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Antimutagenic and Anticancer Effects of *Buchu Kimchi*

Keun-Ok Jung, Kyeoung-Im Lee*, Myoung-Ja Suh and Kun-Young Park†

Department of Food Science and Nutrition, Kimchi Research Institute, Pusan National University, Pusan 609-735, Korea
**Department of Hotel Food Preparation, Yangsan College, Yangsan 626-800, Korea*

Abstract

The antimutagenic effects of *buchu kimchi* and Chinese cabbage *kimchi* and their cytotoxic effects against human cancer cell line were investigated in the *Salmonella typhimurium* system and MTT assay, respectively. Leek and Chinese cabbage were also evaluated in the same system. *Buchu kimchi* was fermented at 15°C for 0, 6 and 9 days and Chinese cabbage *kimchi* was fermented at 15°C for 4 days. *Buchu kimchi* samples showed somewhat higher antimutagenic effects against aflatoxin B_1 (AFB_1) than Chinese cabbage *kimchi* in *Salmonella typhimurium* TA100 strain. There was no difference on the antimutagenic activity according to the length of fermentation. Leek exerted stronger antimutagenicity against AFB_1 than Chinese cabbage in the Ames assay. In MTT assay, 6-day fermented *buchu kimchi* revealed the highest cytotoxicity against AGS human gastric adenocarcinoma cells in which 62% and 82% of the inhibition were observed with the addition of 100 μg, 400 μg/well, respectively. *Buchu kimchi* samples caused 60~70% inhibition on the proliferation of HT-29 human colon adenocarcinoma cells even at 100 μg/well while Chinese cabbage *kimchi* exhibited 60% inhibition against HT-29 at 400 μg/well. Leek exhibited higher antiproliferative effect against both AGS cells and HT-29 cells than Chinese cabbage in MTT assay. From these results, it is considered that *buchu kimchi* has stronger antimutagenic and *in vitro* anticancer effects than Chinese cabbage *kimchi* and the high inhibition rate of *buchu kimchi* probably results from leek, the major ingredient of *buchu kimchi*.

Key words: *buchu kimchi*, leek, antimutagenicity, anticancer effect

INTRODUCTION

Kimchi is a Korean traditional food, which refers to fermented vegetable products (1). The raw materials of *kimchi* are mainly green-yellow vegetables that are known to exhibit antimutagenic and anticancer activities (2-8). *Kimchi* and its ingredients have been investigated to determine chemopreventive properties. Chinese cabbage *kimchi* is the most typical *kimchi*, which is known to exhibit antimutagenic and anticancer activities (9-19). Almost all vegetables cultivated in Korea are involved in *kimchi*, there are many types of *kimchi* depending on the ingredient and preparation method used (1). In these various *kimchi*, *buchu kimchi* is a traditional special *kimchi* of the Kyungsang province, Korea. *Buchu* (Leek, *Allium tuberosum* L.) *kimchi* is prepared with large quantities of red pepper powder and pickled anchovy, therefore, it is known as a good side dish because of the unique flavor of leeks and spicy taste (20-22). Leeks, the major ingredient for *buchu kimchi*, have been used as food or drug for treatment of abdominal pain, diarrhea, hematemesis, snakebite and asthma in folk remedies for a long time (23). Leeks are rich in vitamin A, B_1, and C (24). Leeks belonging to the *Allium* genus contain large amounts of thiosulfinates and organosulfur compounds, which are responsible for the characteristic odor and flavor of allium (25-27). The allyl sulfur compounds are known to inhibit chemically induced tumors (28-33). Leeks also contain high levels of flavonoids (34, 35). Food-derived flavonoids such as the flavonols, quercetin, kaempferol, and myricetin have antimutagenic and anticancer effects *in vitro* and *in vivo* (36, 37). In addition, several studies indicated that high consumption of leek was associated with a reduced risk for colorectal cancer (38-40).

Since the major ingredient of *buchu kimchi* is leek, *buchu kimchi* is a possible food source that exhibits antimutagenic and anticancer effects. However, the details of the antimutagenic and anticancer activity of *buchu kimchi* have been poorly elucidated until now.

In this study, the methanol extracts of *buchu kimchi* were investigated to determine the antimutagenic activity and cytotoxic effects against human cancer cell lines as compared to optimally ripened Chinese cabbage *kimchi*.

