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Radical Scavenging Activity and Cytotoxicity of Maysin (*C*-glycosylflavone) isolated from Silks *of Zea* mays L.

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ABSTRACT: Maysin, a *C*-glycosylflavone, was isolated from the silks of maize, *Zea mays* L. The ESI mass spectrum indicates that molecular weight of maysin is 577M$^+$*m/z*, and the ether-linked sugar is rhamnose, 431M$^+$m/z (MW$^+$-146). The DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity of maysin was higher than that of rutin. However, as compared with its aglycon luteolin, maysin showed the relatively moderate DPPH scavenging activity mainly due to the glycosylation of two sugars moieties, ketofucose and rhamnose. In the *in vitro* cytotoxicity test against the five human tumor cell lines such as lung (A549), ovarian (SK-OV-3), melanoma (SK-MEL-2), central nerve system (XF-489), and colon (HCT-15), maysin exhibited the relatively weaker activities than cisplatin. The ED_{50} values of maysin were 62.24, 43.18, 16.83, 37.22, and 32.09/ml, respectively. Result suggests that maysin is a potential cytotoxicity compound, particularly for human colon, central nerve system, and melanoma tumors.

Keywords: corn silk, maysin, DPPH radical scavenging activity, cytotoxicity

Flavonoids exhibit a wide range of biological activities and antioxidative effects such as free radical scavenges, hydrogen donates, singlet oxgen quenches, and metal ion chelates, because of their hydroxyl groups attached to the phenolic ring structures (Brown and Rice-Evans, 1998; Joseph *et al.*, 1986; Masafumi *et al.*, 2001; Plumb *et al.*, 1998; Recio *et al.*, 1995).

Antioxidants in plants and foods have attracted interest in recent years. Flavonoids given as biological substances in foodstuffs may contribute to the prevention of diseases, although they do not have strong biological activities. Flavonoids also thought to help inhibiting the growth of cancer cells and induce the differentiation of cancer cells. Several dietary flavonoids such as quercetin, luteolin and genistein have been suggested to have cancer chemopreventive effects (Cai *et al.*, 1997). Plants provide a rich source of bioactive substances including flavonoids, and many of them are largely free from adverse effects and have excellent pharmacological actions.

The silk of corn plants has been used as traditional medicine and various purpose in the several countries (Doan *et al.*, 1992; Kim *et al.*, 1996; Martin *et al.*, 1991). In spite of the fact that most traditional drugs have been used for a long time, there is a lack of modern clinical studies demonstrating the efficacy of the drugs and related compounds.

Recently, maysin [2"-O-α-L-rhamnosyl-6-C-(6-deoxy-xylo-hexose-4-ulosyl)luteolin] (Fig. 1) has been reported as one of the important natural antibiotics in corn plants, and has provided a new tool for reducing the damage by the larvae of corn earworm (Byrne *et al.*, 1996; Richard *et al.*, 1992; Snook *et al.*, 1993; 1995; Widstrom *et al.*, 1998). However, in earlier studies on maysin and its analogues of corn silk had been limited to the antibiotic properties to the corn earworm (*Helicoverpa zea* Boddie) and genetic variation in corn plants.

To our knowledge, no previous investigation has been done on the radical scavenging and antitumor activities of maysin isolated from the silk of corn plants. Therefore, we discussed the possibility of corn silk-derived maysin as an agent for radical scavenging activity and cytotoxicity.

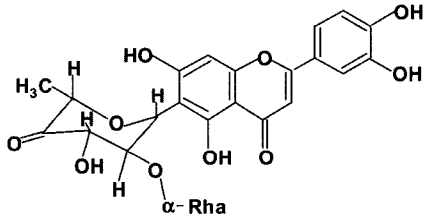

Fig. 1. Chemical structure of maysin.

MATERRIALS AND METHODS

Plant materials

Korean waxy corn variety, Chalok 2, was grown at the field of National Crop Experiment Station, Suwon, Korea. Before the silk-stage, ears of corn were sealed with paper-bag to prevent pollination, and 3-5 days after unpollinated

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corn silks were collected with excising.

