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Full Length Research Paper

Antioxidant, antiproliferative, and *α*-glucosidase inhibitory activities of extracts from *Impatiens textori* Miq

Jinfeng Yang[1#], Ju-Sung Kim[2#] and Myong-Jo Kim[1,3]*

[1]Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, Korea.
[2]Plant Resource Sciences and Environment, College of Applied Life Sciences, Jeju National University, Jeju 690-756, Korea.
[3]Oriental Bio-herb Research Institute, Kangwon National University, Chuncheon 200-701, South Korea.

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***Impatiens textori* Miq is an annual plant used in traditional Chinese medicine to treat superficial infections and fingernail inflammation. In the present study, methanol extract and fractions of *I. textori* Miq were investigated for their antioxidant properties, *α*-glucosidase inhibitory activities, and antiproliferative activities against human gastric (AGS), human cervical (HeLa), human non-small lung (A549), and human colon adenocarcinoma (HT-29) cancer cell lines. Results showed that the ethyl-acetate fraction had high total phenolic and total flavonoid contents of 141.39 ± 1.54 mg GAE/g and 125.93 ± 7.60 mg QE/g, respectively, high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with an IC_{50} of 9.80 ± 0.08 µg/ml, and significant reducing power among all the extracts and fractions, suggesting that *I. textori* Miq is an exceptional source of natural antioxidants. In addition, the ethyl-acetate fraction possessed significant *α*-glucosidase inhibitory activity. The antiproliferative activity of the ethyl-acetate fraction was higher than that of the methanol extract and others fractions. These findings suggest the potential use of this plant as an antitumor agent.**

Key words: *Impatiens textori* Miq, antioxidant, 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power, antitumor, *α*-glucosidase.

INTRODUCTION

Cancer is the second leading cause of death in the Western world (Madhusudan and Middleton, 2005). According to the World Health Organization, more than 10 million new cases of cancer are diagnosed each year worldwide, and statistical trends indicate 15 million new cases in 2020 (Mignogna et al., 2004). Different agents have been described that show a wide range of anticancer effects (Damm et al., 2001; Zou et al., 2006), but the drugs sometimes have unpleasant side effects and complications (Mittal et al., 2004). It is thus necessary to seek alternative medicines, preferably herbal therapies and natural compounds such as *Impatiens textori* Miq. *I. textori* Miq (Balsaminaceae) is an annual plant that grows naturally along water fronts in the mountains in almost every region of Japan, Korea, and northeast China, and it has an attractive purple flower color (Iwaoka et al., 2010; Tatsuzawa et al., 2009). The plant has been used in traditional Chinese medicine for the treatment of superficial infections, fingernail inflammation, detoxification, and carbuncles (Ueda et al., 2005). A recent study showed that ethanol or chloroform extracts of leaves from *Impatiens balsamina* have antitumor activity against the human hepatocellular carcinoma cell line HepG2 (Ding et al., 2008). The allergy-preventive activity of the 35% EtOH extract of *I. textori* Miq flowers was also reported (Emiko et al., 2010). Previous studies have reported several flavones and flavonols in this plant (Ueda et al., 2003). Flavonoids are a large group of polyphenolic compounds that are known

*Corresponding author. E-mail: kimmjo@kangwon.ac.kr. Tel: +82 33 250 6413. Fax: +82 33 253 6413.

# These authors contributed equally to this work.

to have antioxidative activity as well as scavenging effects on reactive oxygen species (Nijveldt et al., 2001). Highly reactive free radicals may cause cardiovascular disease (Simonne et al., 1997), neurodegenerative disease (Joseph et al., 2000), diabetes (Laaksonen et al., 1998), rheumatoid arthritis, cataracts (Taylor, 1992), and aging effects (Halliwell et al., 1995). Grivennikova and Andrei (2006) demonstrated that superoxide is a type of reactive oxygen species (ROS) that is generated within the mitochondria and can result in the induction of additional ROS. Therefore, increasing the level of these ROS creates an environment known as oxidative stress, which may lead to DNA damage and mutations and eventually tumor progression (Hogan et al., 2010). Antioxidants function to prevent the adverse effects of oxygen by capturing free radicals and preventing chronic complications in part through their interactions with ROS (Seifried et al., 2007).

