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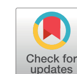
Antioxidant activity of different species and varieties of turmeric (*Curcuma* spp): Isolation of active compounds



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ABSTRACT

There are > 80 species of turmeric (*Curcuma spp.*) and some species have multiple varieties, for example, *Curcuma longa* (*C. longa*) has 70 varieties. They could be different in their chemical properties and biological activities. Therefore, we compared antioxidant activity, total phenolic and flavonoid content of different species and varieties of turmeric namely *C. longa* [variety: Ryudai gold (RD) and Okinawa ukon], *C. xanthorrhiza*, *C. aromatica, C. amada*, and *C. zedoaria*. The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, oxygen radical absorbance capacity (ORAC), reducing power and 2-deoxyribose (2-DR) oxidation assay. Our results suggested that RD contained significantly higher concentrations of total phenolic (157.4 mg gallic acid equivalent/g extract) and flavonoids (1089.5 mg rutin equivalent/g extract). RD also showed significantly higher DPPH radical-scavenging activity (IC_{50}: 26.4 μg/mL), ORAC (14,090 μmol Trolox equivalent/g extract), reducing power absorbance (0.33) and hydroxyl radical scavenging activity (IC_{50}: 7.4 μg/mL). Therefore, RD was chosen for the isolation of antioxidant compounds using silica gel column, Toyopearl HW-40F column, and high-performance liquid chromatography. Structural identification of the compounds was conducted using ^{1}H NMR, ^{13}C NMR, and liquid chromatography-tandem mass spectrometry. The purified antioxidant compounds were bisabolone-9-one (**1**), 4-methyllene-5-hydroxybisabola-2,10-diene-9-one (**2**), turmeronol B (**3**), 5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-1-hepten-3-one (**4**), 3-hydroxy-1,7-bis(4-hydroxyphenyl)-6-hepten-1,5-dione (**5**), cyclobisdemethoxycurcumin (**6**), bisdemethoxycurcumin (**7**), demethoxycurcumin (**8**) and curcumin (**9**). The IC_{50} for DPPH radical-scavenging activity were 474, 621, 234, 29, 39, 257, 198, 47 and 18 μM and hydroxyl radical-scavenging activity were 25.1, 24.4, 20.2, 2.1, 5.1, 17.2, 7.2, 3.3 and 1.5 μM for compound **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8** and **9**, respectively. Our findings suggested that the RD variety of *C. longa*, developed by the University of the Ryukyus, Okinawa, Japan, is a promising source of natural antioxidants.

1. Introduction

Antioxidants are an important part of our regular diet that prevents oxidative cell damage by acting as free radical scavengers (Godic et al., 2014). Free radicals (superoxide radicals [$O_2\cdot^-$], hydroxyl radicals [OH·], and singlet oxygen [$^{1}O_2$]) are producing continuously in the human body by complex redox reactions and play a crucial role in the development of many chronic diseases such as cancer, cardiovascular diseases, arteriosclerosis, diabetes, aging, and neurodegenerative diseases (Pham-Huy et al., 2008). In the normal physiology, specific enzymes in the body such as superoxide dismutase, catalase and glutathione peroxidase usually control the levels of free radicals (Gülçin et al., 2002). However, when the levels of free radicals are higher than those of the endogenous enzymes responsible for clearing them leads to oxidative stress.

To counter the effects of oxidative stress, many people take antioxidants in the form of commercial food additives that are produced synthetically and contain high amounts of preservatives (Shasha et al., 2014). Synthetic antioxidants are also used as food additives to prevent the oxidative deterioration of fats and oils in processed foods (Nanditha

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and Prabhasankar, 2009). However, synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene or tertiary butyl hydroquinone have been reported to produce toxins or act as carcinogens (Grice, 1986; Shasha et al., 2014). In contrary, spices and herbs are rich in antioxidant compounds with no toxic effects (Yanishlieva et al., 2006). Hence, the need for health-promoting natural antioxidants has increased due to limitations on the use of synthetic antioxidants and enhanced public awareness of health issues. Therefore, identifying antioxidant constituents in plant material can be an alternative source of natural antioxidant to ensure sound health.

Turmeric (genus: *Curcuma*) is an herb belonging to the family Zingiberaceae, a yellow spice of high economic importance due to its wide medicinal values. In Indian cooking, it is used in both vegetarian and non-vegetarian preparations as a major spice for the preparation of different types of 'curries'. Numerous studies have been reported the pharmacological activity of turmeric including antioxidant, anti-inflammatory, anti-angiogenic, antibacterial, antifungal, analgesic, immunomodulatory, vasodilatory, antidiabetes, anti-Alzheimer's disease (Ringman et al., 2005; Ramadan et al., 2011; Boaz et al., 2011; Akter et al., 2018a, 2018b). However, the genus *Curcuma* comprises over 80 species and some of the species have multiple varieties, for example, *Curcuma longa* (*C. longa*) has about 70 varieties in India (Sasikumar, 2005). Although some turmeric species have been reported for the antioxidant activity there is a lack of comparative information among the different species and varieties of turmeric. Furthermore, purification of potential antioxidant compounds from *C. longa* has not yet been done. Additionally, RD is a high yielding variety of *C. longa* developed by the University of the Ryukyus, Okinawa and registered by the ministry of agriculture, Japan (registration no. 21485). This is a dwarf and short duration turmeric variety, which provides higher yield (30–40 ton/ha, fresh rhizome) with higher curcumin content, compared to Okinawa indigenous turmeric variety (Okinawa ukon) (Akter et al., 2018a). The rhizome is 5 to 10 cm long, ovoid, cylindrical, branched and deep orange in color. We previously reported that MeOH extract of Ryudai gold (RD) exhibited significantly stronger antifungal activity against *F. solani* than other species and varieties of turmeric (Akter et al., 2018a). Its other pharmacological activities are yet to be explored. Therefore, the aim of this research was to evaluate the antioxidant activity along with the selected phytochemicals (total phenolic and flavonoid) content of different species and varieties of turmeric such as *C. longa* [variety: RD, Okinawa ukon], *C. xanthorrhiza*, *C. aromatica, C. amada* and *C. zedoaria* and purify the active compounds.

