

Evaluation on antioxidative capacity, nephroprotective effect, and DNA damage protection of mixed low potassium vegetables and fruits juice powder in HEK-293 cells

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ABSTRACT

Background: An imbalance between free radical production and antioxidant defenses is one of the multifactorial natures contributing to many disorders. One of them is chronic kidney disease (CKD). The development of novel diets to slow progression or reduces complication of CKD are highly needed. Therefore, the objective of this study was to evaluate the *in vitro* antioxidative capacity and nephroprotective effect of a product, mixed low potassium vegetables and fruits juice powder (MJP) in HEK-293 cells.

Methods: The produced MJP was determined for phenolics and flavonoids content. The antioxidative capacity was examined by DPPH, FRAP and ABTS assay. The cell viability to MJP and hydrogen peroxide (H₂O₂)-treated were determined by MTT assay. The intracellular antioxidative capacity was determined by co-treatment of MJP and H₂O₂-induced HEK-293 cell damage including reactive oxygen species (ROS) production, intracellular enzymes activities, and DNA damage protection.

Results: The MJP at the concentration of 5 and 10 mg/ml showed positive effects on H₂O₂-induced HEK-293 cells protection and significantly increased cell viability. The ROS production measured by 2',7'-dichlorodihydrofluorescein diacetate (DCF) were increased in the H₂O₂ treatment approximately 2 times but significantly decreased in the MJP treatment. The result of intracellular enzymatic antioxidant markers, including superoxide dismutase (SOD) and catalase (CAT) activities were increased while the Malonaldehyde (MDA) level

was attenuated in the co-treatment of MJP and H₂O₂-induced HEK293 cells compared with only H₂O₂-inducing. In the examination of the morphological change due to oxidative stress exhibited less nuclei fragment in the cell of MJP treatment. In addition, the isolated DNA was protected by MJP application.

Conclusion: The produced MJP contains bioactive phenolic and flavonoid compounds. MJP possesses potential antioxidative capacity through the reducing H₂O₂-induced HEK-293 cells damage and increasing the intracellular antioxidant enzymes. In addition, the nephroprotective effects of MJP related to the antioxidant enzymes was involved in the free radical scavenging. Therefore, MJP could be a functional drink for individual limiting potassium and liquid uptake.

Keywords: Antioxidative capacity, DNA damage protection, low potassium vegetables and fruits, nephroprotective, chronic kidney disease

INTRODUCTION

Oxidative stress is defined as an imbalance between endogenous antioxidant defense mechanisms and the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. The high levels of imbalance can cause cell injury and damage through modifications of proteins, lipids, and DNA. Increased oxidative stress is involved in the pathophysiology of many diseases such as cardiovascular diseases (CVDs), neurodegenerative diseases (NDs), macular degeneration (MD), biliary diseases, cancer, chronic kidney disease (CKD) [2]. The cellular antioxidant enzymes play the important role of protecting the cells and organisms from the oxidative damage. For example, superoxide dismutase (SOD) acts as a catalyst to convert superoxide radicals (O₂^{•-}) into oxygen and hydrogen peroxide. Catalase convert hydrogen peroxide (H₂O₂) into water and oxygen to protect the cells from accumulation of H₂O₂ [3]. In case of CKD patients, increased production of ROS, impaired nonenzymatic or enzymatic antioxidant defense mechanisms, and other risk factors including gene polymorphisms, uremic toxins (indoxyl sulfate), deficiency of arylesterase/paraoxonase, hyperhomocysteinemia, dialysis-associated membrane bioincompatibility, and endotoxin can inhibit normal cell function by damaging cell lipids, arachidonic acid derivatives, carbohydrates, proteins, amino acids, and nucleic acids [2,4,5].

Epidemiological studies have found that consumption of vegetables and fruits has a significant role in reducing the risk of cardiovascular disease and other chronic diseases [6]. Current evidence and studies suggests that antioxidant therapy in pre-dialysis chronic kidney patients may prevent progression to end-stage kidney disease (ESKD) [7, 8, 9]. Consumption of antioxidants through diet and supplements is expected to remove ROS from the body system and provide health benefits. Several studies demonstrated that medicinal plants and fruits are a rich source of antioxidant compounds such as phenolics, flavonoids, vitamins, and alkaloids, which decrease the incidence of oxidative stress and associated diseases [10,11]. Previous studies showed that the phytochemicals, especially phenolics, in vegetables and fruits are the major bioactive compounds exhibited potent antioxidant effects [12, 13, 14] and total phenolic contents affects to the antioxidant capacity in vegetables and fruits [15,16].

Chronic kidney disease is a global health problem (11-13%; 200 million) [17]. In Thailand, the prevalence of chronic kidney disease and kidney failure were 17.6% (7.6 million) and 0.9% (70,000) [18]. Patients with kidney failure are subjected to strict restriction on the uptake of potassium and water quantity, , they cannot freely consume fruits, vegetables, and water in the large amounts [19]. Taken together, the development of nutritive dried powder food product

for specific or limit a nutrient potassium and water for CKD patients is necessary. Therefore, the objectives of this study were to evaluate the antioxidative capacity of the product (mixed low potassium vegetables and fruits juice powder (MJP)) and its *in vitro* molecular mechanisms on H₂O₂ induced oxidative stress in HEK-293 cells.

