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Antioxidant and antigenotoxic activities of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* in the Salmonella mutagenicity assay and in HCT116 human colon cancer cells

Daejoong Kwon^a, Sun Yoon^{a,*}, Orianna Carter^b, George S. Bailey^b, and Roderick H. Dashwood^b

^a Department of Foods and Nutrition, Yonsei University, Seoul, Korea 120-749

^b Linus Pauling Institute, Oregon State University, Corvallis, OR 97331-6512, USA

Abstract

Epidemiological studies indicate that consumption of green-yellow vegetables rich in chlorophyll, vitamin C, vitamin E, and carotenoids reduce the risk of cancer. We sought to examine the antigenotoxic and antioxidant properties of chlorophyll-rich methanol extracts of *Angelica keiskei*, *Oenanthe javanica*, and *Brassica oleracea* (kale). In the Salmonella mutagenicity assay, *A. keiskei* caused dose-dependent inhibition against three heterocyclic amine mutagens in the presence of S9, *O. javanica* was antimutagenic only at the highest concentration in the assay (2 mg/plate), and *B. oleracea* showed no consistent inhibitory activity at non-toxic levels. None of the extracts were effective against three direct-acting mutagens in the absence of S9. Extracts of *A. keiskei* and, to a lesser extent *O. javanica*, inhibited two of the major enzymes that play a role in the metabolic activation of heterocyclic amines, based on ethoxyresorufin-*O*-deethylase and methoxyresorufin-*O*-demethylase assays *in vitro*. All three plant extracts were highly effective in assays which measured ferric reducing/antioxidant power, oxygen radical absorbance capacity, and Fe²⁺/H₂O₂-mediated DNA nicking. Finally, using the 'comet' assay, all three plant extracts protected against H₂O₂-induced genotoxic damage in human HCT116 colon cancer cells. These findings provide support for the antigenotoxic and antioxidant properties of chlorophyll-rich extracts of *A. keiskei*, *O. javanica*, and *B. oleracea*, through mechanisms that include inhibition of carcinogen activation and scavenging of reactive oxygen species.

Keywords

Antimutagen; antioxidant; heterocyclic amines; phytochemical; comet assay; DNA breaks

1. Introduction

The human diet contains a great variety of carcinogens, as well as many natural anticarcinogens. Numerous epidemiological studies have shown that intake of green-yellow vegetables rich in chlorophyll, vitamin C, vitamin E, and carotenoids reduce the risk of cancer and other chronic diseases [1–4]. An inverse association between the consumption of vegetables and the formation of epithelial cancers may be stronger for raw vegetables than cooked vegetables [5]. Previous work showed the beneficial effects of green juice made of raw *Angelica keiskei* and *Brassica oleracea* (kale) on nitrite scavenging *in vitro*, growth of cancer cells in culture, and plasma lipid and antioxidant status in smokers [6–8]. A growing interest exists in

*Address for correspondence: Prof. Sun Yoon, Department of Foods and Nutrition, Yonsei University, Seoul, Korea 120-749. Tel.: +82 2 21233119; Fax: +82 2 3653118; E-mail: snkim@yonsei.ac.kr.

establishing the various health benefits of raw green-yellow vegetable extracts, including their potential antioxidant and antigenotoxic properties.

Based on these initial studies, we sought to examine the antimutagenic and antioxidant properties of methanol extracts of *A. keiskei*, *O. javanica*, and *B. oleracea*, including studies with cooked meat heterocyclic amines, which are potent mutagens and multi-organ carcinogens [9]. Heterocyclic amines have been shown to respond *in vitro* and *in vivo* to a broad array of antimutagens and cancer chemopreventive agents, as well as various tumor promoters [10].

2. Materials and methods

2.1. Mutagens and other chemicals

The heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were from Toronto Research Chemicals Inc. (North York, Ontario, Canada), as were the directing-acting mutagens 2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline (*N*-OH-IQ) and 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*N*-OH-PhIP). All other chemicals, including the direct-acting mutagen 4-nitro-1,2-phenylenediamine (NPD), were from sources reported previously [11–13]. Gallic acid and ascorbic acid were purchased from Sigma-aldrich Co. (St. Louis, Missouri, USA).

