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Research Article

Analysis of Compounds of Curcuma Rhizome Using Mass Spectrometry and Investigation of the Antioxidant Activity of Rhizome Extracts

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ABSTRACT

The medicinal plant garden of Hoshi University located in southern Tokyo is home to many medicinal plants, and analysis of odor and measurement of antioxidant activity of cultivated plant have been carried out. In this study compounds originated from *Curcuma* rhizomes, i.e., *Curcuma longa*, *Curcuma aromatica*, *Curcuma zedoaria*, *Curcuma xanthorrhiza*, were investigated and antioxidant activities of rhizome extracts were measured. Volatile compounds originating from the *Curcuma* rhizomes were analyzed using thermal desorption (TD)-GC-MS with solid-phase micro extraction fiber as an adsorption device. *p*-Cymene, 1,8-cineol, β-elemene, and β-caryophyllene were the predominant constituents in most cases. Curcuminoids, which were not identified by TD-GC-MS, were detected using direct analysis in real time time-of-flight MS. Curcumin and demethoxycurcumin were detected from both *C. longa* and *C. xanthorrhiza*. The antioxidant activity of each *Curcuma* species rhizome was confirmed using the electron spin-resonance spin-trapping method with potent scavenging activity against superoxide anion radicals. Extracts from *Curcuma* rhizomes cultivated in the medicinal plant garden exhibited antioxidant activities, and the order of the activity of methanol extracts was: *C. longa*>*C. xanthorrhiza*>*C. aromatica*>*C. zedoaria*. Phenolic compounds, particularly curcumin, are known to possess potent antioxidant activity and are extracted with methanol, thus it reflects the intensity of the antioxidant activity.

Keywords: *Curcuma longa*; *Curcuma aromatica*; *Curcuma zedoaria*; *Curcuma xanthorrhiza*; Component; Thermal desorption-GC-MS; Direct analysis in real time-TOFMS; Antioxidant activity; Electron spin-resonance spectrometry

INTRODUCTION

Curcuma plants belong to the family *Zingiberaceae* and are widely distributed throughout the tropics, particularly in South Asia. Rhizomes of *Curcuma* species located underground have been used for cooking, dyes, healthcare products, and traditional medicines in Asian countries and are endowed with antioxidative properties [1].

In the medicinal plant garden of Hoshi University *Curcuma longa*, *Curcuma aromatica*, *Curcuma zedoaria*, and *Curcuma xanthorrhiza* are cultivated. Turmeric is the rhizome of *C. longa*. The main rhizome is nearly ovoid with a yellow brown to yellow-red color. It contains the greatest volume of curcuminoids, i.e., curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin, compared with other *Curcuma* species. The *C. longa* rhizome is commonly used for cooking such as a spice in curry dishes and for coloring and flavoring. It has also been used for medicinal purposes, e.g. as a cholagogue and as an anti-inflammatory, anti-angiogenic, anti-bacterial, anti-fungal, analgesic, immunomodulatory, vasodilatory, anti-diabetes, and anti-Alzheimer's disease agent [2,3].

Compared with *C. longa*, the activities of other *Curcuma* species have not been thoroughly investigated even though they also show a wide range of medicinal properties. *C. aromatica* has been used in cosmetic formulation and exhibits various pharmacological properties, including anti-inflammatory, antioxidant, anti-angiogenic, and anti-carcinogenic effects as well as anti-bacterial activity. It contains monoterpenoids, sesquiterpenoids, and curcuminoids [4,5]. Zedoary is the rhizome of *C. zedoaria*. It is nearly ovoid and externally grayish yellow brown to grayish-brown in color and is a perennial rhizomatous herb indigenous to Bangladesh, Sri Lanka, and India. Zedoary exhibits anti-inflammatory, anti-babesial, cytotoxic, and anti-fungal activities. In Indonesia, it is used traditionally in the treatment of parasitic organisms. Lotion made from its extract has been used for skin care as an effective whitener [6-9]. *C. xanthorrhiza*, which is native to Indonesia and commonly known as "java ginger" or "*temulawak*," has been shown to exert anti-bacterial, anti-fungal, anti-halitosis, anti-inflammatory, and anti-oxidant activities. In particular, xanthorrhizol (3) exhibits

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strong activity, and inhibitory effects on periodontitis were also reported in Figure 1 [10].