MATERIALS AND METHODS

Preparation of MJP

Vegetables including mungbean sprout (*Vigna radiata* (L.) R. Wilczek), napa cabbage (*Brassica rapa subsp. chinensis* (L.)) and winter melon (*Benincasa hispida* (Thunb.) Cogn.), and fruits including apple (*Malus domestica* Borkh. 'Red Fuji'), watermelon (*Citrullus lanatus* (Thunb.) Matsum. 'Kinaree'), and pineapple (*Ananas comosus* (L.) Merr. 'Batavia / Si Racha') which were purchased from local market in Hatyai, Songkla, Thailand. Juices were extracted by HUROM-H22 extractor and mixed all together in an appreciate amount. The mixed juice powder was made by a spray dryer and kept at -20°C for further analysis. All assays will be performed in at least three individual experiments.

Determination of polyphenol content

Total Phenolics content was determined [20]. Briefly, 12.5 µL of the juice sample (mg MJP powder/mL water) was mixed with 50 µL of distilled water and then mixed with 12.5 µL Folin-Ciocalteu reagent and allowed to stand for 5 min at room temperature. Then, 125 µL of 7% Na₂CO₃ and 100 µL of distilled water were added to the reaction mixture, followed by incubation at room temperature for 30 minutes. The absorbance was detected by a microplate reader at 760 nm and converted to total phenolic (µg gallic acid equivalent, (GAE)/g dry weight).

Total Flavonoids content was determined [21]. Briefly, a 150 µL of juice sample was added to 20 µL of a 5% NaNO₂ solution. The mixture was left to stand at room temperature for 6 minutes, then 20 µL of a 10% AlCl₃ was added and incubated at room temperature for 5 minutes, followed by adding 100 µL of 1 M NaOH. After incubation at room temperature for 30 minutes, the absorbance was measured at 510 nm and the total flavonoids content was expressed as µg quercetin equivalent (QE)/g dry weight.

Determination of antioxidant properties

DPPH assay [22] is based on the scavenging activity of the stable DPPH free radical. Briefly, 20 µL of juice sample was added into 180 µL of 200 µmole/L DPPH (Sigma-Aldrich, St. Louis, MO, USA) dissolved in methanol. The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using the microplate reader with 96-well plates. Trolox calibration solutions was used to generate the standard curve. The radical scavenging activity was expressed as µmole Trolox equivalents (TE)/g dry weight.

FRAP assay [23] was determined with some modifications. A 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (Sigma-Aldrich, St. Louis, MO, USA) in 40 mM HCl, and 20 mM FeCl₃•6H₂O were prepared for stock solutions. The FRAP reagent was prepared by mixing the stock solutions in a ratio of 10:1:1 and incubated in water bath at 37°C. Then, 20 µL of the juice sample was added into 180 µL of FRAP reagent and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 593 nm using the microplate reader with 96-well plates. Trolox was used as standard curve and the results was expressed as µmole Trolox equivalents (TE)/g dry weight.

ABTS assay was followed the previous study [24]. The stock solutions were prepared, 4 mM ABTS solution (Sigma-Aldrich, St. Louis, MO, USA) and 2.5 mM potassium persulfate ($K_2S_2O_8$) solution. The working ABTS solution was then prepared by mixing the two stock solutions in a ratio of 1:1 and allowed to stand in the dark at room temperature for 16 hours. The solution was diluted by mixing a 1 ml of working ABTS solution with 9 ml of distilled water. The juice samples (20 μ L) were mixed with 180 μ L of the diluted ABTS solution and the absorbance was measured at 734 nm after 30 minutes of incubation in the dark at room temperature using the microplate reader with 96-well plates. Trolox was used for standard curve and results was expressed as μ mole Trolox equivalents (TE)/g dry weight.

Cell culture

Human embryonic kidney-293 (HEK-293) cells were cultured using DMEM supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamax and 1% (v/v) penicillin streptomycin solution in a humidified atmosphere of 5% CO_2 at 37°C. All cell culture reagents were purchased from Gibthai Co.,Ltd.

Determination of cytotoxicity of MJP

Cytotoxicity of MJP was determined by the MTT assay according to the method previously [25] with some modifications. Cells were seeded in 96-well culture plates at a density of 5×10^4 cells/well (n=3). Cells were left to adhere on the plate for 24 hours before treated with a different concentration of MJP (0.1–100 mg/ml) for another 24 hours. The viable cells were measured after 24 hours of incubation, by adding 5 mg/mL of MTT solution (Sigma-Aldrich, St. Louis, MO, USA) and further incubating the cells for 2 hrs. The occurred formazan crystals were dissolved with DMSO. The absorbance was measured using microplate reader at a wavelength of 570 nm and 650 nm (reference). The percentage of cell viability was calculated according to the following equation (1):

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100 \quad (1)$$

Examination of toxicity of H_2O_2 in HEK-293 cells

The 5×10^4 cells were seeded in a 96-well culture plate and were allowed to grow for 24 hours, then treated with difference concentrations of H_2O_2 in range from 0.5 to 4 mmole/L for 24 hours. The cells without H_2O_2 treated were the control. The viable cells were measured by adding 5 mg/mL of MTT solution and incubated for 2 hours. The occurred formazan crystals were dissolved with DMSO. The absorbance was measured using microplate reader at wavelength of 570 nm and 650 nm (reference). The percentage of cell viability was determined as equation 1 and IC_{50} of H_2O_2 was used to induce toxicity to the cells for further study.

Determination of neproprotective effect of MJP

Neproprotective effect of MJP was evaluated by the MTT assay [26,27]. Approximately 5×10^4 cells (HEK-293) were seeded in 96 well culture plate in DMEM medium with 10% FBS, 1% L-glutamax and 1% penicillin streptomycin in a 5% CO_2 incubator. After 24 hours of incubation, the cells were exposed to MJP in growth medium to a final concentration of 0.1, 0.5, 1, 5, and 10 mg/mL MJP and 100 μ L of 1.57 mmole/L H_2O_2 , then incubated at 37°C for

24 hours. After incubation, 100 μ L of the medium was removed and 10 μ l of MTT (5 mg/mL) was added in and incubated for 2 hours. Formazan crystals formed and dissolved using DMSO, then the absorbance was measured at 570 nm and 650 nm (reference).

