Natural Red Dyes From *Hibiscus sabdariffa* L. Calyxes Extract For Gamma-Rays Detector

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Abstract. Detection of absorbed dose in materials were irradiated is one method for monitoring irradiation processes. Dyes that are sensitive to ionizing radiation has the potential to used as radiation detectors. This article will report on the fabrication of gamma-rays detector using natural red dyes obtained from *Hibiscus sabdariffa* L. (HS) calyxes extract. The HS extract solution was made using a simple extraction method which was carried out by soaking the dried HS calyxes with double distilled water as a solven for 24 hours. Gammacell irradiator with Cobalt-60 radiation source was used to test the sensitivity of HS extract solution to gamma radiation with a dose of 1-10 kGy. UV-Vis spectrophotometer was used to the characterization of optical properties of HS extract solution. After gamma irradiation, it was observed the color of the HS extract solution faded slightly from dark red to more transparent red color along with increasing doses. The results of UV-Vis characterization showed maximum absorbance of HS extract solution at 523 nm and a decrease in absorbance of 95% after irradiation at a dose of 10 kGy. The HS extract solution is stable in the conditions of storage is not exposed light and at the refrigerator up to 30 days.

1. Introduction

Gamma irradiation is one of the popular methods that been used for industrial sterilization such as medical equipment, medicines, and food products. The success of the sterilization process by gamma irradiation is impacted by the accuracy of the absorbed dose on the irradiated material. Therefore, monitoring of the absorbed dose is a condition that is applied in the irradiation process. The monitoring system of irradiation process with radiochromic dosimeter is an easy and reliable way that can be used at the industrial sterilization because of the flexibility to used in various positions either on packaged or bulk ingredients and its relatively inexpensive to use in a big-scale industrial needs. Furthermore, the discoloration phenomena that present on the radiochromic dosimeter is equivalent to the absorbed dose by the irradiated material.

In the process of fabrication radiochromic dosimeters, dyes are the main ingredients, so determining which materials are sensitive to radiation is a challenge for researchers to find. At present, the use of synthetic dyes are the most common in the fabrication of radiochromic dosimeters because it has various color and easier to get, such as Reactive Red 120 dye was investigated for gamma dosimeters at low levels [1], in addition also reported the use of xylenol blue dye mixed with polyvinyl butyral film for gamma indicators in a dose range 0.25-10 kGy [2], Bromophenol Blue for gamma dosimeters in a high dose measurements [3]. Awad A. Al Zahrany et al., introduced a dosimeter film using methyl red dyes that undergo the color bleeding when exposed to gamma radiation [4]. The
downside of synthetic dyes are not eco-friendly and have toxic effects on humans. In this report, the researcher tries to find an alternative dyes that renewable and more environmentally-safe.

Anthocyanin is one of the natural dyes can be found in plants else than to chlorophyll and beta-carotene. In general, the anthocyanin basic structure consists of two benzene aromatic rings (C₆H₆) namely aromatic rings A and B connected by heterocyclic C rings containing oxygen [5] as shown in Figure 1. Anthocyanin constituent compounds will be affecting the color of the solution such as pelargonidin which has the role of producing orange, red to dark red, while cyanidin has the role of producing orange red, dark red, purplish red to bluish red [6]. The color and stability of anthocyanin are influenced by several factors such as modifications of anthocyanin structure, pH, temperature, light, solvents, enzymes, and the presence of metal ions. *Hibiscus sabdariffa* L. is a plant that is cultivated so that it is easily obtained, especially in Indonesia. HS calyxes extract is used as a dyes in the fabrication of radiochromic dosimeters because it contains anthocyanin compounds with the largest components namely cyanidin and delphinidin which cause red color in the extraction results. The anthocyanin from *Hibiscus sabdariffa* L. has the potential to be used as a primary material of radiochromic dosimeters based on research by P. H. Março et al., 2011 [7], Tri Esti Purbaningtiyas et al., 2017 [8] which reporting about the discoloration of anthocyanin as a reaction of temperature and UV light. Higher energy from gamma radiation compared to UV light is expected to be able to cause discoloration of HS extract solution. The optical properties of the HS extract solution before and after irradiation will be characterized by a UV-Visible spectrophotometer at a wavelength of 300-800nm. The characterize results data will be processed and analyzed by the Origin software to study the dose response of HS extract solutions.

2. Materials and methods

2.1. Preparation of HS extract
The primary material to fabrication radiochromic dosimeters are *Hibiscus sabdariffa* L. (HS) calyxes in dried form was purchased from Indonesian local markets. The HS extract was obtained by soaking 50 grams of dried HS calyxes on 1000 ml of aquabidest and stored in a laboratory tube (Schott-Duran). The extraction has been carried out for 24 hours in 8°C refrigerated temperature without light exposure, next the solution was filtered using filter paper (Whatman # 1). The HS extract solution to be irradiated was prepared in a 5 ml clear glass bottle with a tightly closed lid and covered with aluminum foil.

2.2. Irradiation of HS extract
The irradiation was carried out at Badan Tenaga Atom Nasional (BATAN) irradiation facility, located at Jakarta, Indonesia. The irradiator facility used is Gammacell 220 with a Cobalt 60 radiation source and a dose rate of 4.84 kGy / hour. The observation of the discoloration of HS extract was carried out at 11 measurement spots, which is unirradiated solution (blanko), solution that irradiated at a dose of 1 kGy, 2 kGy, 3 kGy, 4 kGy, 5 kGy, 6 kGy, 7 kGy, 8 kGy, 9 kGy, and 10 kGy.

