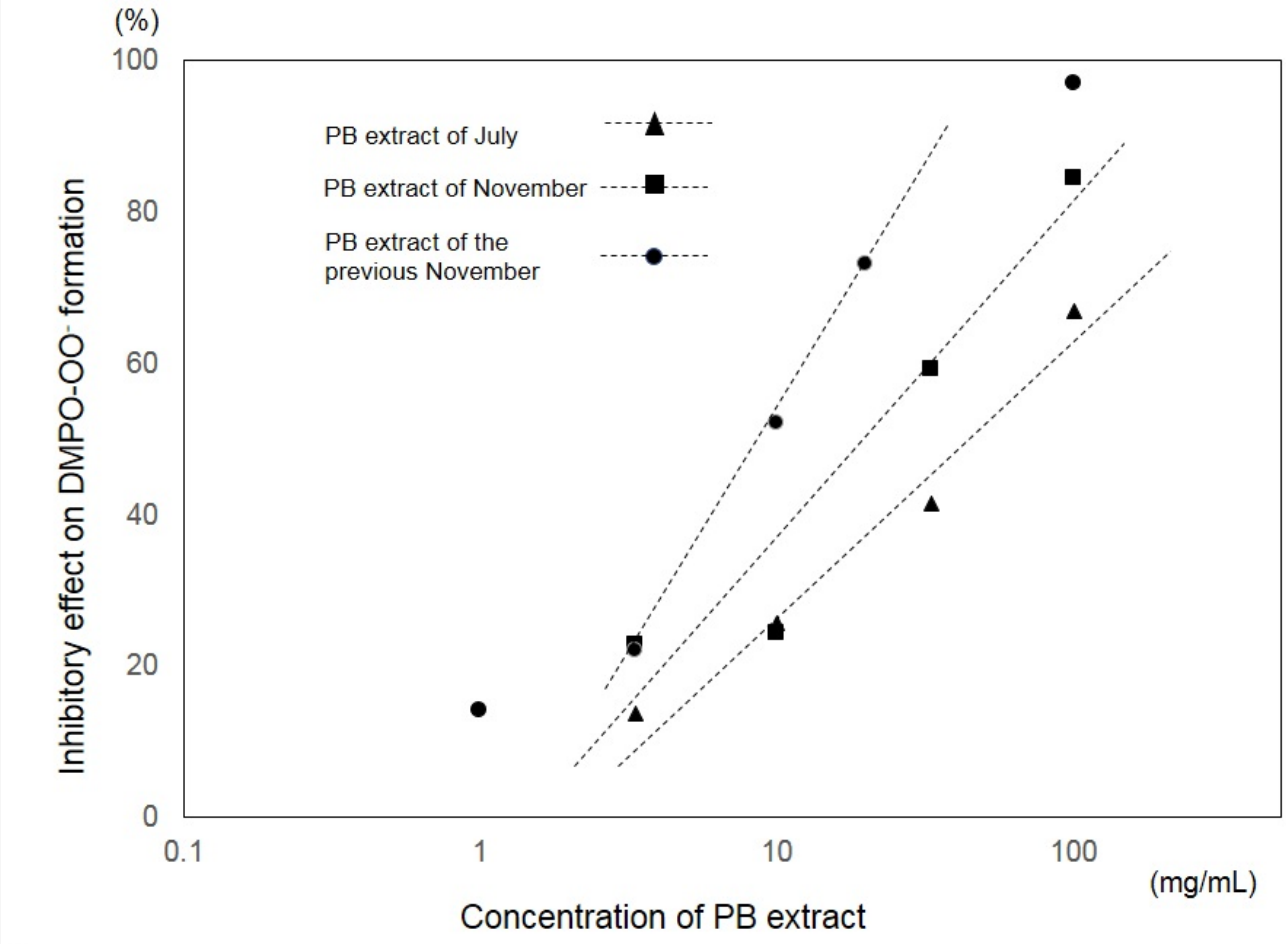

Figure 3: Inhibitory effects of various concentrations of PB extracts from *C. longa* rhizomes harvested in July, November, and the previous November on the formation of DMPO-OO^{-}.

	ID_{50} of extract	ID_{50} of SOD	SOD unit of extract
	(mg/mL)	(U/mL)	(U/mg)
PB extract of July	42.7	16.7	0.39
PB extract of November	20.5	12.1	0.59
PB extract of the previous November	9.0	16.7	1.86
MeOH extract of July	125.3	19.2	0.15
MeOH extract of November	5.1	18.9	3.71
MeOH extract of the previous November	2.9	19.2	6.62

Table 3: ID_{50} values of rhizome extracts and SOD on superoxide anion radical ($O_2^{\cdot -}$) scavenging activity and SOD unit of the extracts.