Extraction, isolation and identification

Collected corn silks (500 g) were immediately soaked with 100% MeOH (1000 ml) and grind with a high-speed homogenizer followed by filtration. Filtrate was concentrated at 35~40°C, dissolved in distilled water (150 ml), and added methylene chloride(CH_2Cl_2, 300 ml) on the concentrated aqueous solution to removed the chlorophyll and lipids, then evaporated to remove methylene chloride. The final concentrate (50 ml) was submitted to preparative reversed phase column chromatography (C_{18}, 55-105 mm, 25 mm× 54 cm) and washed using distilled water (2×250 ml) under nitrogen to remove the sugars and water-soluble pigments. Then the column was eluted by 10, 30, and 50% MeOH (3× 250 ml), and 50% MeOH effluents were combined and evaporated to dryness. The residue was dissolved in MeOH (100 ml) and silicic acid deposited mixture (approximately 30g) was submitted to silicic acid column (25 mm×54 cm) chromatography. Then eluted subsequently by CH_2Cl_2: EtOAc (50:50, 3×250 ml), 100% EtOAc (250 ml), and collected fractions (50 ml, each). The 100% EtOAc effluents (Fraction 16-19) were combined and evaporated to dryness. Final purification was conducted by the C_{18} column (12.7 mm×110 cm) chromatography using 50% MeOH as eluting solvent and obtained 5.1 g of maysin.

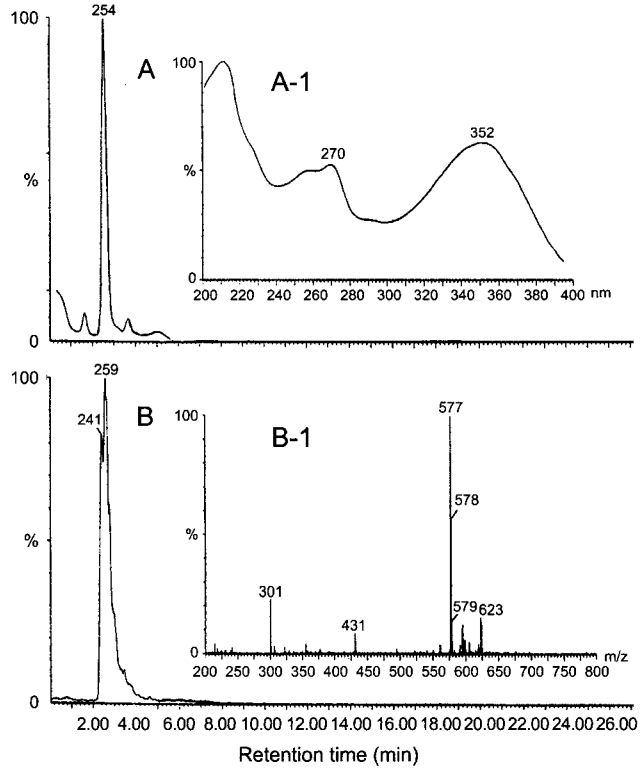

Fig. 2. HPLC chromatogram and ion spectrum of maysin isolated from the silks of Korea waxy corn (*Zea mays* L.). A: HPLC chromatogram of maysin by using Waters X-Terra MS C_{18} column (2.1×150 mm, 3.5 μm); A-1: UV absorption of the peak at 2.54 min. monitored by photodiode array detector; B: ion chromatogram of maysin; B-1: Ion fragmentation spectrum of the peak at 2.59 min.

The purified maysin was dissolved in methanol and electrospray mass spectrometric analysis was performed on a separations module (Waters 2690 alliance) equipped with a photodiode array detector (Waters 996) and a micromass electrospray interface (Micromass ZMD 4000). The ESI mass spectrum was obtained by positive ion mode with 200 to 800*m/z* scan mode. Nitrogen was used as nebulizing gas, source block and desolvation temperature were 150°C and 250°C, respectively. The purity of maysin obtained by the C_{18} column was greater than 90% (Fig. 2A) and showed the λ_{max} of UV absorption at 270 and 352 nm (Fig. 2A-1). The ESI fragmentation pattern in positive ion mode (Fig. 2B-1) indicates that molecular weight (MW^+) of maysin is $577M^+m/z$, and the ether-linked sugar is rhamnose, $431M^+m/z$ (MW^+-146). The structure of the isolated maysin was determined on the basis of ^{1}H-NMR and ^{13}C-NMR spectra recorded by Bruker DPX 400*MHz* NMR spectrometer (German) in DMSO-d_6 at 400 and 100*MHz*, respectively, and confirmed by comparison with literature data (Snook *et al.*, 1995).

