Strong enhancement of antioxidant activity of *Aloe vera* extracts by gamma irradiation

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1. Introduction

The World Health Organization (WHO) has estimated that approximately 80% of individuals rely on traditional medicines [1]. Among over 400 Aloe species, Aloe vera was the most accepted species for various medical, cosmetic and neutraceutical purposes [2]. *Aloe vera* (syn.: *Aloe barbadensis* Miller) was a perennial succulent plant belonging to the Aloeaceae family (sub-family of the Asphodelaceae) [3]. It has been reported that Aloe vera extracts were useful in the treatment of wound and burn healing, minor skin infections, sebaceous cyst, diabetes, and elevated blood lipids in humans [4]. Recent studies have shown that treatment with either Aloe vera crude gel or its extracts, such as acemannan, β-sitosterol, and others, resulted in faster healing of wounds by stimulating fibroblast proliferation, collagen deposition, angiogenesis, and production of growth factors [5].

Ionizing radiation technology has been developed to improve our daily life such as cancer therapy and sterilizing tool due to its unique feature that could be penetrated biomaterials leading to alter their own physical properties. More recently, many studies have attempted to apply the radiation technology to enhance their biological activities [6].

At present, however, very little was known about whether naturally-occurring phenolic compounds of ethanolic aloe gel extracts that were altered their biological activities by ionizing radiation to serve as antioxidant in the body to reduce ROS produced by the stresses. The purpose of the current study was to investigate the influence of gamma irradiation on antioxidant activity of Aloe vera extracts, and open insight new possibilities that gamma ray could be a powerful tool for improving its own biological activities.

2. Methods and Results

2.1 Sample extraction

Aloe vera was purchased from Gochang Aloe farm (Korea). The aloe was peeled and aloe gels were collected and then freeze-dried. The freeze-dried aloe powders (2g) were suspended with 400 ml of 40% ethanol and mixed toughly for 12 hr. The mixture was then centrifuged for 5 min at 10,000 x g. The supernatant was dried and measured the total content of samples. Total content of aloe gel extracts was 3.2 mg/ml.

The HPLC system, consisted of an Agilent 1200 series solvent delivery system, and UV-detector, coupled with a C18 column (YMC-Pack Pro C18; 4.6 × 250 mm), was used to analysis of crude aloe gel extracts.

2.2 Gamma irradiation

The samples were exposed to gamma ray using a cobalt-60 irradiator (MDS Nordion INC, Canada) in the Korea Atomic Energy Research Institute (KAERI; Jeongeup, Korea), from 10 to 100 kGy. Interestingly, we found that the color of ethanolic extracts of aloe gel was changed after gamma irradiation dose-dependently (Fig. 1). The red color had a biphasic pattern: at the beginning, ethanolic extracts of aloe gel was weak yellow in color, and then the red color was appeared at lower doses ($\leq$ 40 kGy), then it begun to become yellow in color in a dose-dependent manner when 40-70 kGy of gamma ray was irradiated. At dose ($\geq$ 80 kGy), the red color was completely disappeared and became yellow in color. Although exact mechanism of which the color changed by gamma irradiation was not known to date, this color alteration might be due to the effects of gamma radiolysis of water and ethanol. Although the exact mechanism of which the color alteration by gamma irradiation was still not clear, results of the recent study suggested that the free radicals produced were capable of demolishing the compounds

2.3 1,1-diphenyl-2-picryhydrazyl (DPPH)-radical scavenging activity

The free-radical scavenging activity was according to a modified method by Lee et al. [6]. The free-radical scavenging activity was calculated as [1-(absorbance of testing solution / absorbance of control solution)] x 100.

We previously have found that antioxidant activity of the ethanolic extracts of red beet (Beta vulgaris L.) hairy root was significantly increased by gamma irradiation up to 20 kGy (unpublished data). In the present study, we investigated the effects of gamma irradiation on the alteration of antioxidant activity of aloe gel extracts. In order to determine the antioxidant activity, DPPH• was used. DPPH• was a stable free

radical with a violet color and it was changed its color to yellow when free radicals have been scavenged [6].