Examination of cell morphology

The Hoechst 33342 was used to examine the cellular and nuclear morphology of cells modified from the previous study [28]. After the co-treatment cells with 0.1, 0.5, 1, 5, and 10 mg/ml MJP and H₂O₂ for 24 hours., the 100 μ l of the medium were removed and cells were stained with 10 μ l of 20 μ g/mL Hoechst 33342, which changed the morphology of what was observed under an IX-71 Olympus fluorescence microscope.

Determination of ROS production

The determination of ROS production on hydrogen peroxide (H₂O₂)-stressed HEK-293 cells was modified previous study [29, 30]. The 5×10^4 HEK-293 cells were seeded in a clear-bottom and black-walled of 96-well plates and allowed the cells attach to the plate for 24 hours. Then, the cells were exposed to final concentration of 0.1, 0.5, 1, 5 and 10 mg/mL MJP and 100 μ L of 1.57 mmole/L H₂O₂ as a co-treatment. After incubation at 37°C for 30 min, 10 μ l of 100 μ M carboxy-2',7'-dichloro-dihydro-fluorescein diacetate (DCFH-DA) (Sigma-Aldrich, St. Louis, MO, USA) probe was added and incubated for another 30 min. At the end of this period, the fluorescence was measured at wavelengths of 485 nm (excitation) and 530 nm (emission) with the fluorescence microplate reader (Varioskan™LUX multimode microplate reader).

Determination of enzymatic antioxidant activity and Lipid oxidation product

HEK-293 cells were cultured in 10 cm culture dish with confluence 80-90 % then the cells were co-treated with final concentration of 0.1, 0.5, 1, 5, 10 mg/mL MJP and 100 μ L of 1.57 mmole/L H₂O₂ for 24 hours. The activities of superoxide dismutase (SOD), catalase (CAT), and the malondialdehyde (MDA) content were measured using assay kits (Bioassayassay kit, USA). All procedures were completely compliant with the manufacturer's instructions.

Examination of DNA damage protection

Fenton's reagent is a solution of hydrogen peroxide and a ferrous iron catalyst used for DNA damage protection assay which was modified method [31]. The sample DNA will be destroyed by Fenton's reagent. Genomic DNA was isolated from HEK-293 cells. A 0.2 μ g of genomic DNA was mixed with 3 μ L of 50 mM phosphate buffer (pH 7.4) and 3 μ L of 40 mM FeSO₄. Then, 5 μ L of 0.1, 0.5, 1, 5 and 10 mg/mL MJP were added and followed by 4 μ L of 1 mM H₂O₂, the mixture was incubated at 37°C for 30 minutes. After that, 5 μ L of 1 mmole/L gallic acid was added immediately to stop the reaction. Finally, the DNA was visualized on 1% agarose gel electrophoresis. The density or intensity of genomic DNA band in each treatment were calculated by CCD, chemiluminescent and infrared fluorescent (Fusion Fx5XT Spectra/Superbright).

Statistical analysis

All data were presented as the mean \pm standard deviation (SD) of three replicate experiments. Analysis of the variance was performed on the data obtained (ANOVA). The data determined significance of difference among mean by Tukey's test.

RESULTS AND DISCUSSION

Mixed low potassium vegetables and fruits juice powder (MJP)

MJP was produced by mixed three vegetables and three fruits including of napa cabbage, mung bean sprout, winter melon, apple, watermelon, and pineapple. These are low/moderate potassium (potassium content < 200 mg / 100 g) plants [35]. The three vegetables and fruits types were weighted in suitable proportions. The extracted juice was obtained and mixed with a minimum amount of carrying agent for spray drying to be powder [32]. The result showed that the yield of MJP powder was 56.17 ± 2.57 %. Typically, the product yield was defined as the ratio of the mass of powder collected after spray drying to the weight of total solids in the feed. The product yield was generally increased as the carrier content increased which was reported in previous studies, tamarind juice [33], and sugarcane juice [34]. However, increasing carrying agents should be considered based on various features of product such as particles characteristic, moisture content, hygroscopicity, solubility, and biological properties.

Total phenolic and flavonoid contents of MJP

The mixed low potassium vegetables and fruits juice powder was expressed for the total phenolic content as μg of gallic acid equivalent (GAE) per gram dry weight (dw) of powder exhibited a total phenolic contents of 906.94 ± 8.97 μg GAE/g dw while the total flavonoid content was expressed as μg of quercetin equivalent (QE) per gram dry weight of powder. The result exhibited total flavonoid content of 132.05 ± 5.23 μg QE /g dw as shown in Figure 1a. Phenolic compound is mainly a bioactive compound in vegetables and fruits which functions to prevent oxidative stress, induce detoxification enzymes, stimulate immune system, decrease the risk of cancers, inhibit malignant transformation and carcinogenic mutations, as well as, reduce proliferation of cancer cells [36- 42].

