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Antimutagenic Effects of Juices from Edible Korean Wild Herbs

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Abstract

The mutagenic and antimutagenic activities of juices from 20 common edible wild herbs found in Korea were investigated using the spore-*rec* assay and Ames test. The juices of *Hemerocallis fulva* and *Capsella bursapastoris* exhibited a little induction or inhibition of mutagenesis in the presence of selected metal ions, but juices of most edible wild herbs did not affect on the mutagenesis in the spore-*rec* assay. In the other hand, all of the juices strongly inhibited the mutagenesis induced by benzo[*a*]pyrene, 2-amino-fluorene, and 3-amino-1,4-dimethyl-5H-pyridol tested on *Salmonella typhimurium* TA98 or TA100 in the presence of S-9 mix. The antimutagenic effects increased as the concentration of the mutagens increase. The results suggest that concentration of samples or types of various mutagen interact to affect the antimutagenic potential of the juices in the TA98 and TA100 strain.

Key words: edible wild herbs, juices, mutagenesis, antimutagenic effects, metal ions

INTRODUCTION

Recently, there has been increased interest in consumption of edible wild herbs because of concern about human health as people income increased. Generally, many processed foods, such as broiled foods, salty foods, fish, fermented vegetables, grains contain harmful components for carcinogenic contamination or occurrence of cancer(1). Such harmful factors are of potential human concern. On the other hand, many researchers have reported the antimutagenic effect of natural food components from plants, fruits, and vegetables(2-5). It has been reported that high correlation between carcinogenecity and mutagenecity is established and significant proportion of mammalian cancers may arise as a consequence of induced somatic mutations(6). Increasing reports on dietary carcinogens and anticarcinogens gave us to clue that the screening on the antimutagenic effects of wild edible herbs are of high potential value in preventing cancer and other disease linked with mutational occurrence.

Edible wild herbs have been traditionally used as food stuffs or medicinal materials without any scientific proof. Consumption of wild herbs depends on the customs and tastes of the each people. The herbs have been usually used as an appetizer or side dishes, and some of them used due to their medicinal effects. However, little works have been reported on the physiological effects of wild herbs on human.

We have previously investigated potent inhibitors of mutagenicity extracted from various heated wild herbs (7) and recently, consumption of fresh edible herbs has rapidly increased due to nutritional value and health concerns. Thus, the purpose of this study was to screen whether fresh juices of twenty species of Korean wild herbs contain the mutagenic properties by themselves or the antimutagenic activities on benzo[*a*]pyrene(B[*a*]P), 2-aminofluorene(2-AF), and 3-amino-1,4-dimethyl-5H-pyrido[4,3-d]indole(Trp-P-1) induced mutagenesis.

MATERIALS AND METHODS

Materials

Twenty edible wild herbs, *Aster tartaricus, Scilla scilloides, Rumex crispus, Hemerocallis fulva, Pimpinella brachycarpa, Yongia sonchifolia, Capsella bursapastoris, Taraxacum platycapum, Sonchus oleraceus, Allium tuberosum, Ligularia fischeri, Aster scaber, Petasites japonicus, Adenophora triphilla, Portulaca oleracea, Synurus deltoides, Ixeris dentata, Plantago asiatica, Amaranthus lividus*, and *Allium monanthum* were used for mutagenic and antimutagenic assay. They were obtained

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from various mountain areas of Hongcheon in Korea. NADP, G-6-P, B[*a*]P, 2-AF, and Trp-P-1 were purchased from the Kangwa company(Japan), and biotin, histidine and other reagents were obtained from Wako Pure Chemical Industries(Japan).

Preparation of edible wild herb juices

Each wild herb was washed with distilled water and drained with paper towels before grinding with pestle in a pre-cooled mortar. Extracts were then obtained using stainless steel press and were centrifuged at 30,000 ×g for 30min at 4°C. The supernatants were filter-sterilized using a 0.45μm membrane filter(Millipore Products Division, Bedford, MA) and the filtered juices were kept at -80°C for further experiments.