Although the medicinal plant garden of Hoshi University is home of medicinal plants, there is a lack of information about odor and functional activity of the plants. Thus, we have investigated the odor, which is composed of several volatile compounds, and analyzed anti-oxidant activities of several plants cultivated in the garden [11-13] because essential oil compositions, odor component, functional activity are affected by the cultivar, time of harvest, and environment.

Although we have already reported about volatile compounds and antioxidant activity of rhizome extract of *C. longa* [12], comparative information among the different *Curcuma* species, i.e., *C. longa*, *C. aromatica*, *C. zedoaria*, and *C. xanthorrhiza*, are required. Our investigations evaluate the differences between *Curcuma* plants species in the volatile compounds, and non-volatile curcuminoids, sesquiterpenoids, and antioxidant activity of extracts from rhizomes of different species. We compared the compounds obtained from *Curcuma* species rhizomes in the medicinal plant garden with those presented in the previous reports [14,15].

For analysis of volatile compounds originating from fresh rhizomes of *Curcuma* plants, thermal desorption (TD)-GC-MS was used. As the device for adsorption of volatile compounds Solid-Phase Micro Extraction (SPME) fiber coated with carboxene (CAR)/polydimethylsiloxane (PDMS) was used. Subsequently analyses of *Curcuma* rhizomes using Direct Analysis in Real Time (DART) Time-of-Flight (TOF) MS was performed. DART is one of the ionization techniques of MS and is conducted in the open air, allowing for the rapid and direct analysis of samples in the gas, liquid, and solid states [16,17]. We expected DART-TOFMS to detect curcuminoids and sesquiterpenoids, which were not detected by TD-GC-MS, without complicated sample preparation prior to measurement.

Antioxidants are important because they prevent oxidative cell damage by acting as free radical scavengers. The superoxide anion

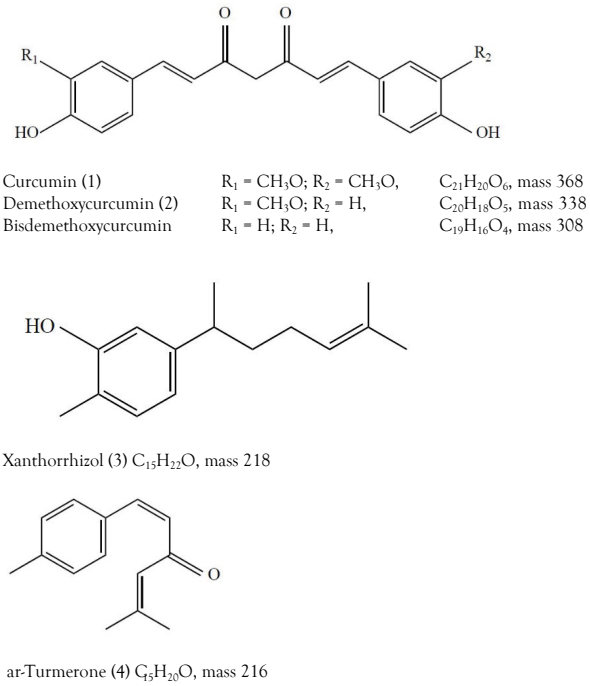

Figure 1: Structures of some anti-oxidant compounds originating from the rhizomes of *Curcuma* species cultivated in the Hoshi University medicinal plant garden identified using DART-TOFMS.

radical ($O_2^{\cdot -}$), hydroxyl radical ($^{\cdot}OH$), and hydrogen peroxide are produced continuously in the human body. The harmful reactive oxygen species may be related to cancer, cardiovascular disease, diabetes, neurodegenerative diseases, and aging. Although endogenous enzymes such as Superoxide Dismutase (SOD) usually control the levels of free radicals, oxidative stress occurs under high levels of free radicals. To counter the effects of oxidative stress, many people take antioxidants. As there are limitations on the use of synthetic antioxidants such as butylated hydroxy anisole, butylated hydroxytoluene, and *tert*-butyl hydroquinone because of their production of toxins or action as carcinogens, identifying antioxidants in natural materials is required. Several plants are rich in antioxidant compounds with no toxic effects. For measuring the antioxidant activity of *Curcuma* rhizomes cultivated in the medicinal plant garden, the Electron Spin-Resonance (ESR) spin-trapping method was used. Superoxide anion radical ($O_2^{\cdot -}$) is trapped by a spin-trapping agent such as 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) to form a spin adduct such as DMPO–OO^-. A decrease in the ESR signal intensity of DMPO-OO^- with an addition of a sample extract to the reaction system reflects the antioxidant activity.