MATERIALS AND METHODS

Preparation of *buchu kimchi* extracts

In *buchu kimchi*, the leeks were cut into 2 pieces and soaked in 20% salt solution for 20 min. at room temperature, then rinsed twice with water. For Chinese cabbage *kimchi*, Chinese cabbage was cut into 4 pieces and soaked in 10% brine for 12 hours at 10°C and then rinsed with tap water. The ingredient ratios of *buchu kimchi* and Chinese cabbage *kimchi* are shown in Table 1. The final NaCl concentration of *buchu kimchi* and Chinese cabbage *kimchi* was 2.5%. Leek(from Kimhae, Korea), Chinese cabbage (from Kimhae, Korea), garlic, radish, spring onion, ginger, red pepper powder, fermented anchovy juice

†Corresponding author. E-mail: kunypark@hyowon.pusan.ac.kr

Table 1. Ingredients ratio of *buchu kimchi* and Chinese cabbage *kimchi*

Ingredients	*Buchu kimchi* (g)	Chinese cabbage (g)
Buchu	100.0	
Chinese cabbage		100.0
Red pepper powder	9.0	3.5
Crushed garlic	5.0	1.4
Crushed ginger	2.0	0.6
Anchovy juice	13.0	2.2
Sugar	2.0	1.0
Radish		13.0
Green onion		2.0
Glutinous rice paste	13.0	
Final salt concentration	2.5%	2.5%

(Miwon, Co.), salt (Chinese cabbage *kimchi* used Chunil salt, *buchu kimchi* used Gueun salt from Sannaedle, Co., Seoul), sugar and glutinous rice powder were purchased from Bujun market in pusan, Korea. The prepared *buchu kimchi* and Chinese cabbage *kimchi* were put into pint jars, pushed down and the lid closed tightly. *Buchu kimchi* was fermented at 15°C for 0, 6 and 9 days and Chinese cabbage *kimchi* was fermeted at 15°C for 4 days. The initial pH of *buchu kimchi* was 5.19 and then decreased to 4.05 after 9 day fermentation. Optimally ripened *kimchi*, the 6 day-fermented *buchu kimchi* and 4 day-fermented Chinese cabbage *kimchi* showed pHs of 4.29 and 4.21, respectively. After fermentation, each *kimchi* samples were freeze dried and powdered, 20-folds of methanol was added to the powdered samples and extracted 3 times. The methanol extracts were evaporated using vacuum evaporator, concentrated, then dissolved in dimethyl sulfoxide (DMSO, Sigma Chemical Co., USA) for the experiment.

Ames mutagenicity test

AFB_1 was purchased from Sigma Chemical Co., St. Louis, Mo. (USA) and dissolved in DMSO. *Salmonella typhimurium* TA100 bacterial strain, histidine requiring mutant was provided by Dr. B. N. Ames, Univ. of California, Berkerly, CA, USA. The genotype of the tester strain was checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor. S9 mixture to activate the direct mutagen, AFB_1, was also prepared by the method of Maron and Ames (41). Mutagenicity test (42, 43) was carried out by a modified plate incorporation test. In the preincubation test, 0.5 ml of S9 mixture (or 0.5 ml of phosphate buffer for direct mutagen, MNNG) was distributed into sterilized capped tubes in an ice bath and then 0.1 ml of test strain cultured overnight ($1\sim2\times10^9$ cells/ml), 0.1 ml of test compound (50 μl of mutagens and 50 μl of methanol extracts) were added. The tubes were vortexed gently and preincubated at 37°C for 20 min. and 2 ml of the top agar supplemented with L-histidine and D-biotin kept at 45°C, were added to each tube and vortexed for 3 seconds. The resulting entire mixture was overlaid on the minimal agar plate. The plates were inverted and incubated at 37°C for 48 hrs and then the revertant bacterial colonies on each plate were counted. Dose response tests (41) of the mutagens on the tester strain was carried out to determine the regions of revealing mutagenicity induced by the mutagens. Toxicity test was also carried out. The methanol extracts used for antimutagenicity test did not show any toxicity on the test strain.

MTT assay

RPMI 1640, fetal calf serum (FCS), trypsin-EDTA and penicillin-streptomycin were purchased from GIBCO Co. (Gaithersburg, MD, USA). AGS human gastric adenocarcinoma cell and HT-29 human colon adenocarcinoma cell were obtained from Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in RPMI 1640 supplemented with 1% penicillin-streptomycin and 10% FCS. Media were changed twice or three times every week. After six or seven days, cultured cancer cells were washed with PBS. The cells were dissociated with 0.05% trypsin-0.02% EDTA and 180 μl/well of cell suspensions (1×10^4 cells/ml) were seeded in 96-well microtitre plates with various concentrations of each *kimchi* extracts(20 μl). After 70 hours incubation, 20 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution(2.5 mg/ml PBS) was added. After additional 4 hours incubation, the supernatant medium was carefully removed. The formazan dye was solubilized by adding 150 μl DMSO to each well followed by gentle shaking. The optical densities were read on an Eliza reader at 540 nm (44-46).

Statistical analysis

Statistical analysis was performed by analysis of variance. Significant differences between treatment means were determined by using Duncan's multiple range test.

