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Antimutagenic Activity of the Methanol Extract and Compounds of *Angelica keiskei* in the *Salmonella* Assay System

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Abstract – The methanol extract of aerial part of *Angelica keiskei* Koidzumi exhibited a strong antimutagenic activity against aflatoxin B_1 (AFB_1), N-methyl-N′-nitro-N-nitrosoguanidine and 4-nitroquinoline-1-oxide in the Ames test with *Salmonella typhimurium* TA100. Cynaroside, isolated from ethylacetate fraction of the methanol extract over silica gel, inhibited the mutagenicity of AFB_1 with an inhibition value of 96% at 1.0 mg/plate concentration and 89% at 0.5 mg/plate concentration. Other compounds, hyperoside, sucrose and luteolin-7-rutinoside, isolated from ethylactate or n-butanol fraction, also showed antimutagenic effect.

Key words – *Angelica keiskei*; antimutagenic; cynaroside; *Salmonella typhimurium* TA 100; aflatoxin B_1.

Angelica keiskei Koidzumi (Umbelliferae) which is used for health food in Korea, has been used as a folklore medicine against many metabolic disease such as hypertension, hepatosis and neuralgia.[1] Of chemical components isolated from the roots of *A. keiskei*, some chalcones have been proved to have antitumor-promoting activity in mouse skin carcinogenesis and antibacterial activity against gram-positive pathogenic bacteria.[2,3] A flavone glycoside from the aerial part of the plant has been shown to be hypocholesterolemic and hypotriglyceridemic in rats with fed high fat and high cholesterol diet.[4]

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Antimutagenic activities of some edible plants have been screened.[5,6] As a part of our continuing search for antimutagenic compounds from edible and medicinal plants, active components in the methanol extract from the aerial part of *A. keiskei* were investigated in the present study.

MATERIALS AND METHODS

Plant material and compounds – *Angelica keiskei* was collected by the author (J.C.P.) in Sunchon on April 25, 1994. The voucher specimen (No. 940425) is deposited in department of Oriental Medicine Resources, Sunchon National University. The air-dried aerial parts of *A. keiskei* (1.3 kg) was ex-

tracted with boiling methanol for 4 hours. The methanol extract was then fractionated into chloroform (125 g), ethyl acetate (24 g), n-butanol (64 g) and aqueous (95 g) fractions. Compounds isolated from the ethyl acetate and n-butanol fractions[4,7] were used in this study.

Mutagen – AFB_1 was purchased from Sigma Chemical Co., St. Louis, MO, U.S.A., and MNNG and 4-NQO were obtained from Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Bacterial strain – *Salmonella typhimurium* strain TA100, which is a histidine-requiring mutant, was kindly provided by Dr. B. N. Ames, University of California, Berkley, CA, U.S.A., and maintained as conditions described by Maron and Ames.[8] The genotypes of the test strains were checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor.

S9 fraction and S9 mix – According to the method described by Maron and Ames,[8] male Sprague-Dawley rats were injected intraperitoneally with Aroclor 1254 dissolved in corn oil (500 mg/kg body weight). Five days after the injection, the rats were sacrificed, their livers were removed and minced in 0.15 M KCl, and then homogenized with a Potter-Elvehjem apparatus. The homogenate was centrifuged at $9{,}000 \times g$ for 10 minutes in a refrigerated centrifuge and the supernatant, S9 fraction, was distributed in 1.8-2.0 ml portions in plastic Nunc tubes, frozen quickly in a bed of crushed dry ice, and stored immediately at -80℃ until use. The S9 for the preparation of S9 mix was thawed at room temperature and placed in a container of crushed ice. S9 mix was prepared as soon as the S9 had thawed. The components of the S9 mix were 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate, pH 7.4, and S9 at a concentration of 0.04 ml per ml of mix. The S9 mix was prepared freshly for each mutagenicity assay.

Mutagenicity test – A modified plate incorporation procedure[9] was employed to determine the effects of the methanol extract on AFB_1, MNNG and 4-NQO-induced mutagenicities, and the fractions and compounds on AFB_1-induced mutagenicity, respectively. In brief, 0.5 ml of S9 mix (or phosphate buffer for MNNG and 4-NQO, respectively) was distributed in sterile capped tubes in an ice bath, and then 0.1 ml of test bacterial suspension from an overnight culture ($1\text{-}2 \times 10^9$ cells/ml) and 0.1 ml of test samples (50 μl of 1 μg/plate mutagen and/or 50 μl of 0.5-5.0 mg/plate samples) were added. After vortexing gently and preincubating at 37℃ for 30 minutes, 2 ml of the top agar kept at 45℃ was added to each tube and vortexed for 3 seconds. The resulting complete mixture was overlaid on a minimal agar plate. The plates were incubated at 37℃ for 48 hours and then the revertant bacterial colonies on each plate were counted. Toxicity tests for the different cells of the samples were also carried out, and the sample concentrations employed for the antimutagenic test did not show any toxicity.