Diabetes mellitus is a congenital metabolic disorder (Abestndara et al., 2004). Diabetes is categorized as either sub-type 1 diabetes or type 2 diabetes. Type 1, usually diagnosed in childhood, is when the body makes little or no insulin. The rapidly increasing prevalence of type 2 diabetes mellitus (T2DM) is a metabolic disease caused by environmental factors, such as increased availability of food and decreased opportunity and motivation for physical activity, and affects genetically susceptible individuals (Park et al., 2005). T2DM is recognized as one of the most common metabolic diseases, and the number of patients is estimated to increase to about 300 million by 2025. The most beneficial therapy for T2DM is one that achieves optimal blood glucose control after a meal (Fang et al., 2008). α-Glucosidase is one of the glucosidases located in the epithelium of the small intestine and functions to reduce the absorption of glucose, thereby inhibiting postprandial hyperglycemia (Yao et al., 2010). Several α-glucosidase inhibitors, including acarbose, voglibose, and miglitol (Scott and Spencer, 2000), are clinically prescribed, but natural products of great structural diversity are still a good source of such inhibitors. Thus, the objectives of this research were: to determine the α-glucosidase inhibitory activity, reducing power, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, total phenolic and flavonoid contents, and antiproliferative activity of *I. textori* Miq.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, gallic acid, butylated hydroxyannisole (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT), phosphate buffered saline (PBS) buffer, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Cisplatin, Streptomycin and Penicillin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, butanol, hexane and ethyl acetate were obtained from Dae Jung chemicals and metals Co. (Seoul, Korea). All reagents were of analytical grade or better.

Extract preparation

The whole plant of *I. textori* Miq were obtained from Geoduri, Chun Cheon city, Korea in July 2010. The plant was identified and authenticated by Dr. Heo Kweon, a taxonomist in the Botanical Garden of Kangwon National University. The shade dried whole plant, was powdered and extracted with 100% methanol. The extract was filtered, and three replications were extracted under the same conditions with new solvent. The filtered extracts were mixed and concentrated using a rotary evaporator. The crude extract was subfractionated into *n*-hexane, ethyl-acetate (EtOAc), *n*-butanol (BuOH; water saturated) and aqueous fractions. The fractions were then stored under refrigeration for further analysis.

For cell studies, 40 mg each of extract, fractions and cisplatin were suspended in 0.04 ml dimethyl sulfoxide (DMSO) each and dissolved in double distilled water as a 10 mg/ml stock solution.

Determination of total phenolic and flavonoid content

Total phenolic content was measured by the method of Folin-Ciocalteu (Singleton and Rossi, 1965). Briefly, 0.1 ml each of the samples at different concentrations was mixed with 0.05 ml of Folin-Ciocalteau reagent and then mixed thoroughly. Three minutes later, 0.3 ml of 20% sodium carbonate ($NaCO_3$) was added, followed by mixing with intermittent shaking. The reaction mixture was then incubated for 30 min at room temperature. Finally, before reading the absorbance of the reaction mixture at 725 nm using a spectrophotometer (V530, Jasco Co., Japan). Measurements were performed in triplicate, and the data were expressed as gallic acid equivalent (GAE) per mg of extract, based on the calibration curve of gallic acid.

The total flavonoid content of the extracts was determined via the colorimetric method as described by Park et al. (1997). An aliquot of 0.2 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate (w/v), 0.1 ml of 1 M potassium acetate, and 4.6 ml of 80% ethanol. After 40 min at room temperature, the absorbance was determined at a wavelength of 415 nm. The total flavonoid contents of the sample were determined by comparison with the optical density values of different concentrations of a standard flavonoid compound, quercetin. This analysis for each sample was analyzed in triplicate, and a calibration curve of quercetin was plotted by plotting the absorbance vs. the concentration of quercetin.

DPPH radical scavenging activity

The DPPH radical scavenging activities of the extracts were determined by the method of Blois (1958) with slight modification. Initially, 4 ml of methanol solution containing 0.1 ml each of the samples at different concentrations was mixed with 1 ml of 0.15 mM DPPH (dissolved in methanol). The reaction mixture was then incubated for 30 min at room temperature. The control contained all reagents without the sample, whereas methanol was used as a blank. All measurements were performed in triplicate. DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm and expressed as the inhibition percentage of free radicals by the sample after calculation using the following formula: (%) inhibition = (1 - absorbance of sample / absorbance of control) × 100. The IC_{50} value (mg/ml) represents the concentration at which the scavenging activity is 50%.

Reducing power assay

Reducing power was measured according to the method reported

Table 1. Total phenolic contents, flavonoid contents, and DPPH radical scavenging activities of the extract and fractions of *Impatiens textori* Miq.

