

Full Length Research Paper

Anticlastogenic effect of aqueous extract from water yam (*Dioscorea alata* L.)

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Dioscorea plants have been widely used as traditional medicine and food for health benefits. Several therapeutic properties such as anti-cough, anti-diabetic, anti-diarrhea, and anti-cancer have been reported. Although the steroidal saponins (such as diosgenin) account for some of Dioscorea's activity, there is little information on the effective components. We have shown here that water yam (*Dioscorea alata* L.) aqueous extract (YAE) inhibited the H₂O₂-CuSO₄ induced damage of calf thymus DNA, and protected cultured human lymphoblastoid cells from CuSO₄-induced DNA damage. This aqueous extract exhibited a major absorption peak at 260 to 262 nm. Upon the addition of CuSO₄, the absorption peak was shifted to 236 nm and it was sensitive to the divalent ion chelator EDTA. The aqueous extract of YAE was also capable of dissociating the complex formation of Cu (II) with tetramethyl murexide (a copper indicator). Thin layer chromatography indicated that it contains one major and several minor anisaldehyde-, sulfuric acid-reactive and highly polar compounds. Based on these findings, we suggest that water-soluble mucilaginous polysaccharides within YAE are likely the effective compounds/candidates, which carry copper-chelating activity, and protect DNA against H₂O₂-CuSO₄ combination or CuSO₄ alone.

Key words: Water yam (*Dioscorea alata* L.), Fenton reaction, metal chelating activity, DNA damage protection, antioxidant.

INTRODUCTION

The metabolism of oxygen and many toxic agents is accompanied by the formation of reactive oxygen species. Reactive oxygen species, such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH[•]) are formed through a process of one-electron reduction of molecular oxygen (O₂). They are generated by a process known as redox cycling, and are catalyzed by transition metals such iron and copper ions that cause DNA damage, protein and lipid oxidation, then finally lead to cell death (Stoys and Bagchi, 1995).

Reactive oxygen species have been implicated in over a hundred human disorders, including arthritis, morrhagic shock, advancing aging, ischemia/reperfusion injury, cardiovascular disorders, Alzheimer's and Parkinson's

diseases, gastrointestinal dysfunctions, cancers and AIDS (Moskovitz et al., 2002; Datta et al, 2000; Martinez-aCayuela, 1995). To minimize potential oxidative damage in vivo, antioxidants from various dietary sources have been studied extensively. Evidences have shown that dietary intake of phytochemicals, including α -tocopherol, ascorbic acid and the flavonoids, have been linked to the maintenance of health and protection from diseases (Giugliano, 2000; Kelly, 1998).

The tubers of some *Dioscorea* species have been used in traditional medicine in China and Zimbabwe for diarrhea, diabetes, skin problems, and rheumatism. They are tonic for the spleen, stomach, lung and kidneys (Dykman et al., 1998; Bensky and gamble, 1993; Iwu et al., 1990; Undie and Akubue, 1986; Gelfand and Mavi, 1985). Those *Dioscorea* species are very important sources of secondary metabolites used in the pharmaceutical industry and general medicine. Diosgenin and related steroidal saponins which provide the steroid

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building-blocks for developing human sex hormones are obtained commercially from the tubers of various *Dioscorea* species (Norton, 1998). The major biological functions of *Dioscorea* extracts include; anti-tumorigenesis and anti-carcinogenesis (Miyoshi et al., 2011; Gao et al., 2002; Hu and Yao, 2002a, b; Dong et al., 2001; Hu et al., 1996), anti-microbial activity (Atindehou et al., 2002; Kelmanson et al., 2000), hypoglycemia (Iwu et al., 1990; Hikino et al., 1986; Undie and Akubue, 1986), anti-hypertension (Liu et al., 2009; Hsu et al., 2002), anti-fatigue (Chiu et al., 2009; Dykman et al., 1998), anti-oxidant (Farombi et al., 2000; Hou et al., 2002, 2001; Araghiniknam et al., 1996), and renoprotection (Lee et al., 2002), according to the clinical supplement trial(s) and animal/cell model system tests. Although evidence indicates that phytochemicals, including saponins (Gao et al., 2002; Hu and Yao, 2002a, b; Dong et al., 2001; Hu et al., 1996), polyphenols (Farombi et al., 2000), storage protein (Hou et al., 2001) and mucilage (Hou et al., 2002), are likely the active components of *Dioscorea* species, the real effective components and their structures have still not been identified yet. Moreover, the molecular mechanisms of its biological functions are still unclear.

The present study was designed to investigate the protective effect of an aqueous extract of yam (*Dioscorea alata* L.) on the catalytic transition metal-driven oxidative DNA damage. The results show that water yam (*Dioscorea alata* L.) aqueous extract (YAE) acts as metal copper ions chelator acting against copper-mediated DNA damage *in vitro* and the water-soluble mucilaginous polysaccharides are likely the protective compound(s)

MATERIALS AND METHODS

Chemicals

Amplex red was purchased from the Molecular Probes (Eugene, OR, USA). All other chemicals were analytical grade and obtained from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany).

Preparation of *Dioscorea* aqueous extracts (YAE)

Tubers of yam (*Dioscorea alata* L., Tainong No. 2) were collected from local cultivation in Taichung, Taiwan. The tubers were peeled off and cut into small pieces and stored at -80°C until the extract was prepared. The small pieces were placed in sterilized MilliQ water (1:4 w/v) and homogenized in a blender (Osterizer, Delray Beach, Florida, USA). The homogenate was centrifuged at 5,000 rpm for 30 min to remove the undissolved materials. The residue was discarded and the supernatant was dried in a ZIBRUS freeze dryer (Vaco 2, Bad Grund, Germany). The freeze-dried extract was stored at -20°C until used for the determination of anti-oxidant activity. Ten percent of the freeze-dried extract powder dissolved in sterilized MilliQ water served as a stock solution.