Spore-*rec* assay

Bacillus subtilis H17 (rec+) and M45 (rec-) strains were cultured according to the methods described by Kada et al.(8). The juices of herbs without metal ions were used as a control. On some of the plates, 10μl of a 2.5mM solution of Al^{3+}, Cu^{2+}, Fe^{2-}, Mn^{2-}, Ni^{2+}, Pb^{2+}, and Zn^{2+} were added to determine the effect of metal ions on the antimutagenesis of the juices. The plates were incubated at 4°C for 15h before incubation at 37°C for 28h and the zones of inhibition were then measured. The experiments were carried out duplicate.

Ames test for antimutagenicity

For the antimutagenecity test of the wild herb juices, a mixed solution containing 0.25ml S-9 mix made from the rat liver S9 product(Organon Technica Corp. Durham, NC, USA), 0.1ml of cell suspension of *Salmonella typhimurium* TA98 or TA100, 0 to 0.3ml of each juice with appropriate aliquot doses of B[*a*]P, 2-AF, and Trp-P-1 was prepared. The prepared sample mixture was preincubated at 37°C for 20min, and then 2ml of molten top agar supplemented with L-histidine and D-biotin at 45°C was added to the mixtures, gently mixed, and then poured onto minimal glucose agar plates. The plates were inverted and incubated at 37°C for 48h. Each set of experiments was performed at least duplicate, and colonies were counted, and the inhibition ratio was recorded using the following formula:

$$\text{Inhibition ratio}(\%) = \{(A - C)/(A - B)\} \times 100$$

A: number of histidine revertants induced by the mutagen alone

B: number of revertants induced in the presence of wild herb juice alone and solvent(negative control)

Table 1. Mutagenesis of juices from edible wild herbs in the presence of metal ions by spore-*rec* assay

Edible wild herb juice	Metal ions							
	None	Al	Cu	Fe	Mn	Ni	Pb	Zn
Adenophora triphylla	−	−	−	−	−	−	−	−
Allium monanthum	−	−	−	−	−	−	−	±
Allium tuberosum	−	−	−	−	−	±	−	−
Amaranthus lividus	−	−	−	−	−	−	−	−
Aster scaber	−	−	−	−	−	−	−	−
Aster tataricus	−	−	−	−	−	−	−	−
Capsella bursapastoris	±	±	−	−	±	−	−	−
Hemerocallis fulva	±	−	±	±	±	±	±	±
Ligularia fischeri	−	−	−	−	−	−	−	±
Ixeris dentata	−	−	−	−	−	−	−	−
Petasites japonicus	−	−	−	−	−	−	−	−
Pimpinella brachycarpa	−	−	−	−	−	−	−	−
Plantago asiatica	−	−	−	−	−	−	−	−
Portulaca oleracea	−	−	−	−	−	−	−	−
Rumex crispus	−	−	−	−	−	−	−	−
Scilla scilloides	−	−	−	−	−	−	−	−
Sonchus oleraceus	−	−	−	−	−	−	−	−
Synurus deltoides	−	−	−	−	−	−	−	−
Taraxacum platycarpum	−	−	−	−	−	−	−	−
Youngia sonchifolia	−	−	−	−	−	−	−	−

−: No inhibition zone. ±: Length of inhibition zone in less than 5mm

+: 5~10mm of inhibition zone, ++: More than 10mm of inhibition zone

C: number of histidine revertants induced by mutagen in the presence of the wild herb juice