2.2. Analysis of total phenolics

The phenolics were extracted twice with 5 mL of extraction solvent (methanol:acetate acid: H₂O at 50:3.7:46.3) from ~100 mg of each dry vegetable powder. The first extraction was carried out with rocking for 5 h and the second was run overnight at 4°C. The total phenolic content of the extracts were assessed with the Folin-Ciocalteu reagent according to the modification for plant materials developed by Singleton et al. [14]. Briefly, the extracts were mixed with the Folin-Ciocalteu phenol reagent, Na₂CO₃ and H₂O and absorbance at 760 nm measured 2 h after incubation at room temperature using a Bekman DU 530 spectrophotometer (USA). The total phenolic content was determined from lyophilized material with the results presented on a dry weight basis and are expressed as μ mol Gallic Acid Equivalents (GAE/g).

2.3. Determination of Vitamin C

Ascorbic acid and total vitamin C (ascorbic acid plus dehydroascorbic acid) were determined by high-performance liquid chromatography (HPLC) using a modification in the procedure of Sanchez-Mata et al. [15]. Two grams of freeze dried vegetables were homogenized with 10 mL of an extraction solution (3% metaphosphoric acid plus 8% acetic acid). The resulting mixture was centrifuged, filtered, and adjusted to 25 mL with distilled water. Filtered sample through a 0.45- μ m membrane filter was analyzed by HPLC. Waters 2690 alliance separation module and a symmetry C18 stainless steel column (250×4.6 i.d. mm) was used for analysis. The solvent system used was an isocratic gradient of a 0.01% solution of H₂SO₄, adjusted to pH 2.5–2.6. The flow rate was fixed at 1.0 mL/min. A Waters 996 UV-visible photodiode array detector was set at 245 nm. Chromatographic data and UV-visible spectra were collected, stored, and integrated using Waters Empower. Identification of the ascorbic acid was carried out by HPLC by comparing the retention time and UV-visible absorption spectrum with those of the standard. The ascorbic acid content was calculated on the basis of a calibration curve and expressed as mg/gram of sample on dry weight basis.

2.4. Preparation of methanol extracts

Methanol extracts of *A. keiskei*, *B. oleracea*, and *O. javanica* (Pulmuone Co., Korea), were prepared as reported before [6–8]. Fresh vegetables were cleaned and freeze dried. The

powdered sample (2 g) was extracted twice with 100 mL methanol at room temperature (~23°C) for 12 h. Extracts were recovered by centrifugation at 1,000 ×g for 10 minutes and filtered through whatman No. 2 filter paper. Filtered extracts were concentrated to dryness under nitrogen gas and stored at -20 °C until they were utilized in the following assays.

2.5. Salmonella mutagenicity assays

Methanol extracts were compared for their inhibitory activities in the Salmonella mutagenicity assay [16]. The basic methodology, using heterocyclic amine mutagens in the presence and absence of S9, was as reported elsewhere [11–13]. In brief, 50 µL of mutagen (or DMSO vehicle alone), 0.1 mL of an overnight culture of *Salmonella typhimurium* strain TA98, 50 µL of plant extract (0.1–2 mg/plate equivalent starting material), and 0.2 mL phosphate-buffered saline or 0.2 mL of 10% Aroclor-induced rat liver S9 mix were preincubated for 30 minutes at 37°C. Following the addition of 2 mL top agar, the mixture was poured onto minimal glucose plates, and His⁺ revertant colonies were counted 2 days later using a Sorcerer Colony Counter (Perceptive Instruments, Haverhill, England). To rule out possible toxic and/or other confounding effects, we routinely confirmed the presence of a normal background lawn of growth, spontaneous counts in the usual range, and high cell viability. No toxicity was detected for data reported here. For purposes of comparison among the different plant extracts and mutagens, data on His⁺ revertants/plate were expressed as ‘percent of control’.