MATERIALS AND METHODS

Plant material

C. longa, *C. aromatica*, *C. zedoaria*, and *C. xanthorrhiza* were purchased from a domestic market in Japan and are cultivated in the medicinal plant garden of Hoshi University located in southern Tokyo. Fresh rhizomes of *Curcuma* species harvested from the garden in November were washed with water, peeled, cut into small pieces or grated prior to analyses.

Chemicals

For TD-GC-MS measurements, the standard reagent β-caryophyllene was purchased from Fujifilm Wako Pure Chemical Corporation (Tokyo, Japan); *p*-cymene, (*E*)-β-farnesene, β-myrcene, and α-terpinolene were from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); and 1,8-cineol was from Sigma-Aldrich (St. Louis, MO, USA). Naginata criteria sample Mix II in dichloromethane, necessary for the determination of retention indices (RIs), was purchased from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). For DART-TOFMS measurement, the standard reagent Polyethylene Glycol (PEG) with average molecular weight of 400 was purchased from Tokyo Chemical Industry Co., Ltd.

For ESR measurement, Hypoxanthine (HPX) and Xanthine Oxidase (XOD) from bovine milk were obtained from Sigma-Aldrich, SOD and sodium dihydrogen phosphate were from Fujifilm Wako Pure Chemical Corporation, DMPO was from Labotec (Tokyo, Japan), methanol (MeOH) and sodium hydroxide were from Kanto Chemical Co., Inc. (Tokyo, Japan). For preparing aqueous solutions water filtered through Autopure WD501UV from Yamato (Tokyo, Japan) was used.

Instrumentation

TD-GC-MS analyses: TD unit equipped with a CIS4 programmed temperature vaporization inlet (Gerstel, Mülheim an der Ruhr, Germany); 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA); and JMS-700 mass spectrometer (JEOL, Tokyo, Japan) was used. 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) was equipped.

DART-TOFMS analyses: JMS-T100LP mass spectrometer (JEOL) in positive-ion mode was used. The DART ion source conditions were needle voltage, 2500 V; electrode, lens 1: 100 V and lens 2: 250 V; helium gas flow, 3 L/min; and temperature, 300°C. The TOFMS was set as follows: 1st orifice lens, 15 V; 2nd orifice lens, 5 V; ling lens, 10 V; peak voltage, 500 V; bias voltage, 27 V; pusher bias voltage, –0.45 V; and detector voltage, 2200 V. PEG 400 was used as a calibration standard.

ESR analyses: JES-RE1X ESR spectrometer (JEOL) was used. The measurement conditions were: magnetic field, 335.7 ± 5 mT; sweep width, 5 × 1; microwave power, 9 mW; modulation width, 0.063 mT; sweep time, 30 s; and time constant, 0.03 s. The signal intensity was normalized as the relative height against the standard signal intensity of the manganese oxide marker.

TD-GC-MS analyses

For adsorption of volatile compounds, SPME fiber coated with a 100-μm-thick film of CAR/PDMS (Supelco, Bellefone, PA, USA) was used as the device. The plant material was harvested, cut into pieces (ca. 1 g per sample), and transferred to a 40-mL vial equipped with a clean pinhole septum (Thermo Fischer Scientific, Waltham, MA, USA) immediately. SPME fiber was fixed in the headspace of the vial in which each rhizome was placed, and headspace sportive extraction was carried out at room temperature for 60 min. The adsorption device was removed and injected into the TD-GC-MS system. The conditions of TD-GC-MS were same as mentioned before [12].

DART-TOFMS analyses

Each rhizome was broken into fine pieces using a grater and then dipped with a glass rod. Then it was placed between the inlet of the MS analyzer and the DART ion source for direct analysis. The ions were observed down to the fourth decimal place using PEG 400 as a calibration standard.

ESR spin-trapping analyses

Each sample solution was prepared by extracting grated rhizome of 0.1 g with either 1 mL of sodium phosphate buffer–water solution (PB; pH 7.8) or with 1 mL of MeOH and was stored at –78°C until further analysis. Superoxide anion radical-scavenging activity (SOSA) was measured by ESR using the same method as mentioned before [11,12,18–20]. The SOSA of the extracts was compared as the SOD equivalent (U/mg) using SOD as a standard.