RESULTS AND DISCUSSION

Before testing for the antimutagenicity of edible wild juices, the mutagenesis of juices by themself was investigated using spore-*rec* assay and Ames test. Table 1 shows the effects of various wild herb juices with metal ions on the mutagenesis in the spore-*rec* assay. Among the twenty wild herbs juices without adding metal ions, only juices from *Hemerocallis fulva* and *Capsella bursapastoris* exhibited a little mutagenesis, while all of other juices did not induce the mutagenesis. The effect of metal ions on the mutagenesis of the juices of edible wild herbs was investigated because foods contain many minerals and these minerals may affect the mutagenic properties. The addition of selected metal ions, such as Ni^{2+}, Zn^{2+}. Al^{3+}, Cu^{2+} and Fe^{2+} to the juices of *Allium tuberosum, Ligularia fischeri, Allium monanthum, Capsella bursapastoris,* and *Hemerocallis fulva* exhibited little induction or inhibition of mutagenesis, while the addition of other metal ions to the juices did not induce mutagenesis as measured by the spore-*rec* assay. Also, all twenty juices by themselves did not influence the spontaneous mutation frequencies in the Ames test(data not shown). This result indicate that juices themselvels did not cause mutagenesis in both assays and addition of metal ions to the juices did not affect on the mutagenesis except for the juices of *Capsella bursapastoris* and *Hemerocallis fulva.*

The juices of *Hemerocallis fulva* and *Capsella bursapastoris* exhibited little mutagenesis in the spore-*rec* assay, but no mutagenesis was observed in the Ames test(data not shown). Thus, the inhibitory effect of wild herb juices on mutagen-induced mutation was investigated. The relationship between the doses of the juices and inhibition of mutagenicity of B[*a*]P is shown in Table 2. The wild herb juices exhibited a dose-related antimutagenic activity in the TA98 or TA100 strains. Most of the juices, with the exception of *Allium tuberosum, Scilla slloides. Sonchus oleraceus,* and *Youngia sonchifolia,* showed more than 79% inhibition of mutagenesis in the *Salmonella typhimurium* TA98 strain treated with B[*a*]P in the presence of 250 μl S9 mix. The juices extracted from *Hemerocallis fulva, Pimpinella brachycarpa, Plantago asiatica, Synurus deltoides,* and *Taraxacum platycarpum* showed more than 80% inhibition in TA100 strain.

Similar results were found in 2-AF or Trp-P-1 induced mutagenesis in the presence of S9 mix, and the inhibitory activity increased as doses of the juices increased in both strains(Table 3 and 4). Three hundred μl of juices from only *Hemerocallis fulva* exhibited more than 90% inhibition in both of the strains treated with 10μg/plate of 2-AF, while juices from *Aster tartaricus, Aster scaber, Capsella bursapastroris, Ligularia fischeri, Ixeris dentata, Pimpinella brachycarpa,* and *Plantago asiatica* showed more than 90% inhibition in the TA98 strain and *Synurus deltoides* in the TA100 strain. For the 300μl treatments, only the juices from *Hemerocallis fulva, Synurus deltoides,* and *Ixeris dentata* showed more than 90% inhibition in both of TA98 and TA100 treated with 0.01μg/plate of Trp-P-1(Table 4). The results indicate that the concentration of juice treatments and the types of mutagens used in the test affect the antimutagenic potential of the wild herb juices in the TA98 or TA100 strains.

Edible wild herbs have played a major role in the livelihood of Korean people with little scientific knowledge as food stuffs or traditionally medicinal materials. Relatively little research has been carried out on the medicinal effects of Korean wild herbs, but compounds isolated from these plants may become a very valuable source of antimutagens and anticarcinogens in the future.

Natural antimutagens or anticarcinogens found in food, especially in plants are most likely safe, inexpensive, and easily obtained. Earlier studies have shown that numerous compounds with antimutagenic properties have been isolated from plants. These include ascorbic, caffeic, chlorogenic, ellagic, ferulic, vanillic acid and catechin gallate. rutin and quercetin(9-12). These components have been used as food additives and in medicines to help prevent carcinogenesis related to active oxygen radicals(13). The present study shows that the juices of edible wild herbs exhibited strong antimutagenic effects. Among these wild edible herbs, some of herbs, such as *Allium monanthum, Capsella bursapastroris, Ligularia fischeri,* and *Synurus deltoides* contain alliin, allicin, palmitic acid, β-carotene, acetylcholin and taraxol(14). These components might exert antimutagenic effect from the juices.