![Figure 1. General Structure of Anthocyanin](image)

The changes in the color of the HS extract solution before and after irradiation are documented with a Canon EOS 75D digital camera in a mini studio. The optical properties of HS extract solutions
are characterized using a UV/Visible spectrophotometer and changes in absorbance of HS extract solution before irradiation ($A_0$) and the absorbance after irradiation ($A_i$) at certain wavelength can be calculated in discoloration percentage using equation 1.

$$\text{% discoloration} = \left(\frac{A_0 - A_i}{A_0}\right) \times 100$$  

In general, the radichromic dosimeters test and characterizes refers to ISO / ASTM 51540:2004 concerning radiochromic dosimeter systems in a liquid form.

2.3. Stability of HS extract
The environmental effects on HS extract solutions were studied to find out the shelf life of HS extract solutions before irradiation and the stability of HS extract solutions after irradiation. The test is carried out by placing the HS extract solution in two different temperature. One on extremely low temperature 8°C at the refrigerator and the other one on extremely high temperature 40°C in an oven. The observation was carried out for thirty days with a various time interval.

3. Results and discussions

3.1 Discoloration of HS extract
The results of HS calyx extraction with aquabidest solvent produce a red solution with a natural pH of 2.52 and a maximum absorbance at a wavelength of 523 nm. Furthermore, the color of the HS extract solution that has been irradiated at dose 1-10 kGy undergoes fading out gradually from dark red to transparent red as shown in Figure 2. This visual observation is also supported by the characterization of optical properties using a UV-Visible spectrophotometer and from Figure 3. appearance a slowly decrease in absorbance of the HS extract solution before and after irradiation. Based on the calculation of the difference in the absorbance spectrum of the HS extract solution before and after irradiation using equation 1, it is known the discoloration percentage ($\text{% discoloration}$) after irradiation with a dose of 1 kGy is around 36% and increases to 47% and 52% after irradiation on a dose of 2 kGy and 3 kGy in sequence. Next, the percentage increase until 95% after irradiation on a dose of 10 kGy.

![Image](image_url)

**Figure 2.** The photograph of HS extract solution before and after gamma irradiation at a dose of 1-10 kGy
Figure 3. The absorbance spectrum of HS extract solution after irradiation up to 10 kGy

3.2 Response Curve of Roselle Extract Solution
The response curve of the HS extract solution is plotted based on the difference in absorbance values before and after irradiation (nett absorbance) at a wavelength of 523 nm towards the absorbed dose. Figure 4. shows the response curve of the HS extract solution was shaped an exponential growth from dose 0 to 10 kGy. In addition, Figure 5. is linear variation of response curve from the natural logarithm of absorbance (ln (A)) versus absorbed dose with correlation coefficient (R$^2$) was 0,982 and slope at -0,27 kGy$^{-1}$ which represents the dose sensitivity of HS extract to the radiation dose. Based on the correlation coefficient value (R$^2$) which is close to 1, it can be concluded that radiation dose has a strong effect towards on changes in the absorbance value of HS extract and this proof that HS extract has the potential to be used as dye on radiochromic dosimeter. However, referring to similar researched that we have reported about liquid radiochromic from roselle extract solution with ethanol as a solvent [9], that the colors fade is slower on HS extract with aquabidest solutions at the same dose level of irradiation. This is caused by the solubility of sugar groups in heterocyclic rings which tend to form anthocyanin compounds which are more stable in water so that needed a higher doses to degradation the color of the solution [10].
Figure 4. Dose response curve of HS extract solution at the dose range 0 to 10 kGy

Figure 5. Linear graph of absorbed dose to ln Absorbance of HS extract solution
3.3 Temperature Effect to Shelf Life and Stability of HS Extract Solution

The stability of HS extract solution before and after gamma irradiation has been observed. During the testing of the color stability, the HS extract solution was stored in the refrigerator at 8°C and in the oven at 40°C, and covered with aluminum foil to prevent light exposure. The absorbance was calculated at 523 nm wavelength during storage period up to 30 days with various time intervals. The stability of unirradiated radiochromic was shown in Figure 6. From this figure, it can be seen that the change in absorbance value of HS extract solution before irradiated and stored in the oven decreased slightly about 6% on the 14th day of observation and after that fell sharply about 72% start from the 21st day of storage up to 30th day of storage. Moreover, the HS extract solution stored in the refrigerated temperature tends to be stable with a gradual decrease in absorbance value of about 3% until the end of the observation period. The storage conditions such as temperature and light are one of the factors that influence the stability of anthocyanins in HS extract. An increase in temperature within a certain time of storage can cause the destruction of anthocyanins. The related research of the stability anthocyanin at various temperature and light conditions has been reported by J. Jenshi Roobha et al., [11] the good stability of the storage conditions were obtained at temperatures less than 30 °C and no exposure light.

![Figure 6. The graphical stability of HS extract before irradiation](image-url)
The stability test was also carried out on the HS extract solution after irradiated at a dose of 4 kGy. From Figure 8, it can be seen that the absorbance of HS extract solution stored in the refrigerator decreased about 1.3% on the 7th day and then decreased slowly to 6.6% on the 30th day. However, the HS extract solution stored in the oven experienced a very sharp change since the observation on the 7th day.

4. Conclusion
Study on the characteristic of HS extract as a gamma radiation detector has been studied using a UV-Vis spectrophotometer and was found that HS extract has the potential to be a gamma-rays detector with a maximum % discoloration of 95% after being irradiated at a dose 10 kGy. Furthermore, there was also found a mighty correlation between the absorbed dose with the change in maximum absorbance at the specified wavelength ($R^2 = 0.98$) and sensitivity of 0.27 kGy$^{-1}$. The unirradiated HS extract has high stability with the condition of storage at a temperature of 10 °C and no exposure light, up to 30 days. While the irradiated HS extract is recommended to observe immediately after irradiation.

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