Antioxidative capacity of MJP

Antioxidative capacity of mixed low potassium vegetables and fruits juice powder determined by DPPH, FRAP and ABTS assay were presented in Figure 1b. These assays were expressed as μmole of trolox equivalent (TE) per gram of dry weight powder. The MJP showed strongest reducing power with antioxidative capacity of 5.16 ± 0.22 $\mu\text{moleTE/g}$ dw by FRAP assay followed by ABTS method showed antioxidant capacity of 5.00 ± 0.02 $\mu\text{moleTE/g}$ dw and DPPH scavenging activity of MJP exhibited 3.58 ± 0.02 $\mu\text{moleTE/g}$ dw. It is known that vegetables and fruits are part of an overall healthy diet. The recommendation is a minimum of 400g of fruit and vegetables per day to prevent chronic diseases due to free radical damage, as well as, alleviation of several micronutrient deficiencies [43]. The USDA recommended an ORAC unit ingestion of about 3000 to 5000 units daily to adequately saturate tissues with antioxidants [44]. Concurrently, this result has shown that the total phenolic and flavonoid quantity existed in MJP were involved in antioxidant activities evaluated using DPPH, FRAP and ABTS methods. The three antioxidant assays exhibited scavenging by electron and hydrogen atom radical, reducing power of Fe^{2+} to Fe^{3+} and hydrogen atom radical, respectively [45]. Moreover, the antioxidative potential of MJP in molecular mechanism was performed as co-treatment with H_2O_2 -induced HEK-293 cell damage and MJP which was reported in different parameters including ROS production, intracellular enzymes activities, and DNA damage protection.

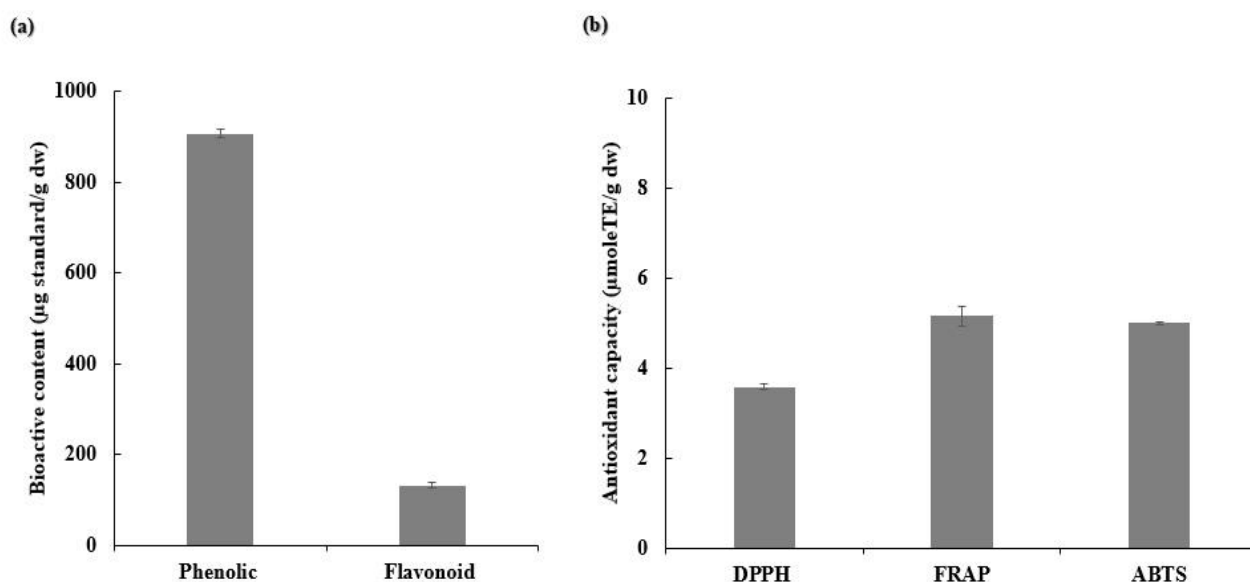


Figure 1. Total phenolic and flavonoid contents in MJP which were expressed as GAE/g dw and QE/g dw, respectively (a) and Antioxidant capacity of mixed low potassium vegetables and fruits juice powder (b). Data are shown as means \pm SD from three individual experiments.

Effect of MJP on cell viability

The effect of mixed low potassium vegetables and fruits juice powder on the viability of HEK-293 cells was shown in Figure 2a. The results showed that concentration of MJP from 0.1 to 10 mg/mL did not significant ($p < 0.05$) affected on cells viability when compared with control that untreated cells. However, at the concentration more than 10 mg/mL of MJP cell viability was less than 80%. This due to MJP has pH 3.78 that which effected to cell survival when the concentration more than 10 mg/mL of MJP. Therefore the 10 mg/mL of MJP was selected for further study.

Effect of MJP on H₂O₂- induced toxicity in HEK-293 cells

The toxicity of hydrogen peroxide (H₂O₂) on HEK-293 cell viability was shown in Figure 2b. The viability of HEK-293 cells decreased significantly ($p < 0.05$) with the increasing concentration of H₂O₂ ranged from 0.5 to 4 mmole/L. The results showed that the half maximal inhibitory concentration (IC₅₀) of H₂O₂ was indicated as 1.57 mmole/L ($R^2 = 0.9972$). Previously reported [25], H₂O₂ concentration 2 mmole/L was affected to 50 % cell viability of HEK-293 cells which was close to our experiment of H₂O₂ concentration. Oxidative stress has been proposed as “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage” [46]. Hydrogen peroxide (H₂O₂) is nonradical reactive oxygen species and (ROS) that changes to highly toxic hydroxyl free radicals (OH \cdot) [3]. The human embryonic kidney (HEK-293) cells were used in the experiments as a demonstrated cell model to determine the antioxidative capacity effects of MJP against H₂O₂-induced toxicity and cells damage. Later, the evaluation of antioxidative capacity in HEK-293 cells was performed by a half maximal inhibitory concentration (IC₅₀) of H₂O₂-induced cells and co-treatment with different concentration of

MJP to determine the nephroprotective effect, cellular morphological changes, intracellular ROS production, enzymatic antioxidant activity (SOD and CAT), MDA and DNA damage protection.