RESULTS AND DISCUSSION

Volatile compounds originating from freshly harvested rhizomes using TD-GC-MS

Odor compositions of *Curcuma* rhizome species cultivated in the medicinal plant garden are shown in Table 1. Volatile compounds were identified by comparison of the mass spectra with those in the National Institute of Standards and Technology (NIST) library (Gaithersburg, MD, USA) and by comparing RIs with those reported in the literature in the Aroma Office database [21]. When authentic standards were available, the compounds were further confirmed by comparing retention times and mass spectra with those of the authentic standards.

p-Cymene, 1,8-cineol, β-elemene, and β-caryophyllene were the predominant constituents in most cases. ar-Curcumene and β-bisabolene were found from both *C. longa* and *C. xanthorrhiza*.

The compounds obtained from *Curcuma* species rhizomes harvested in the medicinal plant garden were compared with those in essential oil obtained by hydrodistillation presented in the previous reports [14,15,22]. In *C. longa* rhizome in the garden β-elemene was newly identified using this procedure. In *C. zedoaria* rhizome limonene, 2-nonanone, camphor, and (*E*)-β-farnesene were newly obtained. In *C. aromatica* rhizome β-myrcene, limonene, 2-nonane, linalool, terpinen-4-ol, β-caryophyllene, α-humulene were newly detected. In *C. xanthorrhiza* rhizome *p*-cymmene, limonene, α-terpinolene, β-caryophyllene, and ar-turmerone were newly identified (Table 1).

Distinctive compounds such as curcuminoids in *Curcuma* rhizomes using DART-TOFMS

Because curcumin (1), the main compound exhibiting antioxidant activity in *C. longa*, was not detected using the TD-GC-MS procedure, DART-TOFMS was carried out subsequently. For investigating the relation between components and antioxidant activities of rhizomes cultivated in the medicinal plant garden, the identification of curcuminoids was necessary.

The measured mass was compared with the calculated mass of the composition formula of compounds previously reported as components of *Curcuma* rhizomes [7,22,23]. Proposed components of *Curcuma* rhizomes from the garden, which were assumed from the difference between the measured mass and calculated mass, are shown in Table 2.

Using this simple procedure, molecular ions of curcumine (1) and demethoxycurcumine (2) were identified only from *C. longa* and *C. xanthorrhiza*, and curcumin (1) was not detected from rhizomes of *C. aromatica* and *C. zedoaria* harvested from the garden. Bisdemethoxycurcumin was not detected in *Curcuma* plants species from the garden. Sesquiterpenoids, e.g. xanthorrhizol (3) and ar-turmerone (4), were also detected using this procedure.

Superoxide anion radical ($O_2^{\cdot -}$) scavenging activity of rhizome extracts

The rhizomes harvested from the garden were extracted with both PB and MeOH, and then each SOSA was measured using ESR. PB or MeOH solutions without sample were used as blanks when the relative percentage of SOSA was calculated. The median inhibitory concentration (IC_{50}) on SOSA was obtained from the relationship between SOSA and concentration of extract.

The results showed that all PB extracts and MeOH extracts from the *Curcuma* rhizomes species cultivated in the medicinal plant garden exhibited antioxidant activity. ESR spectra of DMPO-OO^- observed upon the addition of various concentrations of MeOH extract from *C. xanthorrhiza* rhizome are shown in Figure 2. A reduction in the signal intensity was observed when a concentration of a sample extract increased. All extracts from the *Curcuma* species rhizomes exhibited the same tendency, thus were confirmed as a superoxide anion radical ($O_2^{\cdot -}$) scavenger.

Figure 3 shows the relationship between the relative inhibitory effects of the formation of DMPO-OO^- and logarithm of various

Table 1: Volatile compounds originating from *Curcuma* species rhizomes cultivated in the garden, identified by TD-GC-MS using CAR/PDMS SPME fiber as an adsorption device.