Cell culture

Human peripheral blood samples were drawn from healthy

volunteers with heparinized syringes. Lymphocytes were isolated by centrifugation in a Ficoll-Paque cushion (Pharmacia Biotech, Sweden), washed with phosphate-buffered saline (PBS), and resuspended in RPMI 1640 medium containing 10% fetal calf serum (HyClone, Logan, UT, USA) and 1% PSN antibiotic mixture. The lymphocytes were transformed into immortal lymphoblastoid cell lines using Epstein-Barr virus. The cell cultures were grown in a humidified atmosphere with 5% CO₂ in air at 37°C. The reagents for cell culture were obtained from Gibco BRL (NY, USA).

Ethidium bromide fluorescence binding assay

The ethidium bromide (EtBr) binding assay, based on the formation of a fluorescent complex between double-stranded DNA and EtBr, was used to measure DNA damage (Stoewe and Prutz, 1987). H₂O₂-CuSO₄ damages DNA and inhibits the binding of EtBr to DNA, resulting in a decrease in intensity of fluorescence. Several forms of DNA damage, including strand scission, base oxidation, and base liberation, contribute to the loss of fluorescence.

Therefore, the assay detects a broad range of different DNA lesions. A 2 ml standard reaction mixture contained 20 mM phosphate buffer (pH 7.0), 100 µg/ml calf thymus DNA, 50 µM CuSO₄, and 25 mM H₂O₂. To measure the protection of *Dioscorea* aqueous extracts on DNA damage, various amounts of *Dioscorea* extract were added to the reaction mixture after adding CuSO₄ and before H₂O₂.

The reactions were carried out at 37°C for 10 min and terminated by the addition of a stock solution of 0.5 M EDTA to a final concentration of 10 mM. 4 µl of a 1 mM EtBr solution was added and fluorescence intensity at 590 nm was measured with a Hitachi spectrofluorometer with excitation at 510 nm. The reduction in fluorescence was used as a measure of DNA damage.

Hydrogen peroxide decomposition assay

The H₂O₂ decomposing activity of YAE was assessed by Amplex Red, as described by Zhou and Panchuk (1997). For the fact that Amplex Red is a colorless and non-fluorescent derivative of resorufin, the substrate produces a highly fluorescent product in response to H₂O₂ upon the action of horseradish peroxidase (HRP); as such, the H₂O₂ decomposing activity of extracts can be determined. Briefly, different doses of YAE (0.1 to 100 mg/ml) were incubated with 1 µM H₂O₂ for 5 min at 37°C in 10 mM Tris-HCl buffer, pH 7.0, and then 40 µl 100 µM Amplex Red and 10 µl 100 mU/ml HRP were added to achieve final concentrations of 4 µM and 1 mU/ml, respectively.

Following for additional 5 min incubation at 37°C, the fluorescence intensity of Amplex red was measured using a Hitachi spectrofluorometer at an excitation wavelength of 563 nm and an emission wavelength of 584 nm. The direct quenching effect of YAE on the fluorescent product of Amplex Red was also determined by the addition of YAE after a 5 min incubation of H₂O₂, Amplex Red and HRP at 37°C.

UV/VIS spectral analysis

YAE dissolved in sterilized Milli-Q water was subjected to UV/VIS scanning using a spectrophotometer (JASCO V-530). The copper and iron chelating capacities of the YAE were measured with CuSO₄ and FeSO₄. YAE, 0.5 mg/ml, was either mixed with 50 µM CuSO₄ or FeSO₄, or left without, and incubated at 37°C for 10 min.

After incubation, the absorbance spectra from 200 to 700 nm were recorded and compared on a UV/VIS spectrophotometer. When spectral changes were detectable, 125 µM EDTA was added in order to test the reversibility of the chelation.

Assay of copper/iron chelating capacity

The copper- and iron-chelating capacities of Dioscorea extracts were measured in a hexamine buffer with tetramethyl murexide (TMM) (Shimada et al., 1992). TMM has a maximal absorption at 530 nm and changes to 485 nm in case of forming complex with free Fe (II) or Cu (II). To measure the chelating effect of Dioscorea extracts, 0.5 ml Dioscorea extract and 0.2 ml 1 mM TMM were added to a 0.5 ml mixture containing 30 mM hexamine, 30 mM potassium chloride, and 3 mM FeSO₄ or CuSO₄. After 3 min incubation at room temperature, the absorbance ratio at 485 vs. 530 nm of the mixture was determined on a Jasco UV-visible spectromotometer. The lower the absorbance ratio for 485 vs. 530 nm means the higher the chelating power.

Alkaline comet assay

The method of Singh et al. (1988) was adopted with some modifications, as described previously (Wang et al., 2001).

Thin layer chromatography

YAE and reference standards were applied to a TLC plate (Merck plastic sheets, 20 × 20 cm, Silica gel 60) in bands of 1 cm width. The plates were developed to about 9.0 cm. The solvent system was ethyl acetate: formic acid: water (65:15:20, v/v). Detection was done by evaluation under white (normal) light, or UV light, before or after staining with 10% H₂SO₄ in ethanol for general organic compounds, 1% FeCl₃ in 50% methanol for phenolic compounds, and several spray reagents for saponins, including Liberman-Buchard (5 ml concentrated H₂SO₄, 5 ml glacial acetic acid in 50 ml ethanol), anisaldehyde-sulphuric acid (5 ml anisaldehyde, 1 ml sulfuric acid, and 50 ml glacial acetic acid), antimony trichloride (CHCl₃-saturated antimony trichloride solution), and vanillin-sulfuric acid (15 g vanillin, 2.5 ml sulfuric acid, and 250 ml ethanol). Individual *R_f* for each spot visualized on the TLC plate was measured. A phenolic mixture, including caffeic acid, chlorogenic acid, and tannic acid and a saponin mixture from quillaja bark (saponin content > 25%, Sigma) were used as reference standards.