2. Materials and methods

2.1. Chemicals

Rutin, $AlCl_3$, gallic acid, potassium ferricyanide, $FeCl_3$, 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), hydrogen peroxide (H_2O_2) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Folin-Ciocalteu reagent, sodium carbonate, trichloroacetic acid, methanol (MeOH), ethyl acetate (EtOAc), ascorbic acid, 2-deoxy-D-ribose and *n*-hexane were obtained from nacalai tesque (Kyoto, Japan). Silica gel (63–200 μm) was from Kanto Chemical Co. (Tokyo, Japan). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-Thiobarbituric acid (TBA) and sodium fluorescein were purchased from Sigma-aldrich (Germany). Toyopearl HW-40F was obtained from Tosoh Corporation, Japan. Ethylenediaminetetraacetic acid (EDTA) was obtained from Dojindo Laboratories, Japan.

2.2. Plant material preparation

Different species and varieties of turmeric namely *C. longa* (variety: RD and Okinawa ukon), *C. xanthorrhiza*, *C. aromatica*, *C. amada* and *C. zedoaria* were cultivated in a field of gray soil (coarse sand 3.6%, fine sand 30.9%, silt 24.3%, clay 32.8%, pH 7.4, NO_3-N 0.07%, NH_4-N 0.08%, P 4.6 ng/g, K 42.9 ng/g; Hossain and Ishimine, 2005a) at the Subtropical Field Science Center, University of the Ryukyus, Okinawa, Japan. The average monthly temperature, humidity, and precipitation during the cultivation period were 17–29 °C, 61–83% and 22–369 mm, respectively. Sixty seed rhizomes of each turmeric species were planted on April 15, 2015, to obtained sufficient sample. Common agronomic practices including fertilizer and irrigation were provided according to Ishimine et al., 2004 and Hossain et al., 2005b. Rhizomes were harvested on February 10, 2016, when all the shoots of the species withered completely. The rhizomes were washed, sliced and dried in a hot air oven at 50 °C for 72 h. We got 10, 10.8, 13.1, 9.3, 20 and 9.1% dry powder from fresh rhizomes of RD, Okinawa ukon, *C. xanthorrhiza, C. aromatica, C. amada* and *C. zedoaria*, respectively.

2.3. Extraction and yield of samples

The equal amount of different turmeric powder (20 g) was extracted with MeOH (200 mL) for 2 days at room temperature (25 °C) with continuous magnetic stirring to prevent oxidation by air and shielding from sunlight. The extraction was carried out so that the components were completely extracted and not oxidized. Then the solutions were filtered through double filter paper (Whatman™ No. 1). Fresh MeOH was added into the used plant material and the process was repeated three times. The filtered solutions were dried on a rotary evaporator under reduced pressure at 40 °C. The yield of all extracts was recorded and kept in the refrigerator at 4 °C for experimental analyses.

2.4. Estimation of total phenolic contents

The amount of total phenolic contents (TPC) of the test samples were performed by using a method of Kahkonen et al., 1999. Briefly, 500 μL of Folin-Ciocalteu and 500 μL of distilled water were added to 200 μL of the test samples (1000 μg/mL). After 1 min, 800 μL of sodium carbonate solution (7.5%) was added to the mixture and incubated at room temperature for 30 min. Absorbance was measured at 760 nm by using Biotek Powerwave XS2 Spectrophotometer (Winooski, USA). The TPC was expressed as gallic acid equivalents (GAE) in mg per g extract. The experiment was carried out in triplicate.

2.5. Estimation of total flavonoid contents

Total flavonoid contents (TFC) were estimated according to the method describe by Djeridane et al., 2006. Rutin was used to make a calibration curve. 100 μL of samples (1000 μg/mL) were put in the microplate and 100 μL of 2% $AlCl_3$ was added. The reaction was mixed and stand at room temperature for 15 min. The absorbance of the mixture was measured at 430 nm by using a Biotek Powerwave XS2 Spectrophotometer (Winooski, USA). The TFC was expressed as rutin equivalents in mg per g extract. The experiment was carried out in triplicate.

2.6. Determination of antioxidant activities

2.6.1. DPPH radical scavenging assay

The DPPH radical scavenging assay was estimated according to the procedure of Boskou et al., 2006. 40 μL of DPPH and 80 μL of sodium acetate buffer (0.1 M, pH = 5.5) were added into 80 μL samples at different concentrations (10, 25 and 50 μg/mL). The mixture solution was incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm by using a Biotek Powerwave XS2 Spectrophotometer (Winooski, USA). Trolox was used as positive control in this assay.

The % DPPH radical scavenging activity was calculated using the formula:

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