EFFECT OF GAMMA IRRADIATION ON PHYTOCHEMICAL CONTENT AND ANTICANCER ACTIVITIES OF ROSELLE (*Hibiscus sabdariffa* Linn)

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Abstract *Gamma irradiation is widely used in herbal medicine industries as an efficient preservative method in reducing microorganism contaminants. The aim of this study was to evaluate the effect of gamma irradiation at the doses of 5; 7.5; and 10 kGy on H. sabdariffa ethanolic extract (HS-EE). The Co-60 was used for irradiation the samples. The phytochemical content of HS-EE was carried out by total microorganism analysis using dilution method, TPC by Follin-Cicalteu method, TFC by aluminium chloride colorimetric method, antioxidant activity using DPPH method, TLC profiling on silica gel F254, in vitro anticancer activity using A-549, HUT-78, and MCF-7 cancer cell lines. The irradiation at 10 kGy caused the total bacteria decreased, while dose of 5 kGy could eliminate the total mold. Irradiation at 5 kGy caused TPC, TFC, and antioxidant activity decreased by 5-11%. TLC chromatograms analysis confirmed that one of the compounds contained in HS-EE was quercetin. The HS-EE has the strongest anticancer property against HUT-78 (IC50 10.51 µg/mL) followed by against MCF-7 (IC50 13.39 µg/mL), and A-549 (IC50 14.19 µg/mL). It can be concluded that irradiation at a dose of 10 kGy could remove total bacteria and molds, decreasing the phytochemical content and anticancer activities of HS-EE. It is recommended to increase the active ingredient level in the formulation.*

INTRODUCTION

One of the herbal medicines that have been widely used for its medicinal properties in Indonesia is roselle flower (*Hibiscus sabdariffa Linn*) which contains flavonol as quercetin. It has high antioxidant properties and hypolipidemic effect (1). Clinical trials conducted on cancer patients who were given three grams of *H. sabdariffa* boiled water three times a day for seven consecutive days showed an increased anti-inflammatory. In addition, testing on mice after being given methanol extract helped maintain the IL-1B ratio, excess IL-1B in blood plasma indicated inflammation in the body, one of which could be caused by cancer (2,3). Several studies revealed that *H. sabdariffa* was able to inhibit cancer cells without being toxic to normal cells such as K-562, HepG2, HT-29 (4), breast cancer (MCF-7) (5), lung (A-549) (6), myeloma (7), nasopharyngeal (8), and ovary (9).

As one of the secondary metabolites in *H. sabdariffa*, quercetin is a class of flavonoids that are widely available in plants and fruits and usually available as quercetin glycoside having higher antioxidant activity than vitamins C and E as well as the ability to influence immunity and inflammation (10). In addition, testing on mice after being fed *H. sabdariffa* methanol extract helped maintain the IL-1B ratio, excess IL-1B in blood plasma indicated inflammation in the body, one of which could be caused by cancer (2,3).

The quality of the anticancer properties of *H. sabdariffa* ethanolic extract needs to be maintained before being marketed to consumers. Several methods of preservation are used by the industry to maintain the quality of traditional medicines is gamma irradiation technique (11). Preservation using the gamma irradiation technique has been widely used because of its ability to inactivate pathogens through the destruction of nucleic acids that will extend the shelf life of irradiated products with doses under 10 kGy (12,13).

The current study intends to investigate the influence of gamma irradiation on the sterilization of *H. sabdariffa* ethanolic extract, in addition to evaluating the secondary metabolites content such as total phenolic, flavonoids, quercetin, and antioxidants, as well as anticancer properties against A-549, HUT-78, and MCF-7 from irradiated and unirradiated samples. To our knowledge, there has not been any study on the effect of gamma irradiation on *H. sabdariffa* ethanolic extract and its anticancer properties (A-549, HUT-78, and MCF-7), hence it is considered an update of this study.

EXPERIMENTAL

Material and sample preparation

H. sabdariffa Linn (HS) obtained from PT. Sari Alam Sukabumi was dried in a room at 19°C for ± 2 weeks. The dried HS was then powdered with a size of 80 mesh, followed by measurement of water content using the gravimetric method at a temperature of 105°C until the weight was constant(14).

Extraction and Gamma Irradiation

Extraction was carried out using 96% ethanol by soaking 500 g of HS powder with ethanol (4L). After 24 h, the filtrate was filtered and concentrated using a rotary evaporator at 35°C to obtain a *H. sabdariffa* ethanolic extract (HS-EE). Amount of of HS-EE (10 g) was put into a sample bottle (volume 50 mL) to gamma irradiated with a dose of 0 (control); 5; 7.5; and 10 kGy with two replications using a gamma cell with a ^{60}Co gamma source at a dose rate of 3.163 kGy/h and a source activity of 4.348 kCi.