Statistical evaluation

Results are expressed as mean ± SE from two to three independent experiments. Comparisons were made using Student's *t* test. Differences were considered to be significant at *p* < 0.05.

RESULTS AND DISCUSSION

YAE at 0.5 mg/ml protected the damage to calf thymus DNA induced by H₂O₂-CuSO₄, but did not for that induced by H₂O₂-FeSO₄ (Figure 1A). YAE inhibited the H₂O₂-CuSO₄-induced DNA damage in a dose-dependent manner and the 50% inhibitory activity was estimated to be at a concentration of 0.22 mg/ml (Figure 1B). In addition, the protection of YAE against DNA damage was also noted in a cell model system. YAE alone did not induce DNA strand breaks (Figure 2). A 2 h incubation with 25 μM CuSO₄ or 300 μM FeSO₄ significantly increased DNA damage in human lymphoblastoid cells. A co-treatment of YAE, CuSO₄- or FeSO₄-induced DNA damage was suppressed by 70 and

40%, respectively. Results indicate YAE effectively inhibits copper ion-induced DNA damage in both test tube and cell model systems. However, the protection of YAE on ferric ion-induced DNA damage is only noted on human lymphoblastoid cells. Moreover, the protection of YAE was more profound in copper ion-induced DNA damage as compared to that of ferric ion. We further determined the chelating activity of YAE with copper and iron by UV-visible spectral analysis (Figure 3) and tetramethyl murexide assay (Figure 4). Results revealed that YAE forms metal complex with both Cu (II) and Fe (II) in a similar extent. These findings suggest that the different potent of YAE on suppressing copper and iron-induced DNA damage is not related to its chelating activity with two transient metals. Based on the complexes of metals and phytochemicals including numerous polyphenols differ in redox activity (Sugihara et al., 1999; Yamashita et al., 1999; Moran et al., 1997). It is possible that the redox activity of the complex of YAE-copper is different to that of YAE-iron and thus leads to discrepancy on DNA protection. This speculation can be supported, at least in part, from the finding that EDTA had the different effect on the YAE spectrum in the presence of copper or iron (Figure 3).

YAE exhibited a major absorption peak at 260 to 262 nm. With the co-incubation with 100 μM CuSO₄ and FeSO₄ an apparent modification of the YAE absorption spectrum was detected between 200 and 700 nm (Figure 3). We only displayed the absorption spectrum between 200 to 450 nm, because no significant change in absorption spectrum was observed in the region (450 to 700 nm). With co-treatment of CuSO₄, the peak absorption of YAE at 260 to 262 nm was shifted to 236 nm (Figure 4A), and the increase of absorption was positively related to the CuSO₄ concentration). In contrast, such a change on the spectrum of YAE was not detected by NiSO₄ and CoSO₄. In the presence of EDTA, it is interesting to note the shift of YAE spectra by CuSO₄ was completely inhibited on an equimolar basis (Figure 5). YAE also competitively inhibit the specific binding of tetramethyl murexide (a copper and iron ion indicator) to Cu (II) and Fe (II) (Figure 4B). These results may indicate that YAE contains a homogenous compound with a single copper-binding site and also be a good natural, safe (redox inactive) copper chelator.

Apart from a transition metal chelating activity, the protection of YAE on copper-mediated DNA damage may also result from its hydroxyl radical and hydrogen peroxide scavenging activities. To test this possibility, the direct scavenging of YAE on H₂O₂ was studied in a cell-free system based on the oxidation of Amplex Red to its fluorescent products in the presence of H₂O₂ and horseradish peroxidase. The extent of H₂O₂-initiated Amplex Red oxidation is, thus, negatively related to the amounts of H₂O₂ decomposing substances existed in YAE. As shown in Figure 6, YAE in a dose of 0.1 to 100 mg/ml decreased the fluorescence intensity of activated