2.6. CYP1A1 and CYP1A2 activity

Cytochrome P4501A1 (CYP1A1) and CYP1A2 activities were determined in ethoxyresorufin-*O*-deethylase (EROD) and methoxyresorufin-*O*-demethylase (MROD) assays, respectively [11]. Conversion of substrate (7-ethoxyresorufin or methoxyresorufin) to product (resorufin) was measured at 37 °C in a Hitachi F2500 fluorescence spectrophotometer, with excitation at 530 nm and emission at 585 nm. Assays were performed in duplicate, with various concentrations of plant extract. Controls also were included to verify that changes produced by plant extracts were not from simple quenching of fluorescence.

2.7. ORAC and FRAP assays

The oxygen radical absorbance capacity (ORAC) assay measures the relative efficiency of antioxidants to protect the fluorescent protein β-phycoerythrin (PE) against peroxidation [17]. Peroxyl radicals generated at a constant rate using the thermolabile azo-initiator ABAP (2,2'-azobis 2-methyl-propionamidine dihydrochloride) were related to the water-soluble vitamin E analog Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). The ORAC assay was carried out in a microplate fluorometer Cytofluor 400 (PerSeptive Biosystems, Framingham, MA), with Trolox diluted to a final concentration of 0, 10, 20 and 40 µM, in triplicate. The kinetic change in PE fluorescence was recorded at 2 minute intervals for 2 hours (excitation 485 nm, emission 585 nm).

The ferric reducing/antioxidant power (FRAP) assay determines the ability of extracts to reduce Fe³⁺/tripyriddytriazine complex (Fe³⁺-TPTZ) to its colored form, Fe²⁺/TPTZ [17,18]. In brief, test sample or standard (Trolox, final concentrations of 0, 62.5, 125, 250 and 500 µM) were added to the wells of a 96-well plate in duplicate, followed by addition of pre-warmed Fe³⁺-TPTZ reagent. After 15 minutes incubation at 37°C, absorbance was read at 550 nm and the FRAP value was calculated from the standard calibration curve.

2.8. Plasmid DNA nicking

Conversion of double-stranded supercoiled circular DNA to relaxed or linear ‘open’ DNA was determined based on the methodology previously described [20]. Thus, plasmid pUC19 was incubated with 0.1 mM H₂O₂ and 0.1 mM Fe²⁺ in 150 mM NaCl, pH 7.9, for 30 minutes at

37°C, in the presence and absence of plant extract. Following electrophoresis in 0.8% agarose gel at 100 V for 1 hour and ethidium bromide staining, closed circular, linear, and relaxed forms of pUC19 were quantified on an AlphaInnotech photodocumentation system.

2.9. Comet assay

Antigenotoxic activity of the three plant extracts was studied using the single cell gel electrophoresis ('comet') assay, a sensitive technique that has been applied to studies of DNA damage and its prevention by antioxidants in people, as well as in human colon cancer cells *in vitro* [21–28]. In the present investigation, HCT116 human colon cancer cells from the American Type Culture Collection (Manassas, VA) were grown in McCoy's 5A medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin, at 37°C in a 95% humidified incubator containing 5% CO₂. Confluent cells were trypsinized, re-seeded at a density of 1×10^5 cells in 24-well plates, and cultured for another 48 hours at 37°C. Cells were pre-incubated with or without each plant extract and 0.1 mM H₂O₂ on ice for 30 minutes, they were washed in phosphate-buffered saline (PBS), trypsinized, and suspended in 1% low melting point agarose. Following slide preparation and exposure to ice-cold lysis solution (2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris, 10% DMSO, and 1% Triton X-100) for 1 hour, slides were placed in a submarine gel electrophoresis unit containing 300 mM NaOH and 1 mM EDTA, pH 13. Forty 40 minutes later, electrophoresis was performed at 20 V (300 mA) for 20 minutes, and slides were then immersed in neutralizing buffer (0.4 M Tris-HCl, pH 7.5). After staining with ethidium bromide, 50 cells/slide (3 slides/treatment) were scored using an inverted fluorescence microscope (Olympus IX-70A). Data were expressed relative to positive controls treated with H₂O₂, which were assigned an arbitrary value of 1.0.

2.10. Statistical analysis

Values were expressed as mean $\pm$ standard deviation and analyzed statistically using SPSS statistical package for WINDOWS (version 10.0; SPSS Inc, Chicago). All data were obtained from three separated experiments. *P* values of <0.05 were considered statistically significant.