RESULTS AND DISCUSSION

Methanol extract of this plant exhibited strong antimutagenic activities against aflatoxin B_1 (AFB_1), N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) in the Ames test with *Salmonella typhimurium* TA100 (Table I). The antimutagenic activities were dose dependent at concentrations between 1 and 10%. The extract also showed less antimutagenicity against 4-nitroquinoline-1-oxide

Table I. Effects of methanol extract of *A. keiskei* on the mutagenicity induced by aflatoxin B_1 (AFB_1), N-methyl-N′-nitro-N-nitrosoguanidine(MNNG) and 4-nitroquinoline-1-oxide (4-NQO) in *Salmonella typhimurium* TA 100

Treatment	Concentration (mg/plate)	Revertants per plate		
		AFB_1	MNNG	4-NQO
Spontaneous	-	252±42[a]	81±1	90±2
Mutagen	-	1584±78	998±120	700±18
Mutagen+ MeOH ext.	5.0	492±32** (82)[b]	295±103** (77)	343±59** (59)
Mutagen+ MeOH ext.	2.5	892±15** (52)	300±103** (77)	493±22** (34)
Mutagen+ MeOH ext.	1.25	1120±18** (29)	375±23** (68)	600±12** (16)
Mutagen+ MeOH ext.	0.5	1250±30* (25)	546±40** (49)	621±17** (13)

[a]The values are mean ± S.D. of three replications. Student t-test: significantly different from control (mutagen) group. *: $p<0.05$. **: $p<0.01$. [b]Inhibition (%).

(4-NQO) than against AFB_1 and MNNG with 59% inhibition at 10% concentration.

Since the methanol extract showed a strong antimutagenic activity, the extract was fractionated sequentially with chloroform, ethyl actate and n-butanol. At a dose of 2.5%, chloroform, ethyl acetate and n-butanol fractions inhibited the mutagenicity induced by AFB_1 effectively with an inhibition rate of 80%, 83% and 81%, respectively (Table II). Five compounds, three (adenosine, hyperoside and cynaroside) isolated from the ethyl acetate and two (luteolin-7-rutinoside and sucrose) isolated from n-butanol fraction were tested for antimutagenicity against AFB_1 (Table II). Adenosine did not inhibit the mutagenicity of AFB_1. However, hyperoside, cynaroside, luteolin 7-rutinoside and sucrose were active against AFB_1-induced mutagenicity. Of the four active compounds cynaroside was the most effective agent, showing an inhibition rate of 96% at 1.0 mg/plate concentration and 89% at 0.5 mg/plate concentration, followed by hyperoside, sucrose and luteolin-7-rutinoside. These findings suggest that the four compounds are, at least in part, responsible for the antimutagenic activity of *A. keiskei* against AFB_1. The mechanism of the antimutagenic activity of flavonoids is uncertain presently, but the possible antimutagenic mechanism of flavonoids has been demonstrated as inhibitory action on

Table II. Effect of fractions and compounds isolated from *A. keiskei* on the mutagenicity induced by AFB_1 in *Salmonella typhimurium* TA100

Treatment	Concentration (mg/plate)	Revertants per plate	Inhibition (%)
Spontaneous	-	119±8[a]	
AFB_1	-	1200±122	
AFB_1+$CHCl_3$ fr.	1.25	336±108**	80
AFB_1+EtOAc fr.	1.25	305±1**	83
AFB_1+n-BuOH fr.	1.25	325±30**	81
AFB_1+Aqueous fr.	1.25	1398±269	-
AFB_1+Adenosine	0.5	1211±6	-
	1.0	1175±134	2
AFB_1+Hyperoside	0.5	807±30**	36
	1.0	300±124**	83
AFB_1+Cynaroside	0.5	237±43	89
	1.0	159±2**	96
AFB_1+ Luteolin-7-rutinoside	0.5	1076±71	11
	1.0	809±14**	36
AFB_1+Sucrose	0.5	806±94*	36
	1.0	867±95*	31

[a]The values are mean±S.D. of three replications. Student t-test: significantly different from control (AFB_1) group. *: $p<0.05$. **: $p<0.01$.

DNA-adduct formation through interaction with microsomal activating enzymes.[10]

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