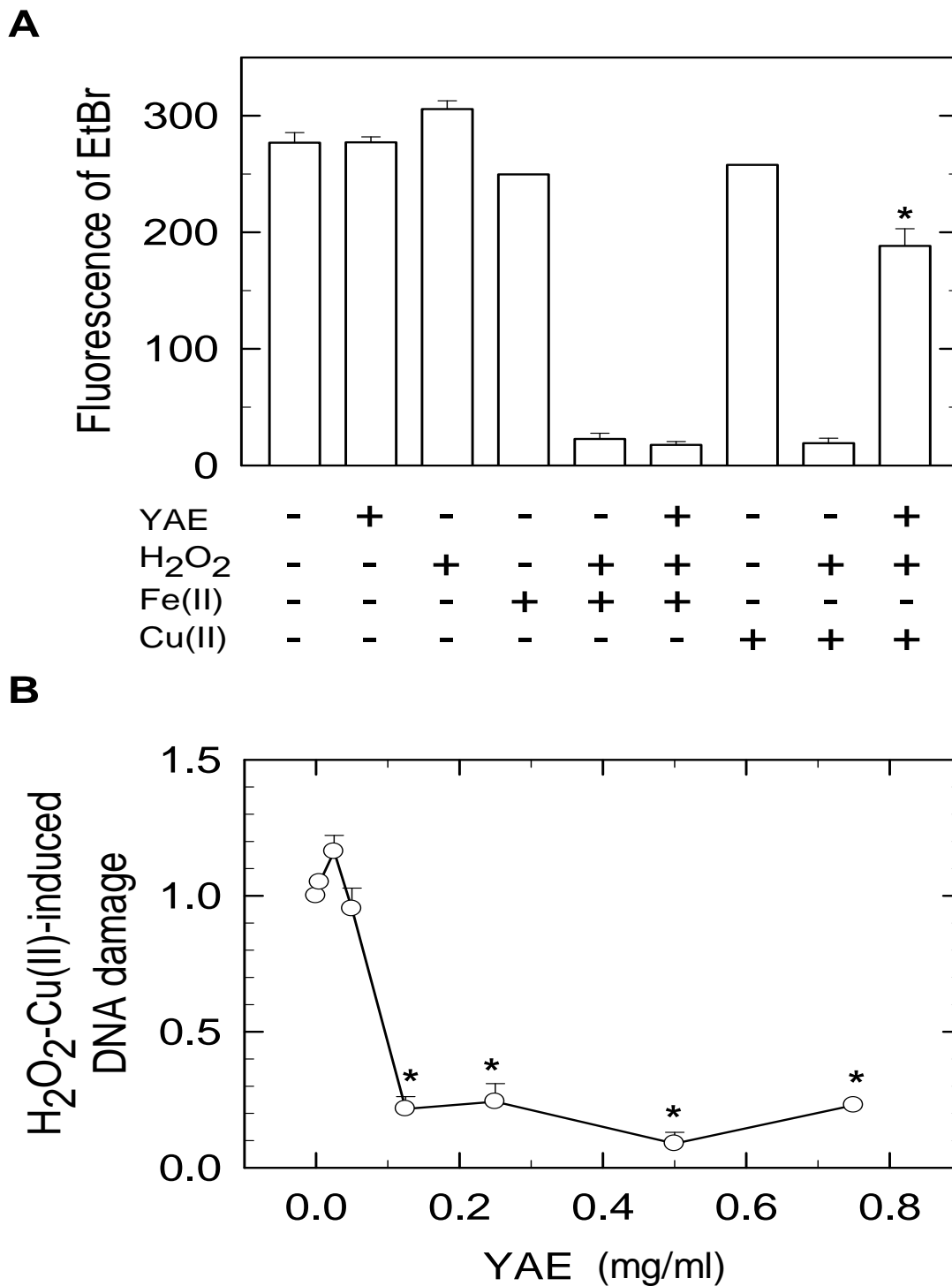


Figure 1. Effect of YAE on CuSO_4 - H_2O_2 - and FeSO_4 - H_2O_2 -induced DNA damage. (A) Calf thymus DNA was incubated with $50 \mu\text{M}$ CuSO_4 or 1 mM FeSO_4 in the presence of 25 mM H_2O_2 with or without YAE (0.5 mg/ml). The extent of DNA damage was evaluated by ethidium bromide fluorescence binding assay as shown in the study's materials and methods. (B) Dose response of YAE on H_2O_2 - CuSO_4 -induced DNA damage. A various amounts of YAE (0 to 0.75 mg/ml) was incubated with DNA, $50 \mu\text{M}$ CuSO_4 , and 25 mM H_2O_2 in 50 mM phosphate buffer, pH 7.2, for 10 min, and then the loss of ethidium bromide-induced DNA fluorescence was detected. In the absence of CuSO_4 , DNA-ethidium bromide gave a measurable maximum amount of fluorescence (control), which is defined as 100%. The degree of fluorescence loss is expressed as percentage of the control and indicates the extent of DNA damage. Values are means \pm SE of three independent experiments. The asterisk indicates a significant difference between the groups with and without YAE ($*P < 0.05$).

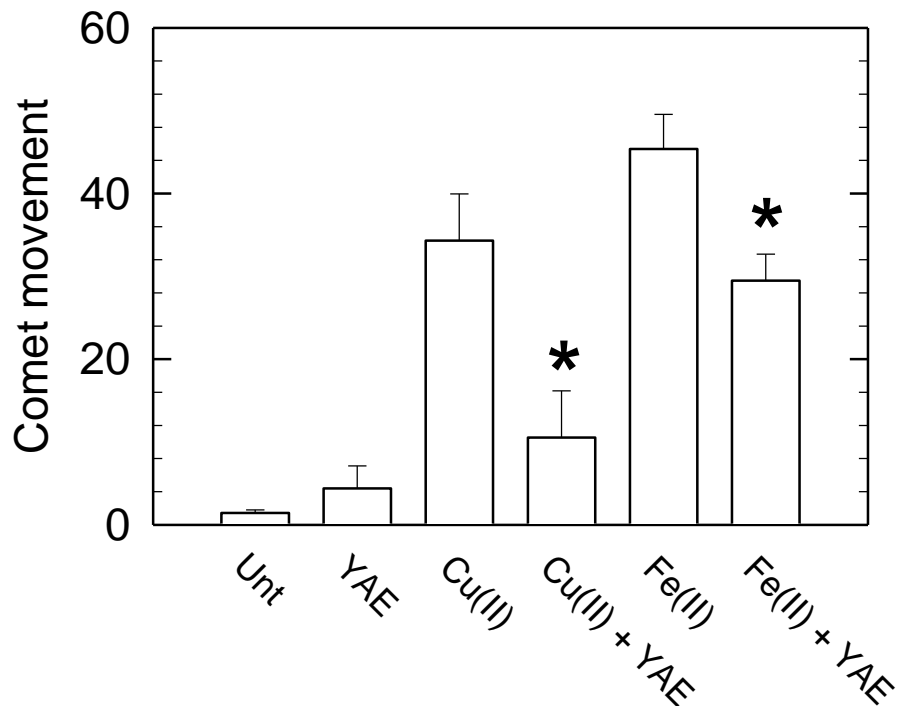


Figure 2. Protection of YAE on CuSO_4 - and FeSO_4 -induced DNA damage of human lymphoblastoid cells. Cells were co-treated with YAE (0.5 mg/ml) and $25 \mu\text{M}$ CuSO_4 or $300 \mu\text{M}$ FeSO_4 in HBSS buffer for 2 h. Cells were washed with phosphate buffered saline and harvested, and then the DNA damage was determined by comet assay. Values are means \pm SE of three independent experiments. *: $P < 0.05$ refers to the differences between metal-treated cells and those co-treated with YAE.