[a] Compounds	[b] RI	[c] RI^{lit}	*C. longa*	*C. zedoaria*	*C. aromatica*	*C. xanthorrhiza*	[d] ID
β-Myrcene	990	990	–	○	○	○	MS, RI, Std
p -Cymene	1024	1023	○	○	○	○	MS, RI, Std
Limonene	1027	1027	–	○	○	○	MS, RI, Std
1, 8-Cineol	1029	1030	○	○	○	○	MS, RI, Std
β-*cis* -Ocimene	1037	1037	–	○	–	–	MS, RI
2-Nonanone	1090	1090	–	○	○	–	MS, RI, Std
α-Terpinolene	1089	1089	○	–	–	○	MS, RI, Std
Linalool	1099	1099	–	–	○	–	MS, RI, Std
Camphor	1143	1143	–	○	–	–	MS, RI, Std
Terpinen-4-ol	1175	1175	–	–	○	–	MS, RI, Std
α-Terpineol	1189	1189	–	○	○	–	MS, RI, Std
2-Undecanone	1292	1292	–	○	–	–	MS, RI
δ-Elemene	1338	1338	–	○	○	–	MS, RI
β-Elemene	1349	1349	○	○	○	○	MS, RI
β-Caryophyllene	1423	1423	○	○	○	○	MS, RI, Std
α-Humulene	1456	1456	–	–	○	–	MS, RI, Std
(*E*)- β-Farnesene	1457	1457	○	○	–	○	MS, RI, Std
ar-Curcumene	1485	1485	○	–	–	○	MS, RI
β-Bisabolene	1512	1512	○	–	–	○	MS, RI
γ-Cadinene	1517	1517	–	○	–	–	MS, RI
δ-Cadinene	1526	1526	–	○	–	–	MS, RI
β-Sesquiphellandrene	1531	1531	–	–	–	○	MS, RI
ar-Turmerone	1663	1664	–	○	–	○	MS, RI

[a]Compounds are listed in order of their elution from a DB-5 column. [b]RI on the DB-5 column, experimentally determined using a homologous series of C9-C33 *n*-alkanes. [c]RI^{lit} taken from previously analyzed compounds in the Aroma Office database [21]. [d]Identification methods: MS, by comparing their mass spectra with those in the NIST library; RI, by comparing RIs with those in RI^{lit}; Std, by comparing retention times and mass spectra with those of available authentic standards.
○, identified; –, not identified.

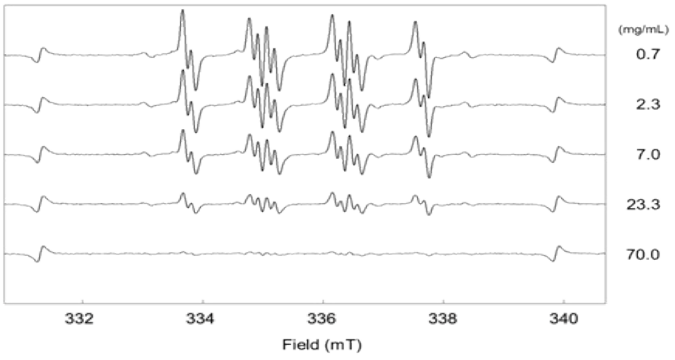

Figure 2: ESR spectra of $DMPOO^-$ observed upon the addition of various concentrations of MeOH extracts from *C. xanthorrhiza* rhizomes.

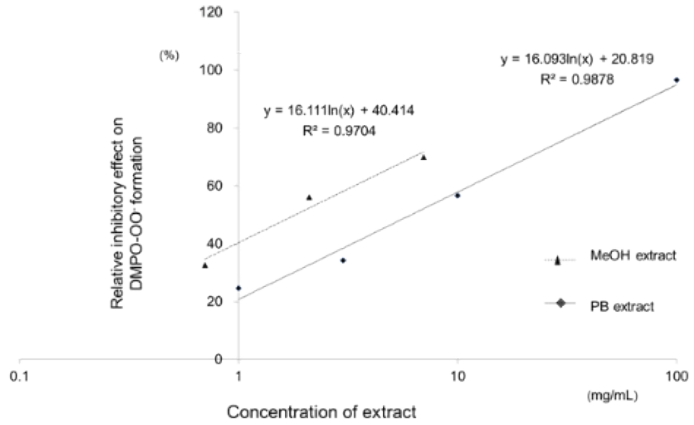

Figure 3: Relative inhibitory effects of various concentrations of MeOH extracts and PB extracts from *C. xanthorrhiza* rhizomes.

concentrations of PB extract and MeOH extract from *C. xanthorrhiza* rhizomes. The relative inhibition of the formation of $DMPO\text{-}OO^-$ increased with the increasing concentration of each extract. The linearity was expressed as $y=16.111\ln(x)+40.414$; $R^2=0.9704$ of MeOH extract, and as $y=16.093\ln(x)+20.819$; $R^2=0.9878$ of PB extract. The MeOH extract tended to exhibit greater scavenging activity against $O_2^{\cdot -}$ than the PB extract. Extracts of *C. longa* exhibited the same tendency. The IC_{50} values of the PB extract and MeOH extract were obtained. It is known that the lower the IC_{50} value, the higher the antioxidant activity.