RESULTS AND DISCUSSION

The methanol extracts from *buchu kimchi* and Chinese cabbage *kimchi* showed antimutagenic effects against AFB_1 in the *Salmonella typhimurium* TA100 strain (Table 2). Chinese cabbage *kimchi* has already been demonstrated to exhibit antimutagenic and anticancer activities (9-19). *Buchu kimchi* samples showed higher antimutagenic effect than Chinese cabbage *kimchi*. The antimutagenic effects of *buchu kimchi* samples increased depending on the concentration. At 0.63 mg/plate, the inhibition rates of *buchu kimchi* samples for AFB_1 were 65-67% while that of Chinese cabbage *kimchi* extract was 53%. The mutagenicity mediated by AFB_1 was inhibited by 78~81% in the presence of 2.5 mg/plate of *buchu kimchi* extracts. It is known that Chinese cabbage *kimchi* exhibits the highest antimutagenic effect on optimally ripened *kimchi* (9, 11,18). But, *buchu kimchi* did not reveal the difference of the antimutagenic activities according to the fermentation periods. These results indicate that besides optimally ripened *buchu kimchi*, fresh and overripened *buchu kimchi* also have considerable antimutagenicity. Park et al (9). had reported that the extracts from Chinese cabbage *kimchi* inhibited the mutagenic-

Table 2. *Effect of the methanol extract from buchu kimchi and Chinese cabbage kimchi on the mutagenicity induced by aflatoxin* B_1 (AFB_1, 0.2 μg/plate) in *Salmonella typhimurium* TA100

Treatment	Revertants/plate (level of methanol extract, mg/plate)		
	0.63	1.25	2.5
Spontaneous	135±4		
AFB_1 (Control)	999±51[a]		
AFB_1+0 day *buchu kimchi*	438±36[c] (65)[1)]	404±7[b] (69)	323±31[bc] (78)
AFB_1+6 day *buchu kimchi*	420±23[c] (67)	374±20[b] (72)	297±11[c] (81)
AFB_1+9 day *buchu kimchi*	427±17[c] (66)	383±14[b] (71)	297±13[c] (81)
AFB_1+4 day Chinese cabbage *kimchi*	541±33[b] (53)	421±13[b] (67)	358±22[b] (74)

[1)]The values in parentheses are the inhibition rates (%)
[a~c]Means with the different letters beside symbols are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

ity against AFB_1 in *Salmonella typhimurium* TA100. They also suggested that vegetables such as Chinese cabbage, leek, garlic and red pepper powder played an important role in the antimutagenicity. The antimutagenic effects of garlic and red pepper powder have been previously reported (6,7,47-49). *Buchu kimchi* was prepared with much larger quantities of garlic and red pepper powder than Chinese cabbage *kimchi* (Table 1). We thought that the difference in the activity between the two samples might be due to the different ingredient ratios and major ingredient, leek and Chinese cabbage. In an effort to identify the difference in the activity between *buchu kimchi* and Chinese cabbage *kimchi*, antimutagenic effects of the major ingredients, leek and Chinese cabbage, were studied in the same system. *Buchu* samples exerted stronger antimutagenic activity than Chinese cabbages (Table 3). At the 2.5 mg/plate concentration, *buchu* extract exhibited 82% antimutagenicity, while Chinese cabbage extract showed 54% of antimutagenicity. These result suggest that the high antimutagenic activity of *buchu kimchi* probably resulted from leek, its major ingredient.

Both *buchu kimchi* samples and Chinese cabbage *kimchi* inhibited the proliferation of AGS human gastric adenocarcinoma cells in the MTT assay (Fig. 1). At 100 μg/well, the antiproliferative effects of 0 day- and 6 day-fermented *buchu kimchi* on AGS cells were stronger than those of Chinese cabbage *kimchi*. 6 day-fermented *buchu kimchi* revealed the highest

Table 3. Effect of the methanol extract from *buchu kimchi* and Chinese cabbage *kimchi* on the mutagenicity induced by aflatoxin B_1 (AFB_1, 0.75 μg/plate) in *Salmonella typhimurium* TA100

Sample	Concentration (mg/plate)	Revertants/plate	Inhibition rate (%)
Spontaneous		115± 5	
AFB_1 (Control)		773±12[a]	
AFB_1+*buchu*	0.63	394± 7[d]	58
	1.25	330±15[f]	67
	2.50	232±13[g]	82
AFB_1+Chinese cabbage	0.63	552±16[b]	34
	1.25	443±16[c]	50
	2.50	353±15[e]	54

[a~f]Means with the different letters beside symbols are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