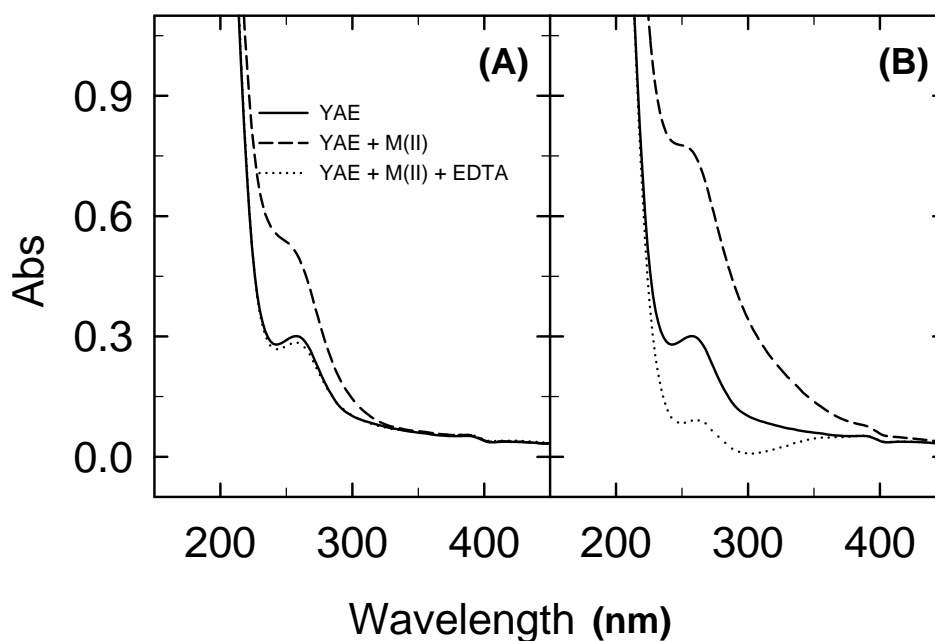


Figure 3. Absorption spectra of YAE: effect of copper (A) and iron (B). The spectra of 0.5 mg/ml YAE alone (—), YAE plus $100 \mu\text{M}$ metal ions (---), and YAE + $100 \mu\text{M}$ metal ions + $100 \mu\text{M}$ EDTA (...) with water were recorded, respectively, at room temperature. One representative picture of three independent experiments is shown.