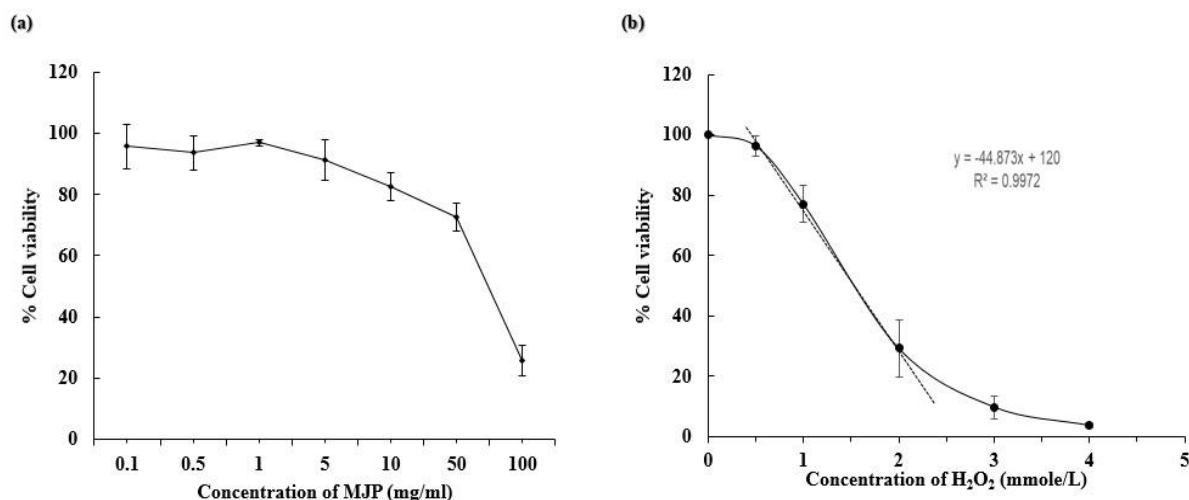


Figure 2. Cytotoxicity of mixed low potassium vegetables and fruits Juice Powder (a) and H₂O₂ (b) in HEK-293 cells. Each point represents a mean \pm SD of three experiments.

Nephroprotective effect of MJP against H₂O₂- induced toxicity

The main purpose of this study is to evaluate the prevention property of MJP against H₂O₂ induce toxicity in HEK-293 cells. The result was shown in Figure 3a, the HEK-293 cells were exposed to MJP ranged of 0.1, 0.5, 1, 5 and 10 mg/mL with or without 1.57 mmole/L of H₂O₂ for 24 hrs. The result showed that cells exposed to only 1.57 mmole/L H₂O₂ showed 54.85% cell death, while the cells that were treated by H₂O₂ and MJP at concentration of 0.1 to 1 did not significantly decreased for 55.11 to 55.94% of cell death. However, the MJP 5 and 10 mg/mL significantly ($p < 0.01$) reduced cell death of 45.09 % and 40.63 %, respectively. The result revealed that MJP at 5 and 10 mg/mL significantly ($p < 0.01$) decreased apoptotic cell death compared with only H₂O₂ treatment. Similar to the present study of jaboticaba fruit peel extracts are antioxidants and exert cytoprotective effects under H₂O₂-induced oxidative stress [47]. The report from other cells such as neuronal and lung epithelial cells were protected from H₂O₂ induced oxidation by *Bacopa monniera*, a traditional medicinal herb that acts as an antioxidant. Therefore, it can be assumed that the antioxidant activity of a high dose of MJP diminished the H₂O₂ toxicity, of which improved the cells to survive [48].

MJP moderate H₂O₂-induced cell morphological changes

The morphological changes of H₂O₂-induced HEK-293 cells observed by a fluorescence microscope were stained with Hoechst 33342 DNA staining dye and phase contrast microscopy. The result shown in Figure 3b, in the H₂O₂-induced cell, the nucleus broke into small fragments and formed apoptotic bodies compared with a control with untreated cells. Moreover, the cells that were treated with H₂O₂ and MJP at the concentration of 5 and 10 mg/mL exhibited less nuclei fragment than only H₂O₂ treated. Especially, at the concentration

of MJP at 10 mg/ml was hardly to observed nuclei fragmentation around nucleus [29], The result showed that ferulic acid did not exhibit such nuclear condensation after pretreatment.