Determination of Microbiological Contamination

Determination of microbiological contamination was performed for each irradiation dose of the HS-EE. One mL of HS-EE (concentration of 1000 µg/mL) with a 10^{-4} dilution then cultivated in nutrient agar medium and incubated for 24 h at 37°C and the number of grew colonies was counted. Meanwhile, for the total number of mold, a 10^{-2} dilution was carried out and planted on PDA medium then incubated for three days at room temperature and the number of grew colonies was counted(15).

Determination of Total Phenolic Content

A total of HS-EE (10 g) was dissolved in 96% ethanol (25 mL). Two mL of the solution was taken, added with aquabidest (5 mL) and 50% Folin-Ciocalteau reagent (0.5 mL), then homogenized on vortex. The solution was incubated for 5 min at room temperature and added 5% Na_2CO_3 (1 mL). It was incubated again for 60 min, then the absorbance was measured using a UV-visible spectrophotometer at λ_{max} 725 nm. The standard used as a comparison was gallic acid(16).

Determination of Total Flavonoid Content

A 4 mg HS-EE was dissolved in methanol (4 mL), then 250 µl of each sample was added distilled water (2 mL) and 5% $NaNO_2$ (150 µL), 10% $AlCl_3$ (150 µL), and 1M NaOH (2 mL), then added distilled water until the total volume was 5 mL, and the absorbance was measured using a UV-visible spectrophotometer at λ_{max} 510 nm(16).

Determination of Antioxidant Activity

The HS-EE (10 mg) was dissolved in methanol (10 mL), then the various concentrations used were 10, 25, 50, and 75 µg/mL. The HS-EE (2 mL) was added with 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.5 mL), then shaken and incubated for 30 min at room temperature. The absorbance of each sample was measured using a UV-Vis spectrophotometer at λ_{max} 515 nm(16,17).

Densitometric Scanning Pattern of TLC Chromatogram

The HS-EE from each sample, and standard quercetin (STD) were analyzed on TLC plates F_{254} using the mobile phase $CHCl_3$:MeOH:H_2O (7 : 3.5 : 1) scanned using a densitometer at λ_{max} 254 nm. The sample spots were compared to the quercetin spot, then the RF value of each sample was determined.

In-Vitro Bioassay against Human Cancer Cell Line

In vitro bioassay was performed on all samples against human cancer cell lines (A-549 lung carcinoma, HUT-78 cutaneous T-lymphocyte, and MCF-7 breast cancer) at the concentrations of 5, 10, 20, 40, and 80 (µg/mL) with dimethyl sulfoxide as a negative control. The medium used were Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS), 1% penicillin, and 1% fungizone. The cells were incubated in 5% CO_2 incubator at 37°C for 72 hours, then were counted using an Olympus microscope at 4000 times magnification(18).

Statistical Analysis

All data are presented as means and standard deviations for three measurements.

Statistical analysis of the significance test of irradiated and unirradiated samples was performed by SPSS 24 software with a 95% confidence level ($p<0.05$) using one way ANOVA.

RESULTS AND DISCUSSION

Water Content and Extraction

Determination of water content is performed before irradiation, the value of water content provides a minimum limit that is still allowed for simplicia. The water content of HS powder is 6.57%. HS extraction using 96% ethanol appeared as viscous extract with a yield of 33.66%. The water content obtained is less than 10% and related to the quality of traditional medicines. The higher water content will be easily contaminated by microbes and fungi. They do not have a longer shelf life due to enzymatic reactions that cause decay in simplicia (11,19,20).

Microbiological Contamination

The total of bacteria and molds was counted as colony forming units (CFU). The effect of gamma irradiation on microbial decontamination can be seen in Table 1. The irradiation dose at 5-10 kGy has reduced the number of bacteria and mold in the samples significantly. The total of bacteria and mold decreased as the irradiation dose increased. Total aerobic plate count (TAPC) decreased significantly at the irradiation dose of 7.5 and 10 kGy no bacteria at all, while total mold count (TMC) at dose 5 kGy was able to kill all the molds in the sample.