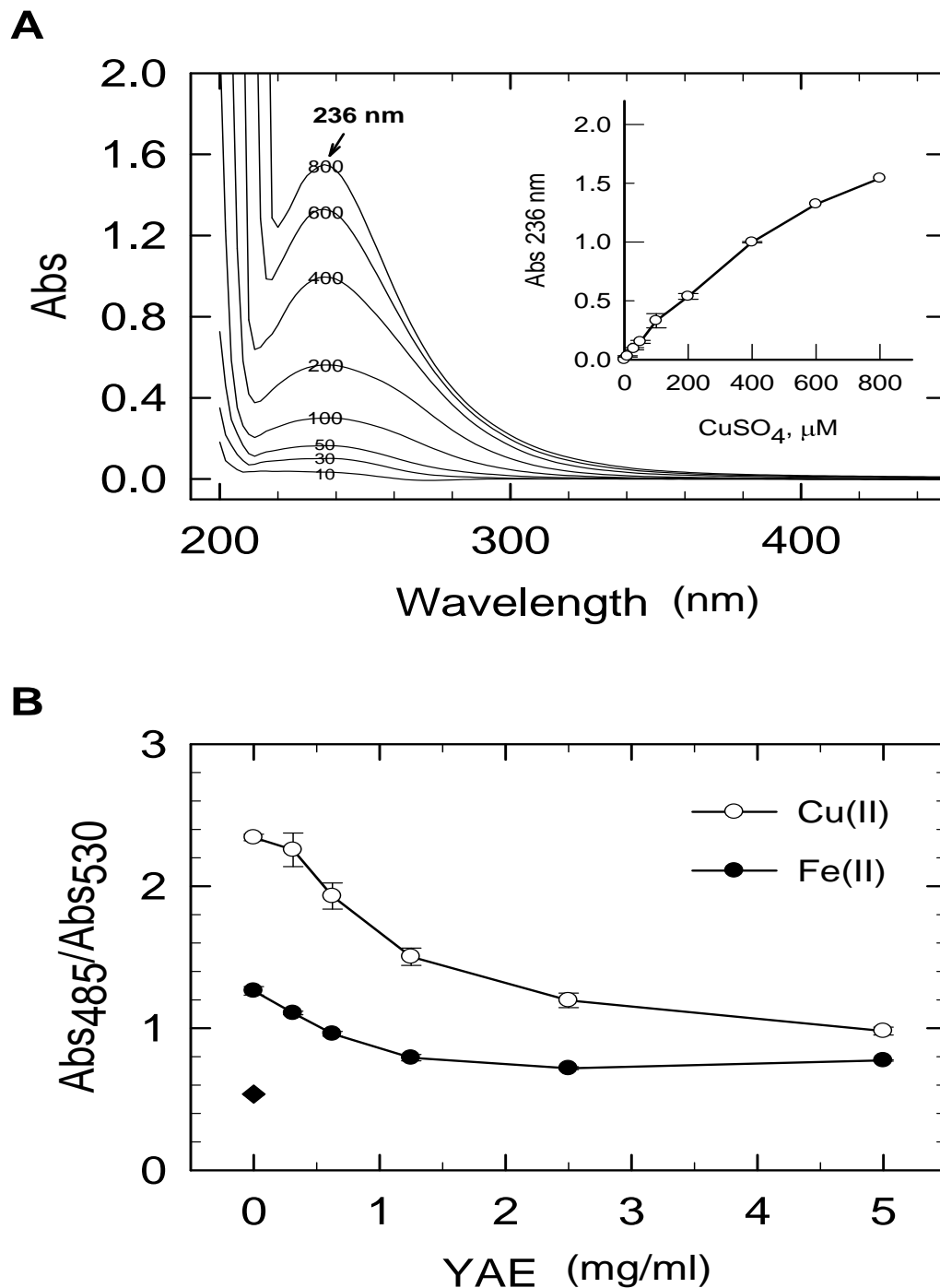


Figure 4. (A). Changes of the spectrum of YAE co-incubation with various concentrations of CuSO_4 . The spectrum of the YAE-Cu complex was monitored immediately following the mixing 0.5 mg/ml YAE with 10-800 μM CuSO_4 using the 0.5 mg/ml YAE as a reference sample. The spectra shown are representative of three independent determinations. The inset shows the interdependence of the YAE-copper complex at 236 nm vs. the concentration of CuSO_4 . (B). Chelating effect of YAE in the presence of CuSO_4 and FeSO_4 . YAE at a level of 0.3125 - 0.5 mg/ml was mixed with 3 mM CuSO_4 (-o-) or FeSO_4 (-•-) and the ratio of absorbance at 485 nm to that at 530 nm was determined by spectrometric assay. Once the sample absorbance ratio at 485 nm vs. 530 nm is lower than that of the control, which does not contain metal ion-binding substance, it is regarded as owning the transition metal-binding ability.) The absorbance ratio of the control was 2.34 ± 0.02 for CuSO_4 and was 1.26 ± 0.03 for FeSO_4 . ♦. the ratio of absorbance at 485 to that at 530 nm of the blank (no metal addition). Values are means \pm SE of three independent experiments and one representative UV/vis absorption spectra of the YAE-Cu complex is shown in panel (A).

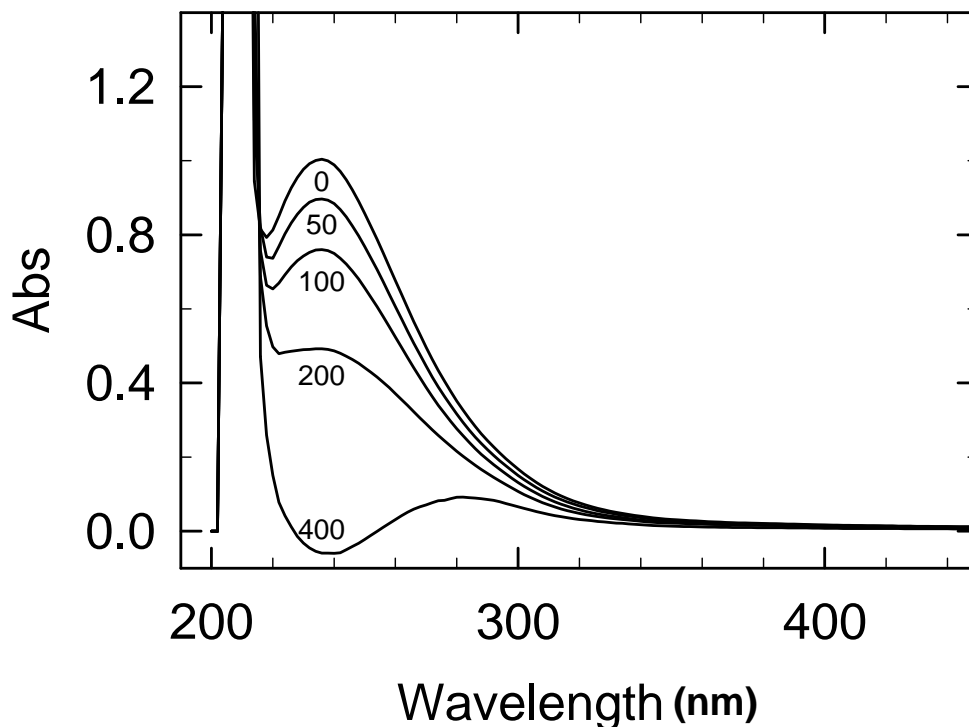


Figure 5. Effect of EDTA concentration on Cu(II)-YAE complex dissociation. YAE (0.5 mg/ml) was pre-incubated with 400 μ M CuSO₄ for 5 min, and then a various levels of EDTA (0-400 μ M) was added. The changes of spectra were recorded immediately at room temperature. One representative UV/Vis absorption spectra of three independent experiments is shown.

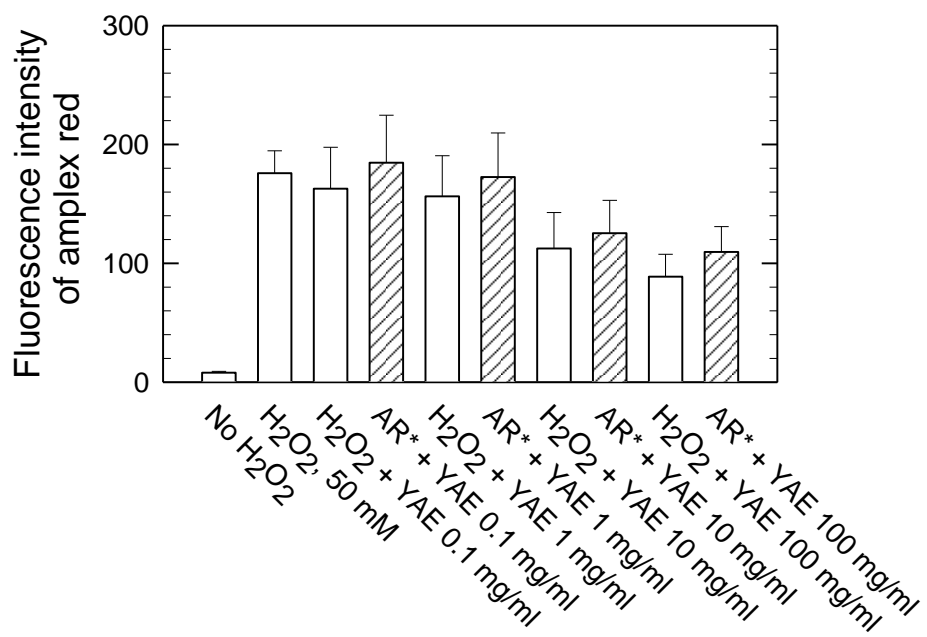


Figure 6. H₂O₂-decomposing activity of YAE. A different dose of YAE was incubated without or with 1 μ M H₂O₂ in 10 mM Tris, pH 7.0, at 37°C for 5 min. After incubation, Amplex Red and horseradish peroxidase were added to make a final volume of 1 ml consisting of 4 μ M Amplex red and 1 mU/ml horseradish peroxidase, and the mixture was incubated at 37°C for an additional 5 min. The change in fluorescence intensity of Amplex Red was determined by spectrofluorimetric analysis as described in Materials and Methods. Values are means \pm SE of three independent experiments.