3. Results

3.1. Antimutagenic activity

Initially, extracts of *A. keiskei*, *O. javanica* and *B. oleracea* were tested for antimutagenic activity against the heterocyclic amines IQ, MeIQx, and PhIP in the presence of an exogenous metabolic activation system (Table 1). No signs of toxicity were observed under the conditions reported here. *A. keiskei* produced dose-dependent inhibition against all three mutagens, whereas *O. javanica* showed a more complex pattern, with slight, non-statistically significant enhancement against two of the mutagens (MeIQx and PhIP) at low concentrations in the assay but antimutagenic activity against all three mutagens at the highest concentration of 2 mg/plate. *B. oleracea* was the least effective of the three plant extracts, and indeed at one or more concentrations in the assay tended to enhance mutagenicity. When ranked in terms of their potencies against three heterocyclic amines in the presence of S9, the relative order of antimutagenic activity was as follows: *A. keiskei* > *O. javanica* >> *B. oleracea*.

Three direct-acting mutagens were next examined in the absence of S9, namely, *N*-OH-IQ, *N*-OH-PhIP, and NPD (Table 2). *A. keiskei* at the highest concentration of 2 mg/plate inhibited *N*-OH-IQ but had no statistically significant effect against *N*-OH-PhIP or NPD. No antimutagenic activity was seen with *O. javanica* or *B. oleracea* against the three direct-acting mutagens, and indeed the former extract produced a slight enhancement of *N*-OH-PhIP mutagenicity and the latter augmented the mutagenicity of *N*-OH-IQ. These data suggested no consistent antimutagenic (or co-mutagenic) effects for the plant extracts against direct-acting mutagens under the assay conditions used here.

3.2. Inhibition of cytochrome P450 activity

Heterocyclic amines such as IQ and PhIP are metabolically activated by CYP1A2, generating *N*-OH-IQ and *N*-OH-PhIP, whereas CYP1A1 catalyzes both *N*-hydroxylation and ring hydroxylation reactions. The activities of CYP1A1 and CYP1A2 were followed in EROD and MROD assays, respectively (Figs 1, 2); *A. keiskei* and *O. javanica* produced concentration dependent inhibition in both assays, whereas *B. oleracea* was the least effective of the three plant extracts tested. At the highest concentration in the assay, inhibition of both EROD and MROD activities was in the relative order *A. keiskei* > *O. javanica* >> *B. oleracea*, which agreed favorably with the order of antimutagenic activity against IQ, MeIQx, and PhIP in the Salmonella assay (Table 1).

3.3. Activity of green vegetable extracts in FRAP and ORAC assays

Although the vegetable extracts lacked any consistent electrophile-scavenging activity against the direct-acting mutagens *N*-OH-IQ, *N*-OH-PhIP and NPD in the Salmonella assay (Table 2), further tests were undertaken for possible antioxidant properties of three vegetable extracts. Total phenolics and vitamin C concentration were determined. *O. javanica* had highest phenolic concentrations than *A. keiskei* and *B. oleracea*. However, vitamin C contents were not significantly different among three samples (Table 3). Antioxidant properties, as defined by their activities in FRAP and ORAC assays were determined (Fig. 3). All three vegetable extracts exhibited concentration-dependent ferric reducing/antioxidant power (Fig. 3A), and oxygen radical absorbance capacity (Fig. 3B). In conformance with the total phenolics, the relative order of antioxidant activities in these assays was as follows: *O. javanica* > *A. keiskei* > *B. oleracea*.

3.4. Inhibition of DNA nicking

The closed circular form of plasmid pUC19 was converted efficiently to the nicked linear form after treatment with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$, and in some experiments the relaxed form also was detected as a minor band (Fig. 4). Each of the plant extracts attenuated the formation of nicked DNA *in vitro*. For example, when the linear form was normalized to the corresponding control treated with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ but no plant extract, *A. keiskei* inhibited DNA nicking by ~50% at 20 $\mu\text{g}/\text{mL}$ (bar chart, Fig. 4A), *O. javanica* inhibited by $p > 70\%$ (Fig. 4B) and *B. oleracea* inhibited by 40% (Fig. 4C). Inhibition of DNA nicking corresponded favorably with the relative order of activity in the FRAP and ORAC assays (Fig. 3).