The quality of herbal medicine may drop because of bacteria, fungi, and insects. Bacteria and fungi can produce a mycotoxin that will contaminate the product. Gamma irradiation treatment can reduce the amount of microbial and mold contamination in HS-EE. Several studies revealed which irradiation at a dose of 0.2 kGy was able to inhibit mold and at dose of 1 kGy was able to kill all molds. The decrease in bacteria and mold is due to structural changes in the microorganism cells. Gamma irradiation can inhibit the growth of bacteria and mold which will directly damage cell membranes and cause the death of cells. The damage will cause a loss of osmotic balance and loss of ions in cells such as loss of protein and ribonucleic acid (21–25).

Total Phenolic Content (TPC)

TPC of unirradiated HS-EE had the highest value compared to irradiated HS-EE, based on one-way ANOVA analysis significantly different between irradiated HS-EE and unirradiated HS-EE (control) with a confidence level of 95% ($p<0.05$). TPC of irradiated and unirradiated HS-EE can be seen in Table 2. At a dose of 5 kGy, the TPC decreased and had the smallest value compared to the other samples. It is known which gamma irradiation reduces the TPC at 5 kGy (26). It was caused by the degradation of secondary metabolites and the breaking of chemical bonds into a low molecular weight, as well as loss of the aromatic ring in the phenolic compound or sample due to gamma irradiation (24). Different conditions occurred at doses of 7.5 and 10 kGy, there was an increase in TPC from the previous dose caused by the release of dissolved phenolic compounds from the glycosidic component due to the breaking of covalent bonds, therefore it would increase the total of phenolic dissolved in the HS-EE (24). In addition, carbonyl bonds and glycosidic bonds are very susceptible to gamma irradiation. Consequently, they will produce radiolysis products (27).

Total Flavonoid Content (TFC)

TFC of HS-EE was expressed as mg quercetin/g extract. Statistically, TFC at the dose of 5 kGy decreased significantly, on the other hand at doses 7.5 and 10 kGy increased (Table 2). There were significant differences ($p<0.05$) between irradiated HS-EE and unirradiated HS-EE (control). The decrease of TPC will affect of TFC in extract directly. The data on TPC and TFC were in accordance with several studies obtained by Yusuf et al, Adhitia et al, and Mun'im et al who revealed at the dose of 5 kGy the TPC and TFC decreased due to gamma irradiation, however increased at the dose of 7.5 kGy (23,28,29). The decrease in TPC and TFC after gamma irradiation also occurred in the extract of Cuscuta chinensis (26), Plucea indica (30), and Peperomia pellucida (28).

Table 1. The effect of gamma irradiation on microbial decontamination

Irradiation dose (kGy)	TAPC (CFU/g)	TMC (CFU/g)
0	$5.9x10^4$	$1.3x10^2$
5	$3.6x10^3$	0
7.5	$3.0x10^1$	0
10	0	0

Antioxidant Activity

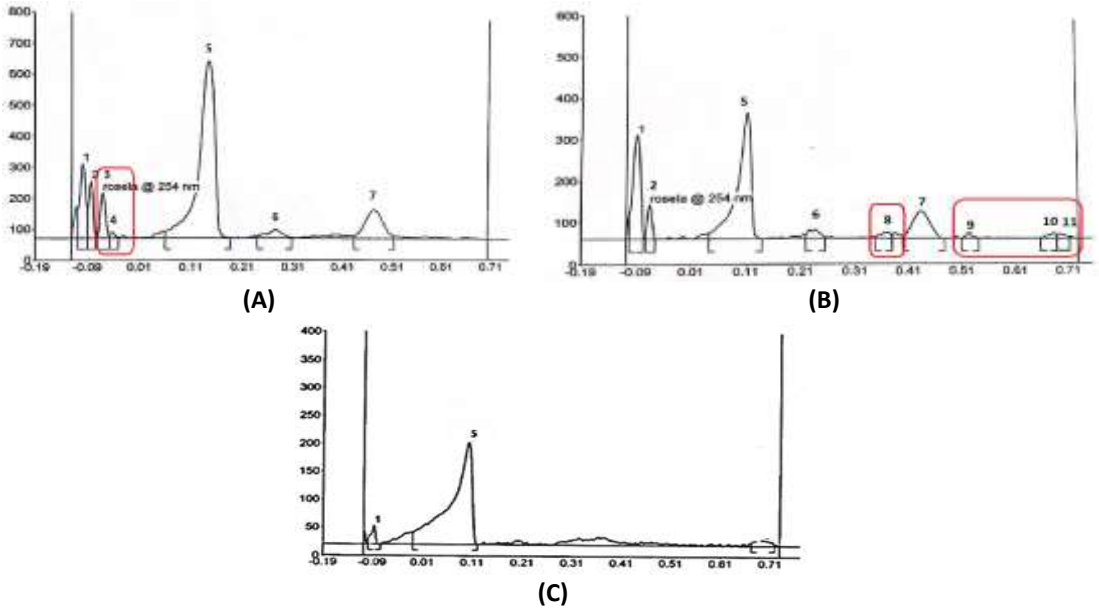
The antioxidant activity analysis are presented as IC50 values. The IC50 value is the concentration required to inhibit 50% of a DPPH radical. A sample with the lowest IC50 value indicates high antioxidant activity (20). Statistically, the results showed a significant different after 5 kGy gamma irradiation. However, there was no significant different after 10 kGy gamma irradiation compared to 7.5 kGy dose (Table 2).