DPPH radical scavenging activity

The DPPH(1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activities of maysin and other flavonoids were assayed by spectrophotometer (Blois, 1958). The 3.2 ml ethanolic solution of DPPH (0.1 mM) was mixed with 0.8ml of flavonoids solution in ethanol, vigorously mixed with vortex mixer for 10 second. After 10 minute, the absorbance of the mixture was measured at 517 against the blank (ethanol). The DPPH radical scavenging activities of maysin and commercially available flavonoids such as rutin, quercetin, and luteolin (Sigma, St. Louis, MO) were expressed by the reduction percentage of DPPH.

Cytotoxicity assay

Five human tumor cell lines such as lung (A549), ovarian (SK-OV-3), melanoma (SK-MEL-2), central nerve system (XF-489), and colon (HCT-15) were used for the *in vitro* cytotoxicity of maysin. The SRB (sulforhodamine B) assay was applied for assessing the cytotoxicity of maysin (Monks *et al.*, 1991; Monks *et al.*, 1990). Cisplatin (control) and maysin dissolved in culture medium were applied to the cul-

ture wells (1-2×10^4 cells/well) followed by incubating for 48 hr at 37°C under 5% CO_2. The culture wells fixed with cold TCA were stained by 0.4% SRB dissolved in 1% acetic acid. The bound dye was destained with 10 mM Tris-base, and the absorbance was measured at 520 nm with a microplate reader. Fifty percent inhibitory dosage (ED_{50}) was defined as the dosage that reduced absorbance by 50% of untreated wells as the control in the SRB assay.

RESULTS AND DISCUSSION

The DPPH assay measured hydrogen atom donating activity and hence provides an evaluation of antioxidant activity due to free radical scavenging. The DPPH radical scavenging activities of maysin and commercial flavonoids were sharply increased as concentrations increased to 50 μg/ml (Fig. 3). Luteolin, quercetin, maysin, and rutin have the *O*-dihydroxybenzene (catechol) structure in their B-rings, thus this property gives an enhanced radical scavenging activity (Plumb *et al.*, 1998; Senba *et al.*, 1999).

Brown and Rice-Evans (1998) reported that luteolin-7-*O*-glucoside extracted from artichoke exhibited the potential role for antioxidative effects, even though less effective than that of luteolin. Maysin and rutin showed relatively moderate radical scavenging activities than their aglycones, luteolin and quercetin.

The relatively moderate DPPH scavenging activity of maysin as compared with its aglycone, luteolin, indicating that the glycosylation of two sugars moieties, ketofucose and rhamnose, was the reason for reducing the activity. In the antibiotic point of view, the sugar residues of maysin and rutin are not important because their aglycones were found to be just as active in protecting the attack of corn earworm (Snook *et al.*, 1993; 1995). The structure difference in flavonoid family results from the variation in the number and arrangement of the hydroxyl groups and the extant of glycosylation of these groups.

Masafumi *et al.* (2001) suggested that not only the numbers of hydroxy group but also the position of hydroxy group

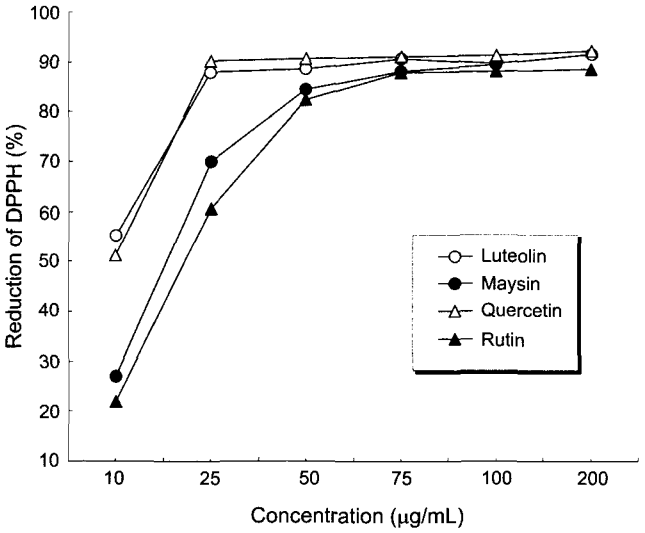

Fig. 3. Comparison of DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging activities among maysin and commercially available flavonoids. The data represents the mean value of replications (n=5), and each concentration is statistically significant at $p<0.01$. Least significant differences (LSD) of each concentration are 0.231, 0.201, 0.162, 0.115, and 0.091, respectively.