Extract and fractions	Total phenolic contnts (mg GAE/g)	Total flavonoid contnts (mg QE/g)	DPPH (IC_{50} (µg/ml))
Methanol extract (M)	37.76 ± 3.36^{c}	19.84 ± 0.30^{d}	23.46 ± 0.15^{d}
Hexane fraction (H)	40.83 ± 3.74^{c}	29.49 ± 2.46^{c}	29.42 ± 0.53^{c}
Ethyl acetate fraction (E)	141.39 ± 1.54^{a}	125.93 ± 7.60^{a}	9.80 ± 0.08^{a}
Butanol fraction (B)	54.20 ± 0.98^{b}	39.58 ± 0.71^{b}	18.52 ± 0.10^{b}
Water fraction (W)	11.83 ± 0.74^{d}	4.01 ± 3.19^{e}	54.02 ± 3.50^{e}
BHA			5.48 ± 0.01
BHT			14.53 ± 0.55

by Oyaizu (1986) with slight modification. An aliquot of each extract (0.1 ml) was mixed with 0.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide, followed by incubation at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) was added, a 0.5 ml aliquot of the upper layer was mixed with distilled water (0.5 ml) and ferric chloride (0.1 ml, 0.1%), after which the absorbance was measured at 700 nm against a blank containing all of the reagents without the tested sample. A higher absorbance indicated higher reducing power. BHT and BHA were used for comparison purposes.

Measurement of α-glucosidase activities

α-Glucosidase inhibitory activities were assayed according to a previously described procedure (Oki et al., 1999) with slight modification. The reaction was initiated with 0.05 ml each of the samples at different concentrations in 0.2 mM phosphate buffer (pH 6.8), followed by incubation at 37°C for 15 min, after which 0.05 ml of enzyme solution was immediately added to the mixture before mixing and incubation at 37°C. Then, 3 mM pNPG (0.1 ml) was added, after which the reaction was stopped by the addition of 0.75 ml of 0.1 M Na_2CO_3. α-Glucosidase inhibitory activity was determined by measuring the release of pNPG at 405 nm. The control (acarbose) contained all reagents without the tested sample. The reactions were conducted in triplicate. The α-glucosidase inhibitory activity was calculated as follows:

Inhibitory ratio % = [1- (As- Ab)/ Ac] × 100

where Ac, As, and Ab represent the absorbance levels of the control, sample, and blank, respectively. The concentration of α-glucosidase inhibitor required to inhibit 50% of α-glucosidase activity under the assay conditions is defined as the IC_{50} value.

Cell culture

Human gastric (AGS), human cervical (Hela), human non-small lung (A549), and human colon adenocarcinoma (HT-29) cancer cell lines were purchased from Korea Cell Line Bank (KCLB, Seoul, Korea). All cultures were maintained in growth medium supplemented with 10% FBS, 1% antibiotics (penicillin and streptomycin, 100 U/ml), incubated at 37℃ in a humidified chamber, 5% CO_2 atmosphere.

Cell viability

Cell viability was assessed by WST-1 assay according to the procedure previously described with slight modification (Worle-Knirsch et al., 2006). Cells were seeded at a density of 1×10^4 cells/100 µl in 96-well plates (SPL, Korea) for 24 h and then treated with (*I. textori* Miq) or reference drug cisplatin (positive control) at 100, 200, or 500 µg/ml. The treatment was repeated in 96-well plates. The cells were further incubated for 24 h at 5% CO_2 37℃ in a humidified chamber. The treated medium was then removed at the end of the incubation period, after which the cells were incubated for 4 h with WST-1 reagent (10 µg /well). Absorbance was read at 450 nm using an ELISA reader multilabel counter (Wallac VICTOR², Japan).

