Pharmacological activities of different species and varieties of turmeric (*Curcuma spp.*)

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ABSTRACT

Turmeric (*Curcuma* spp.) is a rhizomatous perennial herbs with broad spectrum of pharmacological actions including antioxidant, anticancer, antibacterial, antifungal and insecticidal properties. There are more than 80 species of turmeric and 70 varieties/strains of *Curcuma longa*, which may possess different chemical properties and biological activities. Hence, we compared the major active components, antifungal, antioxidant, vasodilatory and phytotoxic effects of three *Curcuma longa* varieties (Ryudai gold; RD, Okinawa ukon, and BK2), *C. xanthorrhiza*, *C. aromatica, C. amada,* and *C. zedoaria*. The content of curcuminoides (curcumin, demethoxycurcumin and bisdemethoxycurcumin) were compared by HPLC and the active compounds were isolated and purified by using silica gel, TOYOPEARL® HW-40F, and high-performance liquid chromatography. The structural identification of the compounds was conducted using ^{1}H NMR, ^{13}C NMR, and liquid chromatography-tandem mass spectrometry.

The chapter I of this thesis compared curcuminoids content and antifungal activity of different species and varieties of turmeric against *Fusarium solani sensu lato* (FSSL). The antifungal activity was measured by the diameter of colonies grown on Petri dish, microscopic observation, and CLSI microdilution methods. The BK2 variety of *C. longa* contained highest concentration of curcumin, demethoxycurcumin, and bisdemethoxycurcumin followed by RD, *C. xanthorrhiza*, Okinawa ukon, and *C. aromatica*.

These compounds were not present in *C. amada* and *C. zedoaria*. Among the different turmeric, RD showed strongest antifungal activity against FSSL. The order of IC_{50} against FSSL was RD (78 to 92 µg/mL) > BK2 (89 to 101 µg/mL) > *C. xanthorrhiza* (98 to 114 µg/mL) > *C. aromatica* (183 to 204 µg/mL) > *C. amada* (183 to 206 µg/mL) > Okinawa ukon (191 to 216 µg/mL) > *C. zedoaria* (354 to 385 µg/mL). Our results found a correlation between the antifungal activity and curcuminiods contents of turmeric. However, RD contained lower curcuminoid than BK2 but showed stronger antifungal activity. Similarly, *C. amada* and *C. zedoaria* had no curcuminoids, but showed antifungal effects which indicated that other compounds present in RD, *C. amada* and *C. zedoaria* could also inhibit the growth of FSSL. Therefore, RD (contain curcuminoids) and *C. amada* (contain no curcuminoids) were chosen for the isolation and purification of antifungal compounds. The purified compounds were turmeronol B, turmeronol A, (*E)*-α-atlantone, dihydrobisdemethoxycurcumin, demethoxycurcumin, and curcumin from RD and zederone and furanodienone from *C. amada*. The IC_{50} of the isolated compounds against the four isolates of FSSL ranged from 116 to172, 127 to 185, 88 to 109, 90 to 112, 74 to 80, 63 to 68, 115 to 129, and 82 to 91 µM for turmeronol B, turmeronol A, (*E)*-α-atlantone, dihydrobisdemethoxycurcumin, demethoxycurcumin, curcumin, zederone and furanodienone, respectively.

The chapter II of this thesis compared antioxidant activity, total phenolic and flavonoid content of different species and varieties of turmeric. 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. The 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). It also showed significantly higher DPPH radical-scavenging activity (IC_{50}: 26.4 μg/mL), ORAC (14090 μmol Trolox equivalent/g extract), reducing power absorbance (0.33) and hydroxyl radical scavenging activity (IC_{50}: 7.4 μg/mL). Therefore, I purified 9 antioxidant compounds from RD namely, 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.

The chapter III clarify the vasodilatory mechanism of turmeric that traditionally used for the treatment of cardiovascular disorders, such as hypertension, and palpitations. A tissue-organ-bath system was used to investigate the vasoactive effects of methanol extracts of turmeric on the isolated porcine basilar artery. All turmeric extracts (20–800 μg/mL) induced concentration-dependent relaxation of the isolated porcine basilar artery without significant difference. No significant differences were observed between the endothelium-intact and denuded arteries. In depolarized, Ca^{2+}-free medium, the turmeric extracts inhibited $CaCl_2$-induced contractions. In addition, propranolol (a non-specific β-adrenoceptor antagonist) slightly inhibited the relaxation induced by turmeric. In contrast, Nω-nitro-L-arginine, indomethacin, tetraethylammonium, glibenclamide and 4-aminopyridine did not affect

turmeric-induced relaxation. These results demonstrated that turmeric induced endothelium-independent relaxation of the porcine basilar artery, which may be due to the inhibition of extracellular and intracellular Ca^{2+} and the partial inhibition of β-adrenergic receptors.

The chapter IV evaluated the plant growth inhibitory activities of two cultivars of *Curcuma longa* (*C. longa*; Ryudai gold and Okinawa ukon) against radish, cress, lettuce and *Bidens pilosa* (*B. pilosa*). The methanol extracts of RD had a significantly higher inhibitory effect on the seed germination and root and shoot growth of the plants than Okinawa ukon. The growth inhibitors from RD were identified as dihydrobisdemethoxycurcumin, bisdemethoxycurcumin, demethoxycurcumin, and curcumin. The IC_{50} of the curcuminoids against the root and shoot growth of *B. pilosa* ranged from 8.7±1.7 to 12.9±1.8 and 15.5±1.8 to 38.9±2.8 μM, respectively.

This thesis concluded that different species and varieties of turmeric contained different levels of curcuminoides, phenolic, flavonoids and other components, and showed different degree of antifungal, antioxidant, relaxation and plant growth inhibitory effects. Some other compounds except curcuminoids present in different turmeric species and strains had antifungal and antioxidant properties. All the nine compounds isolated from the turmeric RD showed antioxidant activities in different levels. The turmeric variety ‘Ryudai gold’ developed by the University of the Ryukyus showed stronger antifungal, antioxidant, relaxation and phytotoxic effects than other turmeric, which could be a potential source of natural compounds with various pharmacological actions.