Subsequently, extracts from rhizomes of *C. zedoaria* and *C. aromatica* were analyzed using the same method. Figure 4 shows the relationship between the relative inhibitory effects of the formation of $DMPO\text{-}OO^-$ and logarithm of various concentrations of PB extract and MeOH extract from *C. zedoaria* rhizomes. The linearity was expressed as $y=14.398\ln(x) + 15.766$; $R^2=0.9863$ of PB extract, and as $y=18.601\ln(x) - 17.878$; $R^2=0.9994$ of MeOH extract. Extracts of *C. aromatica* exhibited the same tendency. Although both of these species exert anti-oxidant activity, their activities were not as strong as those of *C. longa* and *C. xanthorrhiza*.

Table 2: Calculated monoisotopic mass and measured mass of compounds originating from *Curcuma* species rhizomes harvested from the garden using DART-TOFMS.

Compounds	[M+H]	Mass		(mmu) mass difference
		Calcd.	Measd.	
(a) Curcuma longa				
Zingiberene	$C_{15}H_{25}$	205.1956	205.1944	1.2
ar-Turmerone (4)	$C_{15}H_{21}O$	217.1592	217.1589	0.3
Germacrone	$C_{15}H_{23}O$	219.1749	219.1738	1.1
Curcumenol, Isocurcumenol	$C_{15}H_{23}O_2$	235.1698	235.1684	1.4
Demethoxycurcumin (2)	$C_{20}H_{19}O_5$	339.1233	339.1251	1.8
Curcumin (1)	$C_{21}H_{21}O_6$	369.1338	369.1346	0.8
(b) C. xanthorrhiza				
ar-Curcumene	$C_{15}H_{23}$	203.1800	203.1801	0.1
Zingiberene	$C_{15}H_{25}$	205.1956	295.1966	1.0
ar-Turmerone (4)	$C_{15}H_{21}O$	217.1592	217.1599	0.7
Xanthorrhizol (3)	$C_{15}H_{23}O$	219.1749	219.1763	1.4
Demethoxycurcumin (2)	$C_{20}H_{19}O_5$	339.1233	339.1208	2.5
Curcumin (1)	$C_{21}H_{21}O_6$	369.1338	369.1362	2.4
(c) C. aromatica				
ar-Curcumene	$C_{15}H_{23}$	203.1800	203.1776	0.4
Germacrone, α-Turmerone	$C_{15}H_{23}O$	219.1749	219.1722	2.7
(d) C. zedoaria				
Furanodiene	$C_{15}H_{21}O$	217.1592	217.1572	2.0
Curzerenone	$C_{15}H_{23}O$	231.1385	231.1370	1.5
Curcumenone,Curcumenol, Procurcumenol	$C_{15}H_{23}O_2$	235.1698	235.1685	1.3
13-Hydroxycurzerenone, 1-Oxocurzerenone, Curcolone	$C_{15}H_{19}O_3$	247.1334	247.1329	0.5

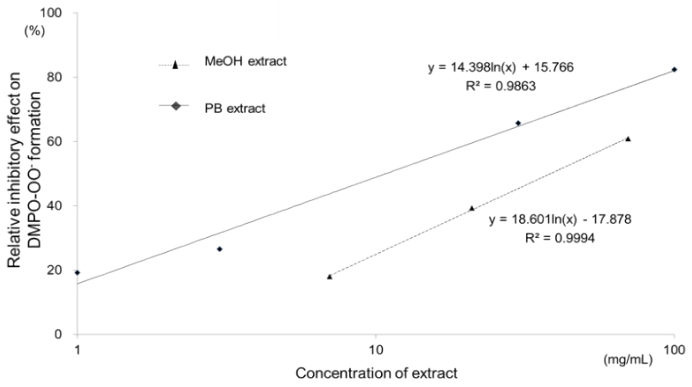

Figure 4: Relative inhibitory effects of various concentrations of MeOH extracts and PB extracts from *C. zedoaria* rhizomes.

Table 3: IC_{50} values of *Curcuma* species rhizome extracts and standard SOD on superoxide anion radical ($O_2^{\cdot-}$) scavenging activity and SOD equivalent values of the extracts.