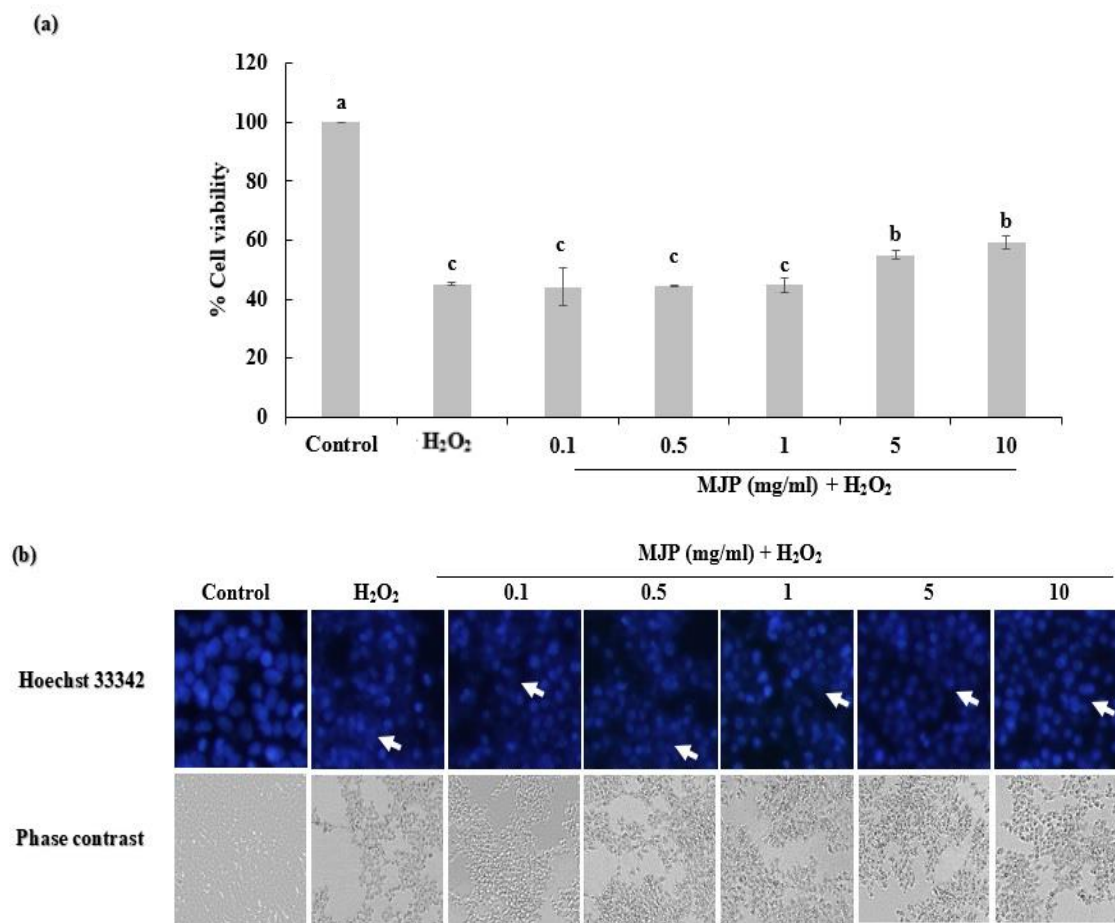


Figure 3. Nephroprotective effect of mixed low potassium vegetables and fruits juice powder. Cells treated with H₂O₂ and H₂O₂ with MJP for 24 h, cell viability data was shown as mean \pm SD (n=3) (a.). Morphological changes of HEK-293 cells observed by fluorescence microscope after staining with Hoechst 33342 and phase contrasted microscopy (100x) (b.) and nuclei fragments showed as arrow.

Effect of MJP on intracellular ROS production.

The reactive oxygen species (ROS) production was quantified using fluorescence microplate reader, the result was shown in Figure 4a. ROS level exhibited 180.65% in the H₂O₂ treated group which was significantly ($p < 0.01$) increased when compare to control normal untreated cell (100%). The HEK-293 cells ROS production was significantly ($p < 0.01$) decreased by MJP at the concentration up to 5 and 10 mg/mL of 133.22 and 129.85%, respectively. However, the MJP at concentrations less than 5 mg/mL were not significant at a different ROS production compared to H₂O₂ treated group.

In order to further investigate the effects of MJP on intracellular ROS production, the ROS molecules produced in different treatments were detected by dichlorodihydrofluorescein (DCF) and visualized under a fluorescence microscope. DCF signals is the fluorescence product occurred by the substrate, hydrolyzed DCFH carboxylate anion was oxidized by intracellular ROS to form DCF. The change in fluorescence intensity ($\lambda_{em/ex} = 530/485$ nm) is directly proportional to the ROS level in the cells. The result showed that there was no

fluorescein signal in the normal cells which were untreated cells while cells were treated by H_2O_2 showed stronger fluorescein of DCF (Fig 4b). Adding MJP in the H_2O_2 -induced cells decreased DCF fluorescein signal especially when increased concentration of MJP up to 10 mg/mL which indicated that MJP reduced ROS production. Additionally, the reported that ferulic acid substance decreased ROS production in HEK-293 cells after exposed to H_2O_2 [28]. This finding reported [30] that the HEK-293 cells of Thai fruits juice significantly decreased the ROS level. Similar to present study [25] found that *Euphorbia bivonae* polysaccharides diminished ROS production in HEK-293 cells compare with only H_2O_2 induced cells.

Effect of MJP on enzymatic antioxidant activity and lipid peroxidation

The effect of MJP on enzymatic antioxidant activities in H_2O_2 induced HEK-293 cells was shown in Fig. 5. Superoxide dismutase (SOD) activity significantly ($p < 0.01$) decreased 39.78% in H_2O_2 treatment compared with control untreated cells. Co-treatment of 10 mg/mL MJP and H_2O_2 significantly increased SOD activity, but not significantly different in MJP concentration less than 10 mg/mL (Figure 5a). Catalase (CAT) activity presented in HEK-293 cells treated with H_2O_2 was also significantly ($p < 0.01$) decreased (78.48%) compared with control cells. However, MJP at the concentration of 0.1- 10 mg/mL exhibited significantly increased CAT activity for 2.81 to 10.04 % compared to only H_2O_2 (Figure 5b). In addition, lipid peroxidation was expressed as MDA contents (by product of lipid peroxidation). The results showed that significantly ($p < 0.01$) increased MDA of 72.23% in H_2O_2 treated group, but in the co-treatment with 0.1-10 mg/mL MJP significantly lower MDA contents than H_2O_2 treated group of 11.74 to 34.71% (Figure 5c) [25, 29, 30]. The most important protection mechanisms against the free radicals or oxidants in the body are endogenous intracellular antioxidant enzymes such as superoxide dismutase (SOD) changes superoxide radical ($O_2^{\cdot-}$) to H_2O_2 and O_2 after that catalase (CAT) convert H_2O_2 in to water (H_2O) and O_2 otherwise H_2O_2 transfer to $HO\cdot$, lead to lipid peroxidation presence malondialdehyde (MDA) [3].