Statistical analysis

The data are expressed as the means ± SD of the values. Statistical significance was determined by analysis of variance (ANOVA). Duncan's multiple range tests were used to determine the significance of differences between the groups. A level of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

Table 1 shows the total phenolic and flavonoid contents of the extract and different fractions of *I. textori* Miq. Total flavonoid content in the samples was as follows: E (125.93 ± 7.60 mg QE/g) > B (39.58 ± 0.71 mg QE/g) > H (29.49 ± 2.46 mg QE/g) > M (19.84 ± 0.30 mg QE/g) > W (4.01±3.19 mg QE/g). Total phenolic content expressed as mg of gallic acid equivalents (GAE) per g of sample was the lowest in the W fraction (11.83 ± 0.74 mg GAE/g). As expected, the E fraction (141.39 ± 1.54 mg GAE/g) had higher phenolic content than the others. Phenolic compounds have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. Scalzo et al. (2005) found that wild strawberries and cultivated strawberries have high phenolic contents as well as strong antioxidant activities. Ethyl acetate extracts from white, yellow, and red onions demonstrate anti-mutagenicity and antioxidant properties related to their phenolic content, including flavonoids (Lin and Tang, 2007). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an

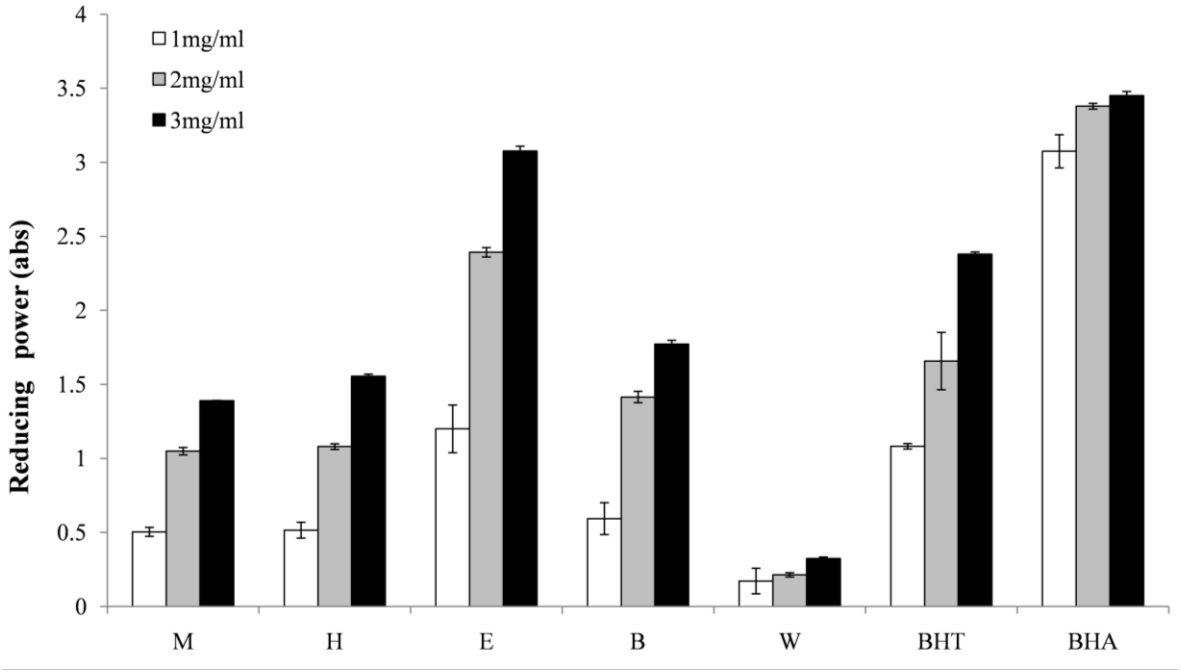

Figure 1. Reducing power of extract and fractions of *Impatiens textori* Miq.

important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Zheng and Wang, 2001). Therefore, the higher content of total phenols in E might account for its higher reducing power and DPPH radical scavenging effect.

DPPH radical scavenging activity

The method of scavenging stable DPPH radical is used in food, plant extracts, and beverages to evaluate antioxidant activity over a short time (Djilas et al., 2009), and the concentration of antioxidant required to reduce or "inhibit" 50% of DPPH radical is a widely used measure of antioxidant activity (Ramírez-Mares et al., 2010). In this study, the scavenging abilities of extract and various fractions of *I. textori* Miq against DPPH radical are illustrated in Table 1, and the results are normalized and expressed as IC_{50} values (mg/ml) for comparison. The results of our experiments demonstrated that the extract and all of the fractions tested possessed radical scavenging activity. E fraction was found to have the highest active DPPH radical scavenging activity, and it was superior to the positive control, BHT. On the other hand, the lowest DPPH radical scavenging activity was observed in W fraction. In addition, we found that the DPPH antioxidant capacity corresponded to the TP content. Reynertson et al. (2005) reported seven fruits of the Myrtaceus family as good antioxidant sources using DPPH assay. A good linear correlation was established between TP and DPPH antioxidant capacity (R^2 = 0.931). These conclusions are in agreement with those of Chirinos et al. (2010), who reported that the antioxidant potential of camu camu fruit is related to its phenolics content. These results suggest that antioxidant capacity is correlated with phenolic content. Thus, the E fraction of *I. textori* Miq has strong DPPH radical scavenging activity.