Extract	IC_{50} of extract (mg/mL)	IC_{50} of SOD (U/mL)	antioxidant unit of extract (U/mg)
C. *longa* in PB	8.50	16.70	1.96
C. *aromatica* in PB	5.77	4.15	0.72
C. *zedoaria* in PB	10.78	4.15	0.38
C. *xanthorrhiza* in PB	6.13	4.15	0.68
C. *longa* in MeOH	2.90	19.20	6.62
C. *aromatica* in MeOH	35.41	8.71	0.25
C. *zedoaria* in MeOH	40.17	8.71	0.22
C. *xanthorrhiza* in MeOH	2.19	8.71	3.98

SOD was used as a standard, and the activity of each rhizome was shown as the SOD equivalent (U/mg) for comparison. The results are shown in Table 3. Although all extracts exhibited antioxidant activity, the MeOH extract of *C. longa* exhibited the greatest activity among the extracts investigated.

The potency order of the MeOH extract was C. *longa*>C. *xanthorrhiza* >C. *aromatica* >C. *zedoaria*. The results indicate the potent anti-oxidant activity of the MeOH extracts of C. *longa* and C. *xanthorrhiza*. The total phenolic and flavonoid contents present in the rhizome are correlated with the anti-oxidant activity. High levels of these compounds in the MeOH extracts indicated strong anti-oxidant activity. The phenolic hydroxyl group, methoxyl group, and the 1,3-diketone are important structural features for the activity. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-1-hepten-3-one, and 3-hydroxy-1,7-bis(4-hydroxyphenyl)-6-hepten-1,5-dione present in C. *longa* contributed to the potent anti-oxidant activity [3]. Some sesquiterpenoids, e.g. xanthorrhizol (3) in C. *xanthorrhiza* and ar-turmerone (4), also exhibited anti-oxidant activity. The phenolic hydroxyl group on the bisabolene skeleton as seen in xanthorrhizol (3) is also an important structural feature. ar-Turmerone (4) has anti-oxidant activity, although its activity is weaker than those of the compounds mentioned previously.

As shown in Table 2, curcumin (1), demethoxycurcumin (2), xanthorrhizol (3), and ar-turmerone (4) were detected from the rhizomes harvested from the garden using DART-TOFMS. The

order of antioxidant activity of the MeOH extracts, i.e., the strongest *C. longa* followed by *C. xanthorrhiza*, could be due to the high levels of curcuminoids, ar-turmerone, and xanthorrhizol present.

Lower antioxidant activities of *C. zedoaria* and *C. aromatica* may depend on the lower levels of phenolic and flavonoid contents in both of them compared with *C. longa* and *C. xanthorrhiza* [3]. Using DART-TOFMS, curcumin (1) was not detected from the rhizomes of *C. zedoaria* and *C. aromatica* cultivated in the medicinal plant garden. Its absence resulted in less potent antioxidant activity of the MeOH extracts of both species.

The rhizomes of *Curcuma* species cultivated in the garden exhibited antioxidant activity. Curcumin (1), demethoxycurcumin (2), and ar-turmerone (4) were identified from *C. longa* and *C. xanthorrhiza*, and xanthorizol (3) was identified from *C. xanthorrhiza* using DART-TOFMS. Phenolic compounds extracted with MeOH play an important role in antioxidant activity [24,25]. The presence of high levels of curcuminoids and other compounds in MeOH extracts reflect the potency of antioxidant activity.

CONCLUSION

Curcuma rhizomes, i.e., *Curcuma longa*, *Curcuma aromatica*, *Curcuma zedoaria*, *Curcuma xanthorrhiza*, cultivated in the medicinal plant garden of Hoshi University were evaluated. Volatile compounds were identified using TD-GC-MS with SPME fiber as an adsorption device, and comparative results between *Curcuma* species were presented and the results were compared with those previous reported. Curcuminoids, which are main active compound in *C. longa* rhizome and were not detected by TD-GC-MS, were detected using DART-TOFMS. Curcumin and demethoxycurcumin were identified from *C. longa* and *C. xanthorrhiza* without complicated preparation prior to measurement. Antioxidant activities of extracts from the rhizomes were investigated using ESR spin-trapping method. The MeOH extracts of *C. longa* and *C. xanthorrhiza* rhizomes exhibited greater antioxidant activity than others. The presence of high levels of curcuminoids and other compounds in MeOH extracts reflect the potency of antioxidant activity.

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