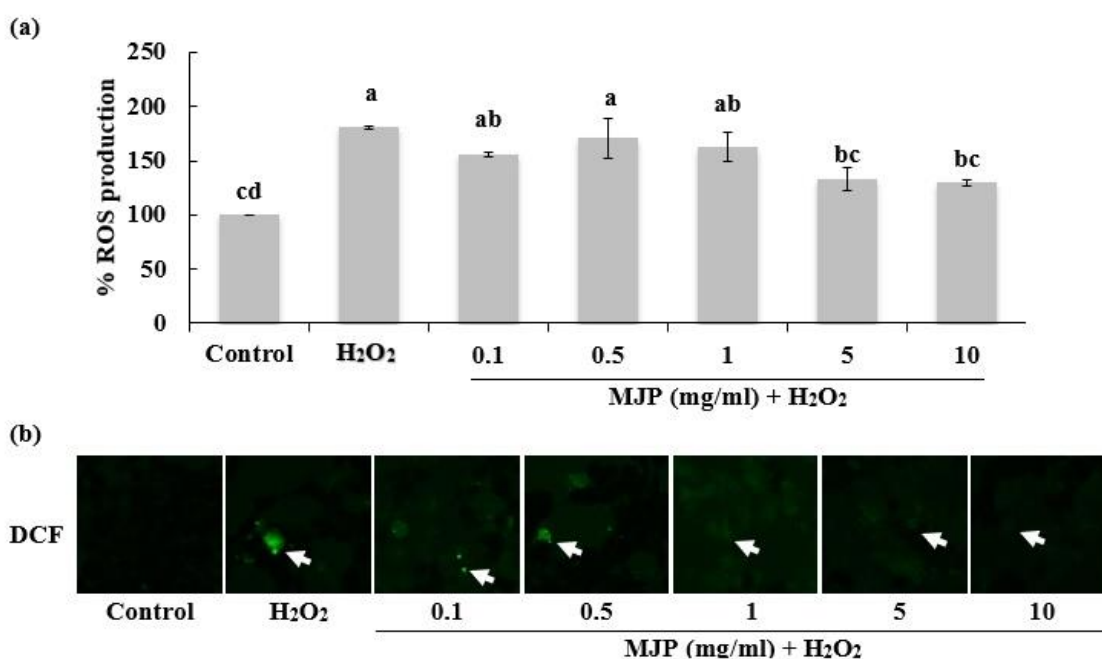


Figure 4. Effect of MJP on H_2O_2 -induced ROS production in HEK-293 cells. Cells treated with H_2O_2 and H_2O_2 with MJP for 30 minutes. Cells were incubated with DCFH-DA. (a.) The intracellular

ROS production was expressed as a percentage of control and shown as mean ± SD (n=3). A value same superscript is not significant (p < 0.05). (b.) DCF represented intracellular ROS observed by fluorescence microscope (100X)

This result exhibited MJP enhanced SOD and CAT to protect the cells from oxidation, and the same way MJP decreased lipidperoxidation that showed MDA decreased. This result may occur by inhibition of MJP that scavenged HO· or inhibited fenton’s reaction, more understanding DNA damage protection was examined.

DNA damage protection of MJP

The agarose gel electrophoresis of DNA damage protection was shown in Figure 6a and the calculated intensity DNA band from the gel was shown in Figure 6b. The DNA damage was induced by Fenton’s reagent, a solution of hydrogen peroxide (H₂O₂) with ferrous iron as a catalyst. The genomic DNA extracted from HEK-293 cells showed up as a dark band on agarose gel electrophoresis (lane 1 in Figure 6a and control in Figure 6b). The DNA incubated with Fenton’s reagent was shown in lane 2 and 3, which lack of DNA in both lanes implied that the genomic DNA has been thoroughly degraded. As in the concentration of MJP was increased in lanes 3 to 7 (0.1, 0.5, 1, 5 and 10 respectively), the presence and darkness of the DNA band were increased, as well as, the increased of calculated intensity indicating MJP can infer a protective effect against Fenton’s agent-induced DNA damage. The result which agreed with the previous study. This DNA survival can be explained by reducing level of ROS production which cause of cell injury and damage [31].