Table 1. Cytotoxic activity of maysin for each tumor cell line (Net growth as prcent of control).

Compound	Conc. (μg/ml)	Tumor cell lines*				
		A549	SK-OV-3	SK-MEL-2	XF-498	HC-T15
Cisplatin	0.03	93.69	100.19	93.42	98.90	104.04
	0.10	80.46	91.02	75.51	58.81	104.63
	0.30	60.71	88.53	67.51	28.49	99.71
	1.00	25.00	28.88	48.70	-28.75	72.63
	3.00	-62.44	-29.34	-69.44	-86.47	30.01
	10.00	-54.07	-44.85	-64.86	-66.95	-91.58
	ED_{50}	0.43	0.62	0.91	0.18	1.84
Maysin	0.3	98.07	100.52	98.25	97.94	103.66
	1.0	95.74	98.53	93.38	99.79	105.17
	3.0	93.13	102.51	88.19	92.81	94.56
	10.0	92.38	96.86	63.21	79.05	84.81
	30.0	81.78	69.62	38.81	57.02	51.65
	100.0	20.31	2.36	- 21.64	13.63	1.73
	ED_{50}	64.24	43.18	16.83	37.22	32.09

*A549 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF-498 (human central nerve system) and HCT-15 (human colon); 100: Growing than control, 100: Control, -100: Total killing state; ED_{50}: Effective dosage for reducing absorbance by 50% of untreated wells as the control in the SRB assay.

are important for mediating potent activity. Results showed that quercetin which have 3,5,7,3',4'-pentahydroxyl group exhibited the slightly higher DPPH radical reduction activity than luteolin which having 5,7,3'4'-tetrahydroxyl group, while the activity of the maysin (luteolin-*C*-glycoside) was higher than that of the rutin (quercetin-*O*-glycoside).

Thus, it was suggested that the C-3 hydroxyl group in A-ring (phloroglucinol) also have a scavenge activity for DPPH radical. The scavenging activity of maysin (luteolin-*C*-glycoside) was statistically higher ($P<0.01$) than that of the rutin (quercetin-*O*-glycoside). For all these reasons, the antioxidant activity assessments of maysin by measuring their activity to scavenge the DPPH indicated that the maysin is a potent antioxidant obtained from the silk of corn plants.

Five human tumor cell lines such as A549 (human lung), SK-MEL-2 (human melanoma), SK-OV-3 (human overian), HCT-15 (human colon), and XF-498 (human central nerve system) were used for the *in vitro* antitumor activity test of maysin. The SRB assay (Monks *et al.*, 1991; Rubinstein *et al.*, 1990) was used to the measurement of the antitumor activity of maysin against human tumor cell.

Table 1 shows the *in vitro* antitumor activity test for maysin. The effective growth inhibition against human tumor cell lines was achieved with relatively low concentrations of cisplatin and maysin, but cisplatin showed stronger cytotoxicity than that of maysin. Cisplatin is used in the treatment of ovarian, bladder, head and neck, and non-small cell lung cancers. It has been thought that cisplatin may led to cell death due to the formation of cisplatin adduct but its effectiveness is restricted because administration of the drug often lead to resistance of the tumor to the drug (Barry *et al.*, 1990; Zamble and Lippard, 1995).

Maysin showed relatively weaker activities than cisplatin against human tumor cell lines, but it has been generally acknowledged that plant extracts having cytotoxicity at <40 μg/ml may be useful for developing antitumor agents. The ED_{50} values of maysin on the five human tumor cell lines such as human lung (A549), ovarian (SK-OV-3), melanoma (SK-MEL-2), central nerve system (XF-489), and colon (HCT-15) were 62.24, 43.18, 16.83, 37.22, and 32.09 μg/ml, respectively.

Result suggests that maysin is a potential cytotoxicity compound, particularly for human colon, central nerve system, and melanoma tumors. The biological activity and metabolic pathway of flavonoid in plants are still complicated because a number of substances are included in various proportions in plants.

However, the study on radical scavenging and antitumor substances including flavonoids in crops and medicinal plants will be more important because such beneficial substances might be applied for treatment and prevention of human diseases.

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