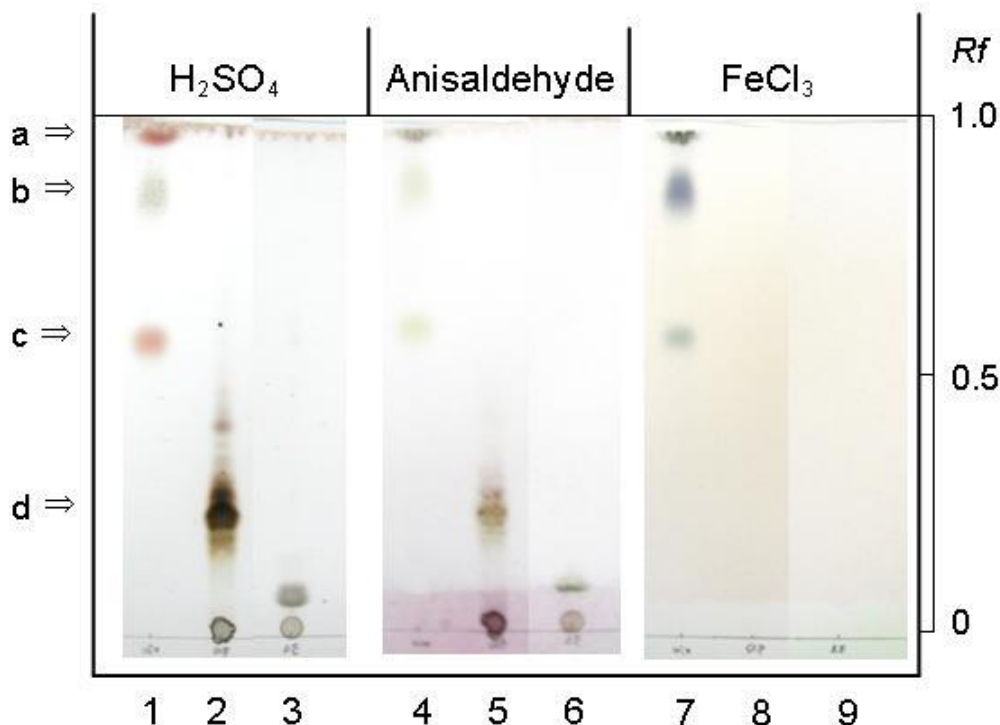


Figure 7. The TLC chromatograms of YAE. YAE and reference standards of phenolic compounds and quillaja saponin were analyzed by TLC, and the separated spots were visualized by spraying with H_2SO_4 (lane 1, 2, and 3), anisaldehyde (lane 4, 5, and 6), and FeCl_3 , (lane 7, 8, and 9) as described in Materials and Methods. The TLC chromatograms were photographed by a digital camera (Nikon Coolpix 990) under white light. Lane 1, 4, and 7: phenolic standard compounds (a. caffeic acid, b. tannic acid, c. chlorogenic acid); lane 2, 5, and 8: saponin standard compound (d. quillaja saponins); lane 3, 6, 9: YAE. One representative picture of two independent experiments is shown.

Amplex Red in a dose-dependent manner. When H_2O_2 was removed from this system, a similar change of the fluorescence intensity by YAE was also noted. This suggests the inhibition of YAE on copper-mediated DNA damage is unlikely related to its H_2O_2 -decomposing activity.

The numerous biological activities of *Dioscorea* species have been attributed to their rich in phytochemicals including phenolics, saponins, polysaccharides, and mucilages. Based on the aromatic characteristic, phenolic compounds show intense absorption in the UV region of the spectrum. For instance, most of the benzoic acid derivatives display their maxima at 246 to 262 nm (Harbone, 1989; Torres et al., 1987). The maximum UV/visible absorption ranged in the 200 to 300 nm as noted in Figure 4 indicate that the presence of polyphenolics in the YAE is likely. However, a low level of polyphenols, 5.0 mg tannic acid equivalents (TAE)/g-lyophilized powder was determined by Folin-Ciocalteu reagent in YAE. At the same time, no detectable polyphenolics were detected in YAE by TLC (Figure 7, lanes 9 vs. 7). Moreover, polyvinylpyrrolidone, a polyphenolics remover (or chelator), was found to be lack of effect on YAE suppressed copper- H_2O_2 -induced DNA

damage. These findings suggest the role of polyphenolics in YAE protection on DNA damage is minor or negligible. In addition to polyphenolics, other possible active components were further detected by spraying TLC plates with anisaldehyde or sulphuric acid which are generally used as visualized reagents for sugars, steroids, and terpenes. As noted, a major spot with R_f value of 0.10 under visible light was detected in YAE (Figure 7, lanes 3 and 6). We, thus, proposed the active candidates of YAE are anisaldehyde-, sulphuric acid-reactive and highly polar substances. Recently, crude and purified plant derived saponins have been demonstrated to be potent on chelating copper/iron ions in aqueous media (Chen et al., 2008; Gulcin et al., 2006; Yoshiki et al., 2005). Furthermore, the *Dioscorea* saponin extracts have been successfully applied in removing heavy metals including copper from solid waste (Hong et al., 2000). Besides saponins, *Dioscorea* species are rich in polysaccharide mucilage (Wanasundera and Ravindran, 1994; Tsai and Tai, 1984). Similar to saponins, mucilage polysaccharides from bacteria and algae are good biosorption materials for removal toxic heavy metals, including copper ions (Lombardi et al., 2005; Iyer et al., 2005; Ozdemir et al., 2003). These