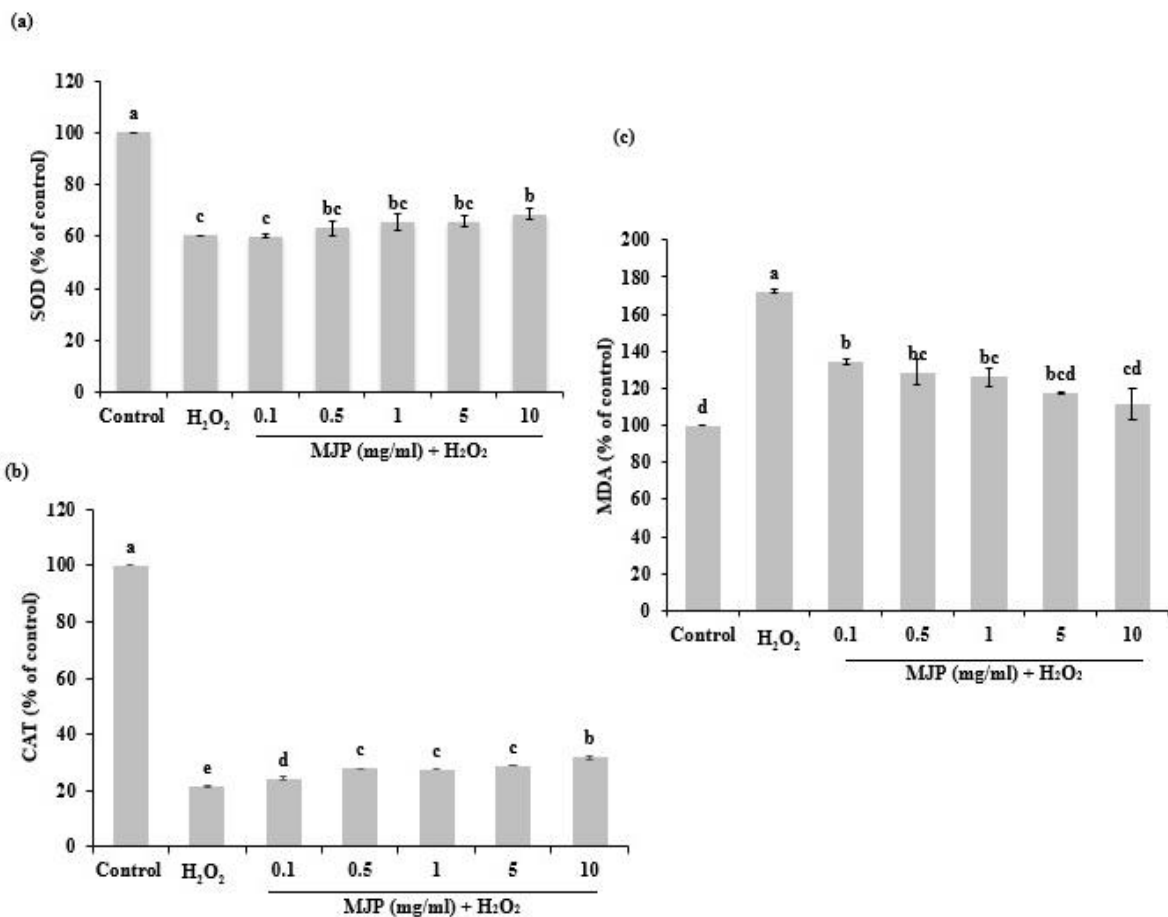
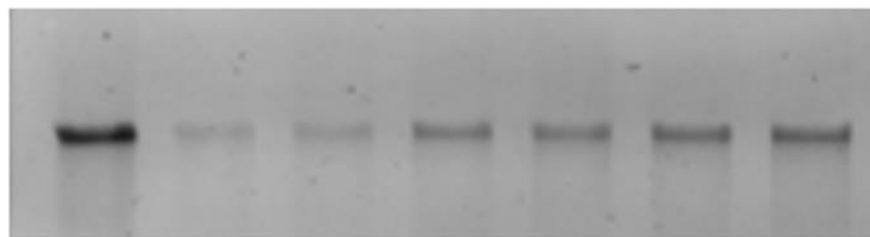


Figure 5. Effect of MJP on superoxide dismutase (SOD), catalase (CAT) activity and malondialdehyde (MDA) production in H₂O₂-induced HEK-293 cells. Data represents mean \pm SD. The difference letters on the bars are significantly difference ($p < 0.05$) according to Turkey's test.

(a)

Lane	1	2	3	4	5	6	7
Fenton's reagent	-	+	+	+	+	+	+
MJP (mg/ml)	-	-	0.1	0.5	1	5	10



(b)

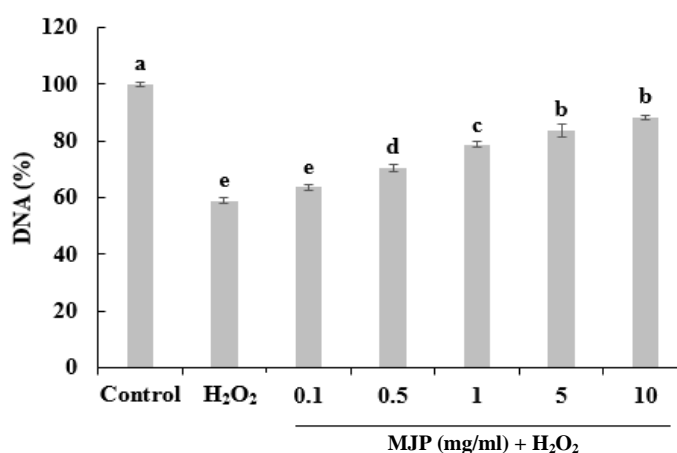


Figure 6. Visualization of the DNA damage protection in the present and absence of MJP on agarose gel electrophoresis (a) and the quantified band intensity for the genomic DNA (b). Lane 1, DNA incubated without Fenton's reagent; Lane 2, DNA incubated with Fenton's reagent; Lanes 3-7, DNA incubated with Fenton's reagent and MJP of 0.1, 0.5, 1, 5 and 10, respectively.

CONCLUSION

The present study was accomplished antioxidative capacity of mixed low potassium vegetables and fruits juice powder (MJP) on molecular mechanism in HEK-293 cells against the hydrogen peroxide toxicity. The treated cells responded to cytoprotective effects and DNA damage protection of MJP, reduced ROS production, induced intracellular enzymatic antioxidant activity such as SOD and CAT, and reduced the end product of lipid peroxidation (MDA). Taken together, the results provided comprehensions of antioxidant effects of MJP which could take as a novel functional juice powder for individual limiting potassium and liquid uptake.

List of Abbreviations: MJP, mixed low potassium vegetables and fruits powder; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde

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