3.5. Antigenotoxic effects in human colon cancer cells

Finally, since plant extracts inhibited DNA nicking induced by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ *in vitro*, we treated HCT116 human colon cancer cells with H_2O_2 and examined the genotoxic damage using the 'comet' assay (Fig. 5). The median tail moment in controls treated with H_2O_2 alone (no vegetable extract) was assigned an arbitrary value of 1.0 in order to compare among the three treatment groups; extracts of *A. keiskei* (Fig. 5A) and *O. javanica* (Fig. 5B) produced significant, dose-dependent protection, whereas *B. oleracea* was effective only at the highest concentration tested of 20 $\mu\text{g}/\text{mL}$ (Fig. 5C). Little damage was detected in untreated cells.

4. Discussion

The present investigation has demonstrated that chlorophyll-rich methanol extracts of *A. keiskei*, *O. javanica* and *B. oleracea* exhibited antimutagenic and antioxidant activities *in vitro*. These three vegetable species were chosen for study because of their widespread use in popular health drinks, such as Korean green juice, and because they are known to contain high levels of antioxidants and phytochemicals, such as chlorophyll, β -carotene, vitamin E, vitamin C, Se and Zn [6–8,29]. Under the present conditions, *A. keiskei* was most effective against the

three indirect-acting heterocyclic amines IQ, MeIQx and PhIP in the Salmonella assay, as well as being most inhibitory in the EROD and MROD assays, but had no effect against direct-acting mutagens in the absence of S9. These findings imply that one or more constituents in *A. keiskei* interfered with the enzymes which metabolically activate heterocyclic amines, without affecting the direct-acting mutagens, such as by electrophile-scavenging [10]. Although *O. javanica* and *B. oleracea* were less effective than *A. keiskei* under the present conditions, we cannot exclude the possibility that other cultivars of these three plants might exhibit greater inhibitory activity.

Indeed, in prior studies [6–8], extract of kale (*B. oleracea*) was more effective than *A. keiskei* in preventing the oxidation of linoleic acid during storage, which is indicative of antioxidant activity, as well as exhibiting higher nitrite scavenging *in vitro*. We confirmed the high antioxidant activity of all three plant extracts in ORAC and FRAP assays, and demonstrated inhibition of H₂O₂-induced DNA damage, assessed directly using pUC19 *in vitro* and indirectly using the comet assay in HCT116 colon cancer cells. In contrast to the results obtained in the Salmonella assays, *O. javanica* was generally the most potent of the three plant extracts in the various assays used here in order to assess antioxidant properties.

Although, as mentioned above, different cultivars are likely to exhibit different inhibitory potencies in the various assays, in the present investigation *A. keiskei* was most effective against heterocyclic amine mutagens, whereas *O. javanica* had the highest antioxidant potential. These findings suggest that, indeed, a ‘cocktail’ of plant extracts is likely to prove more beneficial than an extract containing a single plant species, since this would likely provide for complementary protective mechanisms. This is one rationale used for health drinks containing a cocktail of plant species, such as Korean green juice, which has high levels of phytochemicals with demonstrated cancer inhibitory activity, such as chlorophylls, indoles and isothiocyanates [1–4,20–32]. Future studies should examine the possible synergistic protective effects of *A. keiskei*, *O. javanica* and *B. oleracea* in combination, and assess the optimal ‘blend’ of these three plant extracts with respect to antimutagenic *versus* antioxidant effects.

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Fig. 1. Inhibition of 7-ethoxyresorufin O-deethylase (EROD) activities by *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* extracts. Inset: Percent inhibition vs. concentration of each vegetable extracts. Percent inhibition was calculated from the initial slopes, in which each vegetable extracts addition was compared with the control. Data points and error bars indicate mean $\pm$ S.D. ($n = 3$). Symbols: $\bullet$ control, $\circ$ 25 $\mu\text{g/mL}$, $\blacktriangledown$ 50 $\mu\text{g/mL}$, ∇ 100 $\mu\text{g/mL}$, $\blacksquare$ 200 $\mu\text{g/mL}$.