Reducing power assay

Reducing power can serve as a significant measure of the antioxidant activity of polyphenols (Duan et al., 2007). Thus, it is necessary to determine the reducing power of the extract and fractions of *I. textori* Miq to elucidate the relationship between antioxidant effect and reducing power. All extracts were measured spectrophotometrically by reading the absorbance at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. As shown in Figure 1, the extracts were tested at the 1.0, 2.0 and 3.0 mg/ml levels. All of the samples showed some degree of reducing power.

However, all extracts displayed increased reducing power with increasing concentration. In addition, E fraction had higher absorbance values compared with the other extracts. On the other hand, W fraction appear to have lower reducing power. This result is in good correlation with our total phenolic content results, in which we demonstrated that E fraction possessed high phenolic content. Phenolic compounds have been proposed to be responsible for donating an electron to

Table 2. α-Glucosidase inhibitory activities of the extract and fractions of *Impatiens textori* Miq.

Extract and fractions	IC_{50} (μg/ml)
Methanol extract (M)	21.64 ± 0.32^{d}
Hexane fraction (H)	18.81 ± 0.48^{c}
Ethyl acetate fraction (E)	8.56 ± 0.30^{a}
Butanol fraction (B)	13.67 ± 1.02^{b}
Water fraction (W)	-
Acarbose	3.00 ± 0.23

free radicals, which terminates the radical chain reaction by converting free radicals to more stable products (Wang et al., 2008). Shimada et al, (1992) reported that antioxidative activity is concomitant with the development of reductones. Therefore, the significant antioxidative activity of E fraction from *I. textori* Miq may be related to its reducing power. These results are in agreement with the results of Odabasoglu et al. (2005) who found that the methanol extract of *Peltigera rufescens* has the highest reducing power.

α-Glucosidase activities

Primarily, α-glucosidase inhibitory activities are expressed as IC_{50} values as summarized in Table 2. Among these, W fraction did not have potent inhibitory activity towards α-glucosidase, M extract (IC_{50} values of 21.64 ± 0.32 μg/ml) had the lowest activity, and E fraction (IC_{50} values of 8.56 ± 0.30 μg/ml) had the strongest inhibitory activity. These results suggest that the α-glucosidase inhibitory activities of the extract and different fractions of *I. textori* Miq were very different. These different inhibitory activities may be attributed to significant differences in phenolic content among the fractions. As reported previously, polyphenols or classes of polyphenols may have other beneficial effects, independent of their antioxidant capacities, by directly influencing the activities of key enzymes.

There have been reports that polyphenolic fractions from plants can cause insulin-like effects in glucose utilization (McDougall et al., 2005). Many researchers have investigated polyphenolic extracts from a number of plants with α-glucosidase inhibitory activity (Matsui et al., 2001). Recently, Zhang et al. (2007) determined that polyphenol-rich extracts from *Ascophyllum* inhibit glucosidase and show promising anti-diabetic effects in mouse models.

Cell viability

To evaluate the effects of *I. textori* Miq on cell viability of AGS, HeLa, A549, and HT-29 cancer cell lines, we performed WST-1 assay. The cells were treated with various concentrations of *I. textori* Miq at 100, 200, and 500 μg/ml for 24 h. Figure 2 shows that there were significant differences in cell viability between the extract and different fractions. Treatment of these cells with *I. textori* Miq resulted in significant dose-dependent inhibition of cell growth. In addition, the E fraction significantly inhibited the growth of cancer cells compared to other fractions. This was most likely due to the presence of phenolics, which were responsible for the strong anticancer activities. According to a study by Cai et al. (1997), polyphenolic compounds, especially flavonoids, are well-known as dietary antioxidants. These compounds have important effects on cancer chemo-prevention and play a key role in reducing cancer cell proliferation (Chinery et al., 1998; Sergediene et al., 1999). Until now, much research has been carried out on their potential to induce apoptotic cell death in various cancer cell lines, including human stomach cancer and human colon adenocarcinoma (Yoshioka et al., 2000). Hence, this study provides a basis for the discovery and development of *I. textori* Miq as a potential anticancer agent.