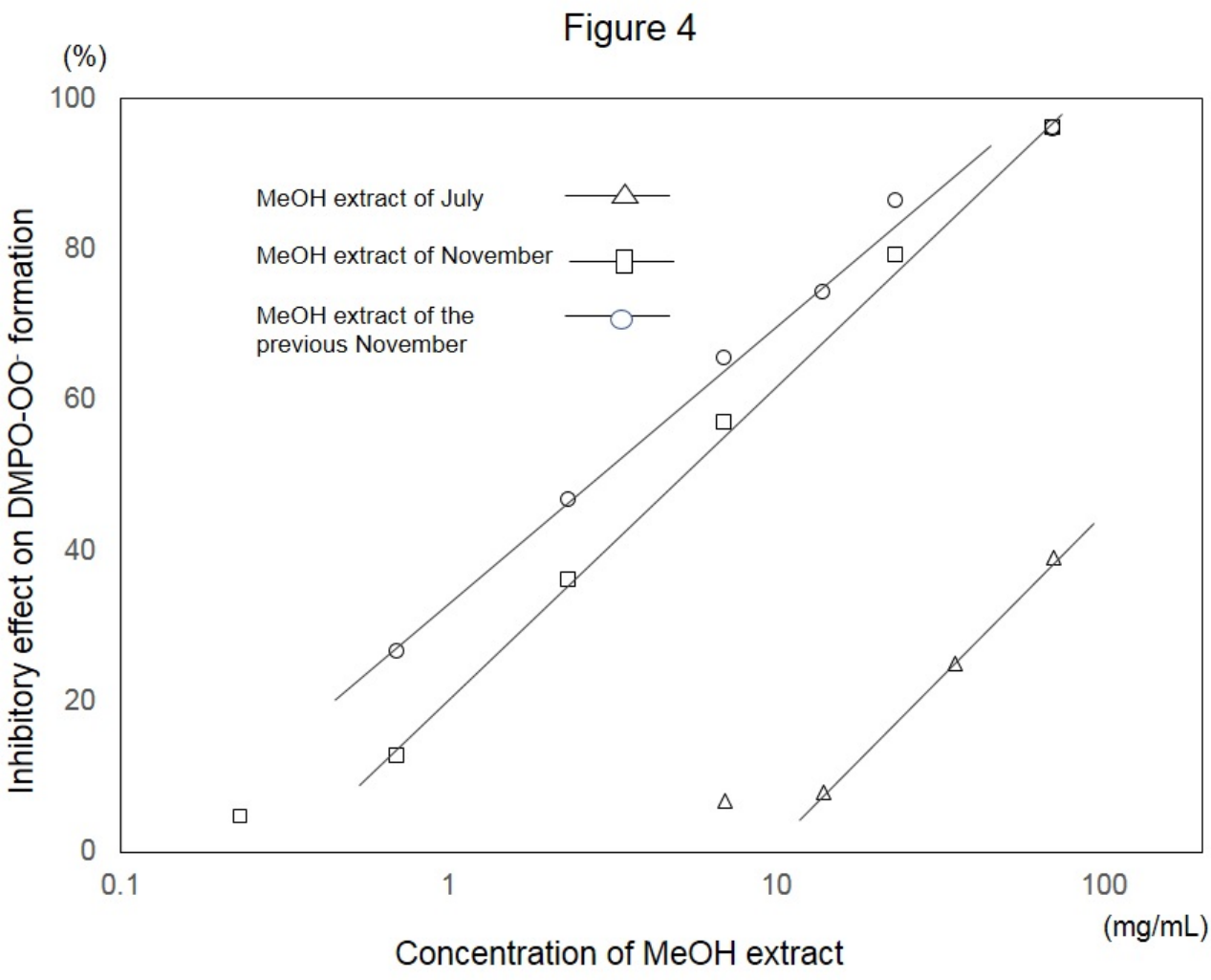

Figure 4: Inhibitory effects of various concentrations of MeOH extracts from *C. longa* rhizomes harvested in July, November, and the previous November on the formation of DMPO-OO^{-}.

Summary

This study focused on the odor components and antioxidant activity of *C. longa* plant cultivated in a medicinal plant garden in southern Tokyo. Although the scientific and family names of all plants in the garden are clearly presented, no information is provided on the fragrances and antioxidant activity. Thus additional information about the fragrances of plants cultivated in the garden is now required.

Volatile compounds were detected using TD-GC-MS, and qualitative differences were observed in different growth phases when the rhizomes were young (in July) and ready for harvest (in November). α-Phellandrene and γ-elemene were obtained from the young rhizomes in July, and ar-turmerone from ripened rhizomes in November. 3-Hexen-1-ol was additionally obtained from *C. longa* leaves. The volatile compounds obtained from *C. longa* roots were the same as those from the rhizomes.

Water and MeOH extracts from *C. longa* rhizomes exhibited effective antioxidant activity as determined using the ESR spin-trapping method. Extracts from ripe rhizomes ready for harvest exhibited greater antioxidant activity than those obtained from young rhizomes.

The demand to develop odor profiles of plants cultivated in the garden is increasing along with the rise in applications of volatile compounds to improve human health.

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