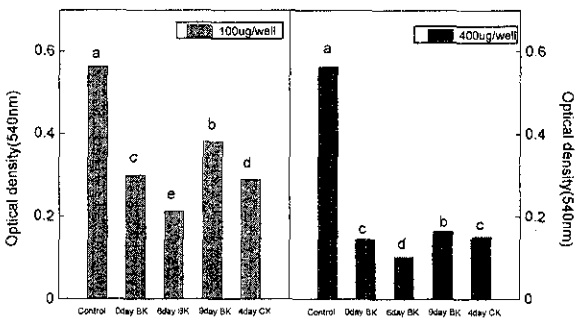

Fig. 1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of the methanol extract from *buchu kimchi* (BK) and Chinese cabbage *kimchi* (CK) against AGS human gastric adenocarcinoma cells.
[a~c]Means with the different letters beside data are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

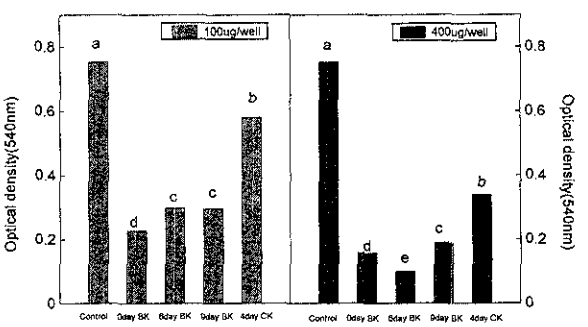

Fig. 2. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of the methanol extract from *buchu kimchi* (BK) and Chinese cabbage *kimchi* (CK) against HT-29 human colon carcinoma cells.
[a~c]Means with the different letters beside data are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

cytotoxicity against AGS, where 62%, 82% of inhibition were observed with addition of 100, 400 μg/well, respectively. Both *buchu kimchi* samples and Chinese cabbage *kimchi* also had inhibitory effects on the proliferation of HT-29 human colon adenocarcinoma cells (Fig. 2). *Buchu kimchi* samples caused 60~70% inhibition of HT-29 proliferation even at 100 μg/well, while Chinese cabbage *kimchi* exhibited 60% inhibition against

Table 4. Inhibitory effect of the methanol extract from *buchu* and Chinese cabbage on the growth of AGS human gastric adenocarcinoma cells and HT-29 human colon carcinoma cells in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Sample (μg/well)		OD_{540}	
		AGS	HT-29
Control		0.757 ± 0.009^{a}	0.800 ± 0.010^{a}
Buchu	100	0.108 ± 0.012^{d} (86)[1)]	0.298 ± 0.023^{d} (63)
	200	0.027 ± 0.008^{e} (96)	0.192 ± 0.013^{e} (85)
Chinese cabbage	100	0.461 ± 0.019^{b} (39)	0.535 ± 0.004^{b} (33)
	200	0.253 ± 0.017^{c} (67)	0.477 ± 0.011^{c} (40)

[1]The values in parentheses are the inhibition rates (%)
[a-e]Means with the different letters beside symbols are significantly different at the 0.01 level of significance as determined by Duncan's multiple range test.

HT-29 cells at the high level of 400 μg/well. It is interesting that 0 day-femented(fresh) *buchu kimchi* showed the highest cytotoxicity against HT-29 with 100 μg/well addition while 6 day-fermented (optimally ripened) *buchu kimchi* had the highest cytotoxicity at the concentration of 400 μg/well. At 100 μg addition to the system, the inhibition rate shown by 0 day-fermented *buchu kimchi* samples on HT-29 proliferation were 70% while 60% of inhibition was observed with the addition of 6 day-fermented *buchu kimchi* samples. In contrast, 0 day-fermented *buchu kimchi* at the addition of 400 μg to the system inhibited the HT-29 proliferation by 79%, however, the inhibition rates of 6 day-fermented *buchu kimchi* were 87% at the same concentration. It could be shown that, besides optimally ripened *buchu kimchi*, fresh *buchu kimchi* also exhibit considerable cytotoxicity against HT-29 cells in MTT assay, whereas over-ripened *buchu kimchi* and Chinese cabbage *kimchi* were only sightly to moderately cytotoxicity against HT-29 cells. From the above data, significant cytotoxic effect was exhibited by *buchu kimchi* samples on both AGS and HT-29 cell lines ($p<0.01$). In addition optimally ripened *buchu kimchi* showed a higher cytotoxicity effect against AGS and HT-29 than optimally ripened Chinese cabbage *kimchi*.

To determine the antiproliferative effect of the major ingredients, leek and Chinese cabbage, the MTT assay using AGS and HT-29 was carried out. The methanol extracts from *buchu* showed significantly higher inhibitory effect on the proliferation of both AGS and HT-29 than those from Chinese cabbage ($p<0.01$, Table 4).

Thus, it can be concluded that *buchu kimchi* samples showed stronger antimutagenic and *in vitro* anticancer effects than Chinese cabbage *kimchi*. The high inhibition rate in the *buchu kimchi* probably results from leek, the major ingredient of *buchu kimchi*. Further studies on identifying active compounds of of *buchu kimchi* and their mechanisms are needed.

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