The results show that the juice of *Hemerocallis fulva* has the strongest antimutagenic effect on the B[*a*]P

Table 2. Inhibitory activities of juices from edible wild herbs on B[*a*]P[1)] induced mutagenesis with S9 mix in *Salmonella typhimurium* TA98 and TA100

Sample	Dose[2,3)] (μl/plate)	Inhibition(%)[4)]		Sample	Dose (μl/plate)	Inhibition(%)	
		TA98	TA100			TA98	TA100
	50	66.2	69.4		50	90.1	68.3
Adenophora	100	76.6	74.5	*Plantago*	100	92.0	79.1
triphylla	200	83.3	77.5	*asiatica*	200	93.1	79.7
	300	88.6	77.2		300	93.3	80.8
	50	10.2	14.5		50	39.8	57.9
Allium	100	20.1	21.2	*Petasites*	100	62.9	68.2
tuberosum	200	20.2	29.1	*japonicus*	200	88.6	73.1
	300	21.5	30.7		300	88.9	78.5
	50	8.3	19.8		50	76.2	75.5
Allium	100	11.7	31.9	*Pimpinella*	100	88.2	78.9
monanthum	200	24.4	51.9	*brachycarpa*	200	89.8	83.2
	300	90.0	59.3		300	94.3	83.5
	50	32.0	32.2		50	6.2	30.1
Amaranthus	100	51.3	57.3	*Porulaca*	100	43.6	45.0
lividus	200	73.6	57.8	*oleracea*	200	58.3	60.3
	300	79.4	65.4		300	79.7	72.2
	50	56.2	72.9		50	34.1	16.6
Aster	100	82.3	78.1	*Rumex*	100	54.4	32.5
scaber	200	87.9	78.4	*crispus*	200	79.9	32.3
	300	90.9	78.4		300	85.1	52.7
	50	72.6	25.5		50	41.5	32.6
Aster	100	85.5	30.4	*Scilla*	100	46.3	39.4
tataricus	200	92.9	42.4	*scilloides*	200	60.8	60.6
	300	96.6	43.8		300	62.2	65.2
	50	55.2	61.2		50	8.2	20.3
Capsella	100	62.8	63.3	*Sonchus*	100	31.4	38.2
bursapastoris	200	78.0	71.4	*oleraceus*	200	43.7	49.6
	300	82.1	72.6		300	61.6	58.8
	50	53.5	51.6		50	75.6	75.0
Ligularia	100	74.9	60.9	*Synurus*	100	89.3	80.4
fischeri	200	75.6	63.7	*deltoides*	200	93.4	83.5
	300	79.6	62.6		300	93.2	85.7
	50	42.3	44.2		50	46.2	55.4
Ixeris	100	70.6	52.1	*Taraxacum*	100	71.3	70.1
dentata	200	85.5	62.3	*platycarpum*	200	84.5	79.3
	300	85.6	62.7		300	87.0	80.7
	50	58.4	30.6		50	22.3	30.2
Hemerocallis	100	70.3	83.9	*Youngia*	100	40.5	40.3
fulva	200	94.1	90.1	*sonchifolia*	200	70.1	42.5
	300	96.0	92.3		300	71.5	49.4

1)The dose on plates treated with B[*a*]P was 20μg/plate

2)The average number of spontaneous revertants/plate on TA100 and TA98 strains was approximately 140±20 and 22±6, respectively

3)The average number of revertants/plate on the B[*a*]P-treated TA100 and TA98 strains was approximately 560±30 and 175±20, respectively

4)The inhibition ratio of B[*a*]P induced revertants was calculated by the formula shown in materials and methods

Table 3. Inhibitory effects of juices from edible wild herbs on 2-AF[1] induced mutagenesis with S9 mix in *Salmonella typhimurium* TA98 and TA100