Fig. 2. Inhibition of methoxyresorufin O-demethylase (MROD) activities by *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* extracts. Inset: Percent inhibition vs. concentration of each vegetable extracts. Percent inhibition was calculated from the initial slopes, in which each vegetable extracts addition was compared with the control. Data points and error bars indicate mean $\pm$ S.D. ($n = 3$). Symbols: $\bullet$ control, $\circ$ 25 $\mu\text{g/mL}$, $\blacktriangledown$ 50 $\mu\text{g/mL}$, ∇ 100 $\mu\text{g/mL}$, $\blacksquare$ 200 $\mu\text{g/mL}$.

Fig. 3. Antioxidant capacity of *A. keiskei*, *O. javanica* and *B. oleracea* extracts. **A** ferric reducing/antioxidant power (FRAP) and **B** oxygen radical absorbance capacity (ORAC) was determined relative to Trolox, a water-soluble vitamin E analog. Results are given as mean $\pm$ SD. Wedge symbol: increasing concentration of each extract (1, 5, 10, 20 µg/mL).

Fig. 4.

Inhibition of DNA nicking by *A. keiskei*, *O. javanica* and *B. oleracea*. pUC19 was incubated in the absence (–) or presence (+) of H₂O₂/Fe²⁺ in order to convert closed circular plasmid to nicked linear and/or relaxed forms. The linear form of DNA was quantified relative to controls treated with H₂O₂/Fe²⁺ but no plant extract, and expressed as ‘percent control’ to facilitate comparison among groups. Wedge symbol: increasing concentration of each extract (0, 2, 4, 10, 20 μg/mL). **P* < 0.05 and ***P* < 0.01 by ANOVA.

Fig. 5.

Antigenotoxic effects of *A. keiskei*, *O. javanica* and *B. oleracea* in HCT116 human colon cancer cells. Cells were incubated in the absence (–) or presence (+) of 0.1 mM H₂O₂ and DNA damage was quantified via the comet assay. Median tail moment in positive controls treated with H₂O₂ but no plant extract was assigned an arbitrary value of ‘1.0’ in order to facilitate comparison among treatment groups. Wedge symbol: increasing concentration of each extract (0, 1, 5, 10, 20 µg/mL) **P* < 0.05 and ***P* < 0.01 by ANOVA.

Table 1

Antimutagenic activities of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* extracts against three heterocyclic amines

	His+ revertants/plate		
	IQ ^a	MeIQx ^b	PhIP ^c
<i>A. keiskei</i>			
Control (-) ^d	50.33 ± 5.03	38.00 ± 7.94	50.33 ± 5.03
Control (+) ^e	1962.00 ± 139.15	966.67 ± 57.00	1663.00 ± 20.78
0.1 mg/plate	1858.67 ± 105.55	960.67 ± 52.55	1447.00 ± 10.20**
1.0 mg/plate	1558.00 ± 54.37**	692.67 ± 48.00**	1321.00 ± 11.30**
2.0 mg/plate	1090.33 ± 164.27**	391.67 ± 58.94**	747.67 ± 61.52**
<i>O. javanica</i>			
Control (-)	34.33 ± 5.51	45.67 ± 3.21	30.00 ± 14.73
control (+)	1078.00 ± 171.76	766.00 ± 75.45	1376.67 ± 123.14
0.1 mg/plate	1067.00 ± 77.49	737.33 ± 17.62	1422.33 ± 166.17
0.5 mg/plate	725.00 ± 101.82*	828.00 ± 59.09	1587.67 ± 55.23
1.0 mg/plate	1006.67 ± 87.67	695.00 ± 48.66	1303.00 ± 138.39
2.0 mg/plate	604.67 ± 183.47**	541.67 ± 45.49**	1111.00 ± 137.02*
<i>B. oleracea</i>			
control (-)	34.33 ± 5.51	45.67 ± 3.21	30.00 ± 14.73
control (+)	1078.00 ± 171.76	766.00 ± 75.45	1376.67 ± 123.14
0.1 mg/plate	1234.33 ± 240.56	638.33 ± 68.24	1372.00 ± 57.86
0.5 mg/plate	1173.67 ± 231.23	880.00 ± 184.47	1468.67 ± 166.43
1.0 mg/plate	1149.33 ± 96.93	695.33 ± 43.25	1513.33 ± 82.44
2.0 mg/plate	818.33 ± 76.16	677.33 ± 92.50	1577.00 ± 299.60

^a 2-amino-3-methylimidazo[4,5-f]quinoline.