Conclusion

In summary, we demonstrated that the ethyl-acetate fraction of *I. textori* Miq possesses strong antioxidant activities and antiproliferative activity against AGS, Hela, A549, and HT-29 cancer cell lines. The effect of the ethyl-acetate fraction of *I. textori* Miq on cancer cells was significant, as the extract and other fractions did not exhibit similar inhibitory activity. In addition, the ethyl-acetate fraction of *I. textori* Miq exhibited good α-glucosidase inhibitory activity.

According to this study, a significant relationship was observed among antioxidant, antitumor, and α-glucosidase inhibitory activities, indicating that phenolic compounds could be major contributors. Further studies should identify active constituents in *I. textori* Miq and elucidate its inhibitory mechanisms in these cancer cells.

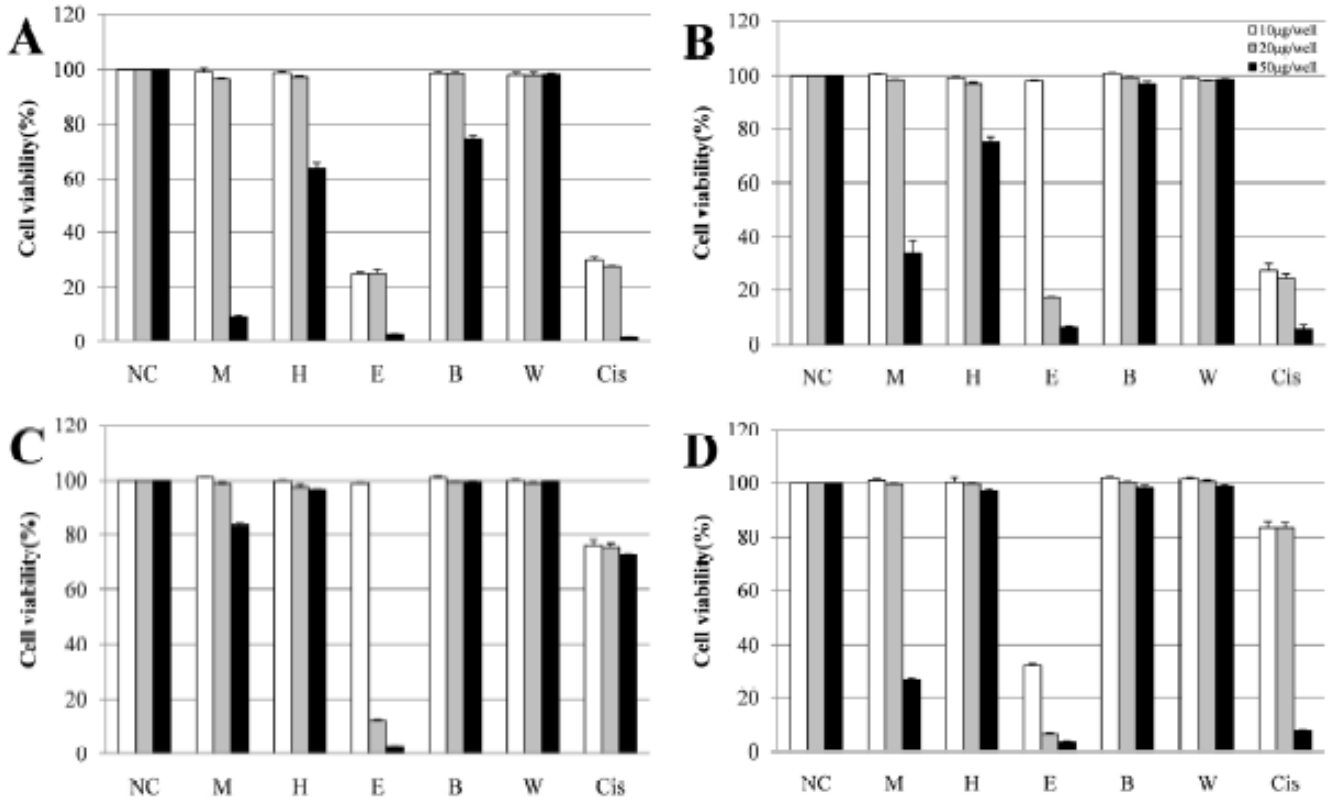

Figure 2. Antiproliferative activities of *Impatiens textori* Miq on AGS, HeLa, A549, and HT-29 cancer cells.

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