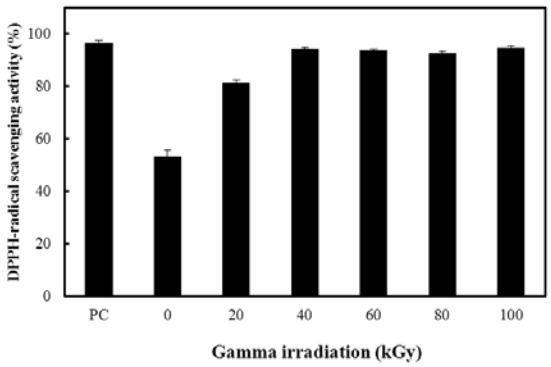

Fig. 1. Effects of gamma irradiation on DPPH radical scavenging activity of ethanolic extracts of aloe gel.

We found that DPPH radical-scavenging activity was remarkably accelerated up to approximately 80% in gamma irradiated samples at the dose 20 kGy, and then antioxidant activity was reached to 100% and retained in irradiated samples (20-100 kGy), as we expected (Fig. 1). Therefore, the optimum gamma irradiation dose which could be enough to enhance their antioxidant activity was about 40kGy in aloe gel extract. In comparison, ascorbic acid was used as a positive control.

In general, increased radical-scavenging activity by gamma irradiation was known to be co-related with new compounds formed by gamma irradiation. To determine whether the observed increased radical-scavenging activity by gamma irradiation was due to a new compound formed, gamma irradiated samples were subjected to HPLC for detecting new compounds. While only one peak 1 was found in normal aloe gel extracts, two peaks were detected after gamma irradiation; one peak was a peak found in normal sample (peak 1), and another peak was a novel peak (peak 2), newly generated by irradiation (Fig. 2A). The formation of the new peak was increased dose dependently, and reached a plateau at 40 kGy gamma irradiation. However, the peak 1 was decreased by increasing a peak 2. The newly formed compound was shown a good correlation with enhanced its antioxidant activity. Next, LC-MS was used to identity of the new peak detected. It was found that molecular weight of a small fragment was 132.0 (Fig. 2B). These results were implying that improved antioxidant activity by gamma irradiation was most likely due to the compound generated by gamma irradiation, and the newly formed compound might be a fragment of peak 1.

3. Conclusions

In conclusion, gamma irradiation in aloe gel extract would be boosted antioxidant activity through the formation of a new compound. Based on these results, increase in antioxidant activity of aloe gel extract by gamma ray can be applied to various industrial area especially cosmetic resource, food stuff, and pharmaceutical source.

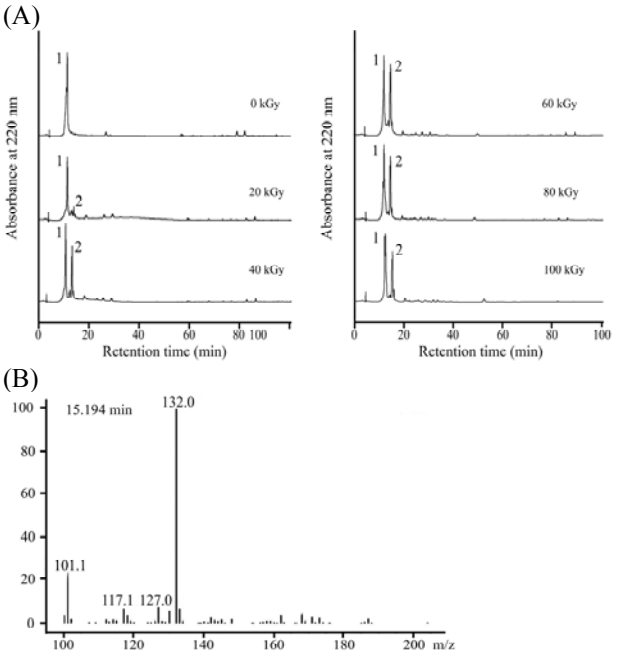

Fig. 2. HPLC chromatograms of ethanolic extracts of aloe gel (A) and LC/MS spectra of the peak 2 generated by gamma irradiation (B).

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