Sample	Dose[2,3] (μl/plate)	Inhibition(%)[4]		Sample	Dose (μl/plate)	Inhibition(%)	
		TA98	TA100			TA98	TA100
Adenophora triphylla	50	30.1	50.2	*Plantago asiatica*	50	87.1	67.1
	100	37.5	61.4		100	90.3	77.2
	200	49.5	70.3		200	92.2	79.0
	300	56.7	77.6		300	97.5	80.2
Allium tuberosum	50	9.5	41.0	*Petasites japonicus*	50	30.2	41.5
	100	18.3	52.4		100	42.4	50.7
	200	60.5	61.5		200	50.3	51.8
	300	80.1	73.2		300	59.7	61.7
Allium monanthum	50	32.3	27.4	*Pimpinella brachycarpa*	50	75.5	68.5
	100	46.4	38.3		100	82.4	72.5
	200	71.7	52.2		200	90.3	99.5
	300	82.6	68.6		300	98.2	82.4
Amaranthus lividus	50	32.4	27.2	*Porulaca oleracea*	50	16.5	15.2
	100	45.0	55.4		100	25.6	20.1
	200	52.4	62.5		200	60.9	58.0
	300	60.2	67.4		300	76.5	63.7
Aster scaber	50	76.6	58.6	*Rumex crispus*	50	30.2	15.5
	100	84.5	62.4		100	40.5	25.5
	200	90.4	70.6		200	60.1	30.4
	300	95.6	79.4		300	67.7	37.6
Aster tataricus	50	70.5	75.5	*Scilla scilloides*	50	40.5	20.2
	100	89.2	80.3		100	66.5	35.5
	200	88.3	87.2		200	78.4	40.5
	300	90.5	68.4		300	86.4	47.5
Capsella bursapastoris	50	50.2	62.0	*Sonchus oleraceus*	50	13.1	12.7
	100	73.4	72.2		100	22.3	21.5
	200	88.1	78.4		200	39.4	31.0
	300	92.4	80.7		300	65.6	40.7
Ligularia fischeri	50	80.1	50.1	*Synurus deltoides*	50	10.1	51.0
	100	85.3	57.2		100	21.0	69.5
	200	90.4	60.4		200	84.0	80.0
	300	97.3	67.2		300	87.5	93.5
Ixeris dentata	50	25.6	30.6	*Taraxacum platycarpum*	50	8.8	20.2
	100	58.4	63.0		100	15.9	39.0
	200	82.5	66.4		200	55.6	50.0
	300	90.1	70.6		300	81.6	65.4
Hemerocallis fulva	50	70.1	80.5	*Youngia sonchifolia*	50	30.4	17.5
	100	82.5	85.4		100	42.8	35.4
	200	90.0	91.3		200	68.1	62.0
	300	99.1	99.2		300	85.2	69.4

[1]The dose on plates treated with 2-AF was 10μg/plate

[2]The average number of spontaneous revertants/plate on TA100 and TA98 strains was approximately 140±20 and 22±6, respectively

[3]The average number of revertants/plate on the 2-AF-treated TA100 and TA98 strains was approximately 260±30 and 1300±120, respectively

[4]The inhibition ratio of 2-AF induced revertants was calculated by the formula shown in materials and methods

Table 4. Inhibitory effects of juices from edible wild herbs on Trp-p-1[1] induced mutagenesis with S9 mix in *Salmonella typhimurium* TA98 and TA100