YAE-HS, %	-	-	-	-	-	0.3125	0.625	1.25
YAE, %	-	0	0.3125	0.625	1.25	-	-	-
Cu-H₂O₂	-	+	+	+	+	+	+	+

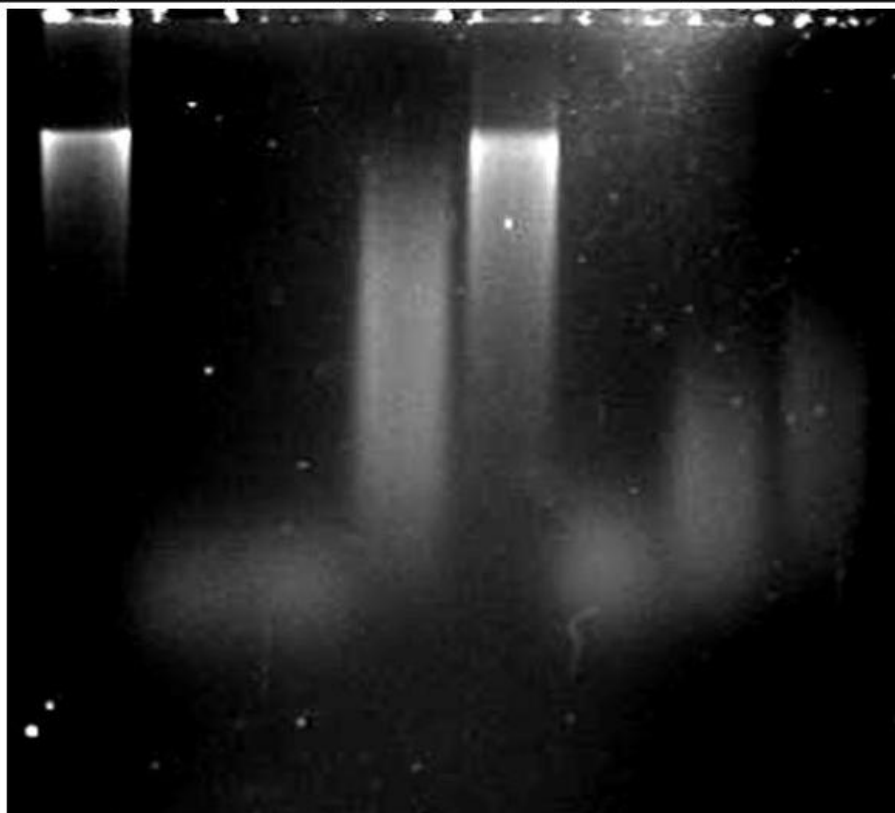


Figure 8. Protection effects of yam extracts contained high- and low-saponin contents on copper-catalyzed DNA damage. Calf thymus DNA was incubated with CuSO₄ (0.05 mM) and H₂O₂ (3 mM) for 2 hours in the absence or presence of aqueous extracts derived from two different *Dioscorea* species with low (*D. alata*, YAE) and high (*D. japonica*, YAE-HS) saponin contents. After incubating, electrophoretic migration profile of DNA was assayed using agarose gel electrophoresis as described in our previous paper (Wang et al., 2007). One representative picture of three independent experiments is shown.

findings suggest saponins and water-soluble mucilaginous polysaccharides, but not polyphenols, are the most likely active components of YAE on ameliorating transient metal initiated DNA oxidative damage. To assess further the role of saponin and mucilage molecules in YAE on copper binding, we compared the protective effects of aqueous extract from species of *Dioscorea japonica* contained high saponin content with YAE on copper-catalyzed DNA damage *in vitro*. The result showed that although the total saponin contents in the aqueous extract varied by 6.9-fold from the *D. japonica* (48.9 mg beta-escin equivalent/g dry powder) to *Dioscorea alata* (7.1 mg beta-escin equivalent/g dry powder), high-saponin content extract from *D. japonica* did not provide better protection for copper/H₂O₂-induced

DNA damage in calf thymus DNA *in vitro* (Figure 8). This outcome further indicates that mucilage polysaccharides are probably the most important copper chelator in YAE. Although the structure of mucilage from *D. alata* is not been elucidate yet, yam tuber contained mucilages reported to be a water-soluble mannan-protein complex (Tsai and Tai, 1984). Based on the example of heavy metal binding polymers from yeast cell wall, both protein and polysaccharide component of mucilage may play a crucial role in the chelation of copper (II) ion. Further, an understanding of the structure, composition and properties of copper-chelating of isolated mucilages from *D. alata* might aid in development of new biosorbents materials for copper removal, or functional food for health from mucilages of *D. alata*.

Conclusion

This is the first time that a hypothesis involving in the relationship between water-soluble mucilage, copper chelating ability, and inhibition of copper catalyst Fenton reaction-induced DNA damage in aqueous extracts (YAE) of yam (*D. alata*) has been studied. Furthermore, in the transgenic model animal studies, several reports have clearly demonstrated that a copper chelator supplement could; (1) markedly inhibit beta-amyloid accumulation in Alzheimer's disease (Cherny et al., 2001), (2) delay onset of disease and extend survival of familial amyotrophic lateral sclerosis (Hottinger et al., 1997) and (3) act as therapy in Wilson and Menkes' diseases (Sugawara et al., 1999; Tanaka et al., 1990). In conclusion, this study also opens a new view on the potential efficacy of the *Dioscorea alata* L. aqueous extracts (YAE) in the management of copper-mediated oxidative disorders, such as Willson/Menkes diseases, neurodegenerative diseases (Alzheimer's and Parkinson's) and diabetes.

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