^b 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline.

^c 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

^d Negative control; DMSO vehicle alone group.

^e Positive control; mutagens treated group.

** $P < 0.01$ relative to positive control group.

* $P < 0.05$ relative to positive control group.

Table 2

Antimutagenic activities of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea* extracts against two directing-acting heterocyclic amines and mutagens

	His+ revertants/plate		
	NPD ^a	N-OH-IQ ^b	N-OH-PhIP ^c
<i>A. keiskei</i>			
Control (-) ^d	28.33 ± 7.02	28.33 ± 7.02	14.00 ± 5.29
Control (+) ^e	3086.00 ± 161.11	2378.00 ± 219.97	1114.67 ± 113.59
0.1 mg/plate	2655.33 ± 95.00	1791.00 ± 292.74 [*]	1047.67 ± 68.49
1.0 mg/plate	2568.00 ± 174.11	1654.00 ± 257.03 ^{**}	1014.67 ± 201.53
2.0 mg/plate	2192.00 ± 216.52 [*]	691.67 ± 72.61 ^{**}	1050.00 ± 148.92
<i>O. javanica</i>			
Control (-)	77.00 ± 30.64	16.00 ± 7.00	22.67 ± 6.35
Control (+)	2479.67 ± 161.92	2553.33 ± 226.62	1338.00 ± 53.84
0.1 mg/plate	2206.67 ± 260.16 ^{**}	2514.33 ± 90.78	1106.33 ± 150.98
0.5 mg/plate	1973.67 ± 139.33 ^{**}	2620.67 ± 131.07	1181.33 ± 40.27
1.0 mg/plate	2016.67 ± 115.79 ^{**}	2514.33 ± 90.78	1370.00 ± 150.44
2.0 mg/plate	2062.67 ± 44.66 ^{**}	2826.67 ± 543.57	1676.33 ± 477.63
<i>B. oleracea</i>			
Control (-)	77.00 ± 30.64	16.00 ± 7.00	22.67 ± 6.35
Control (+)	2479.67 ± 161.92	2553.33 ± 226.62	1338.00 ± 53.84
0.1 mg/plate	2062.67 ± 44.66	3476.33 ± 158.47 ^{**}	1262.33 ± 89.95
0.5 mg/plate	2016.67 ± 115.79	3383.33 ± 204.59 ^{**}	1204.67 ± 26.35 [*]
1.0 mg/plate	1973.67 ± 139.33	4101.67 ± 338.92 ^{**}	1280.00 ± 41.76
2.0 mg/plate	2206.67 ± 260.16	5128.33 ± 125.01 ^{**}	1488.67 ± 85.64 [*]

^a 4-nitro-1,2-phenylenediamine (NPD).

^b 2-hydroxyamino-3-methylimidazo[4,5-f]quinoline.

^c 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine.

^d Negative control; DMSO vehicle alone group.

^e Positive control; mutagens treated group.

^{**} $P < 0.01$ relative to positive control group.

^{*} $P < 0.05$ relative to positive control group.

Table 3

Total phenolics and total ascorbic acid concentrations of *Angelica keiskei*, *Oenanthe javanica* and *Brassica oleracea*

	Moistures(%) ^a	Total phenolics (umolGAE/g) ^b	Ascorbic acid(mg/g) ^c
<i>A. keiskei</i>	92.08 ± 0.43	675.00 ± 17.68	13.00 ± 0.06
<i>O. javanica</i>	94.42 ± 0.40	1070.00 ± 81.32	12.37 ± 2.62
<i>B. oleracea</i>	92.76 ± 0.46	510.00 ± 10.61	11.48 ± 0.13

^a Moisture, lipids and protein content were determined using the standard AOAC methods 7(33).

^b Total phenolics are expressed as μ mol Gallic Acid Equivalents (GAE/g) based on dry weights.

^c Ascorbic acid contents are based on dry weights.