Sample	Dose[2,3] (μl/plate)	Inhibition(%)[4]		Sample	Dose (μl/plate)	Inhibition(%)	
		TA98	TA100			TA98	TA100
	50	27.0	52.2		50	92.5	52.1
Adenophora	100	32.5	60.5	*Plantago*	100	94.1	54.5
triphylla	200	40.4	69.6	*asiatica*	200	95.5	57.0
	300	56.4	77.8		300	96.3	59.7
	50	42.0	6.0		50	48.4	31.5
Allium	100	53.4	11.8	*Petasites*	100	72.4	42.6
tuberosum	200	75.8	32.7	*japonicus*	200	79.5	47.7
	300	90.1	50.7		300	83.5	50.4
	50	49.1	11.4		50	76.4	40.1
Allium	100	67.3	20.6	*Pimpinella*	100	82.4	62.3
monanthum	200	80.3	29.5	*brachycarpa*	200	90.5	70.3
	300	98.2	34.5		300	99.2	76.9
	50	10.5	82.7		50	64.5	23.0
Amaranthus	100	29.6	84.0	*Porulaca*	100	81.0	42.0
lividus	200	53.2	86.3	*oleracea*	200	89.0	60.5
	300	79.3	90.5		300	91.2	65.4
	50	70.6	28.3		50	67.5	60.1
Aster	100	80.7	52.3	*Rumex*	100	82.4	68.3
scaber	200	91.5	59.2	*crispus*	200	90.3	30.4
	300	97.6	61.7		300	67.7	80.2
	50	70.3	52.5		50	70.3	52.2
Aster	100	82.2	60.5	*Scilla*	100	87.2	60.3
tataricus	200	90.4	69.4	*scilloides*	200	92.5	70.4
	300	98.2	73.4		300	99.3	77.5
	50	70.0	22.5		50	13.4	4.87
Capsella	100	81.4	30.1	*Sonchus*	100	30.2	12.0
bursapastoris	200	89.7	37.0	*oleraceus*	200	75.6	22.0
	300	95.2	45.8		300	85.4	30.8
	50	78.4	21.4		50	93.2	92.5
Ligularia	100	82.4	32.4	*Synurus*	100	94.5	93.0
fischeri	200	91.5	38.3	*deltoides*	200	95.0	94.0
	300	98.2	40.6		300	95.0	94.5
	50	21.4	90.0		50	76.4	32.4
Ixeris	100	40.1	92.4	*Taraxacum*	100	81.0	41.6
dentata	200	72.5	94.0	*platycarpum*	200	90.0	47.8
	300	90.1	95.4		300	92.2	55.8
	50	70.4	82.4		50	50.6	10.0
Hemerocallis	100	85.5	89.3	*Youngia*	100	62.0	15.4
fulva	200	90.6	95.3	*sonchifolia*	200	72.3	18.7
	300	99.3	100		300	83.4	20.7

[1]The dose on plates treated with Trp-p-1 was 0.5μg/plate

[2]The average number of spontaneous revertants/plate on TA100 and TA98 strains was approximately 120±10 and 28±8, respectively

[3]The average number of revertants/plate Trp-p-1-treated TA100 and TA98 strains was approximately 960±100 and 1200±100, respectively

[4]The inhibition ratio of Trp-p-1 induced revertants was calculated by the formula shown in materials and methods

and 2-AF induced mutagenesis in the TA98 and TA 100 strain and *Synurus deltoides* has the strongest on Trp-P-1 induced mutagenesis compared with other juices of wild edible herbs. Also, the antimutagenic effects increased as doses of the juices increased in the both strains and TA98 strain was more sensitive than TA100 strain. This difference of sensitivity may come from different genetic base pair arrangement between TA98(frameshift type mutant) and TA100(base substitution type mutant).

Reduced gastric cancer risk in a case-controlled study was associated with increased intake of raw vegetables and citrus fruits(15). The reduction in risk was associated with raw but not with cooked vegetables and with fresh but not with dried or preserved fruits. These might be due to loss of active components by physical damages. Similar expectation could be followed in the wild edible herbs and some juices of the raw edible herbs exhibited much stronger antimutagenic effect than heated herb juices at the same dose level(data not shown).

This is a preliminary study on the antimutagenic activity of the juices of edible wild herbs commonly used as diet, food additives, and medicines in Korea. This results have prompted further studies, including extracts from various organic solvents, heat, and pH stability of extract and identification of the main active substances in the edible wild herbs, and these experiments are now in progress.

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