The results of the antioxidant analysis showed a correlation with the total phenolic and flavonoids. The presence of hydroxyl groups in phenolic compounds related to free radical scavenging activity by DPPH. In contrast to other studies, El-Beltagi et al. reported that irradiated dates fruits at a dose of 5 kGy increases the antioxidant activity (31). The effect of gamma irradiation on the activity and secondary metabolites content whether decrease or increase is caused by variations and types of plants, environmental conditions, geography, solid or powder samples, the composition of secondary metabolites, solvents used during extraction, extraction procedures and irradiation doses (32). In addition, gamma irradiation will produce a free radical that will reduce its antioxidant activity due to a decrease of phenolic compounds, hence it will affect the antioxidant activity of the extract (30,33). The relationship between total phenolics, antioxidants, and flavonoids depends on their molecular structure where the number of OH groups and the position of the OH groups on the benzene ring will allow electrons delocalization in the double bond. In general, the number of OH groups influences the antioxidant activity and flavonoids (34).

Table 2. The effect of gamma irradiation on TPC, TFC, and antioxidant of HS-EE

Irradiation dose (kGy)	TPC (mg GAE/g Extract)	TFC (mg QE/g Extract)	IC_{50} of Antioxidant Activity (µg/mL)
0	5.17±0.09	3.11±0.09	26.67±0.16
5	4.60±0.01*	2.20±0.04*	28,54±0.11*
7.5	4.90±0.02*	2.89±0.20	27.23±0.81*
10	4.93±0.05*	2.61±0.22*	27.46±0.19*

* Significantly different ($p<0.05$) compare to 0 kGy, Each value is median ± standard deviation (n=3)



(A) **(B)** **(C)**

Figure 1. Densitometric scanning pattern of TLC-chromatogram of HS-EE at λ 254 nm: (A) control , (B) 7.5 kGy, and (C) quercetin

Densitometric Scanning Pattern of TLC Chromatogram

The analysis using a densitometer of each sample contained quercetin (peak No. 5) as compared with the quercetin standard (Figure 1C) and was suspected to be responsible for the activity of HS-EE. The control sample contained seven peaks detected on the TLC plate with the main peak being No. 5. However, in the chromatogram pattens of the irradiated sample, the area and height of the quercetin peak decreased. In addition, in the 7.5 and 10 kGy irradiated samples, some peaks (No. 3&4 Figur 1A) were lost and new peaks (No. 8, 9,10 and 11 Figur 1B) appeared.

Among flavonoid compounds, quercetin is one of the flavonoid group that have the potential as an antioxidant. The study was carried out according to Zhen et al, the main flavonol compound in H. sabdariffa extract was 26% quercetin, while the other content was a kaempferol compound of 6% (35). Marfak et al. revealed that at a dose between 2 to 14 kGy would degrade the quercetin compounds significantly (36).

***In-Vitro* Bioassay Against Cancer Cells**

In vitro bioassay against cancer cells was carried out to determine whether the quercetin in HS-EE has potential as an anticancer agent. Inhibition activity on cancer cells is expressed by the value of IC50 (inhibition concentration capable of killing 50% of cancer cells) (37). Figure 4 showed the relationship between the percent of cell viability of each cancer cells (A-549, HUT-78, and MCF-7).

The higher sample concentration will decrease the cell viability. Based on the cell viability value of each cancer cells, it could be stated which HS-EE can inhibit cancer cells. The concentration of about 20 µg/mL from each sample was able to decrease cell viability by 50%. Table 4 showed that the R value obtained from each sample is closed to 1. It can be concluded which the R value is almost linear and good correlation between sample concentration and anticancer activity of samples against cancer cell lines (A-549, HUT-78, and MCF-7) (6,38,39).

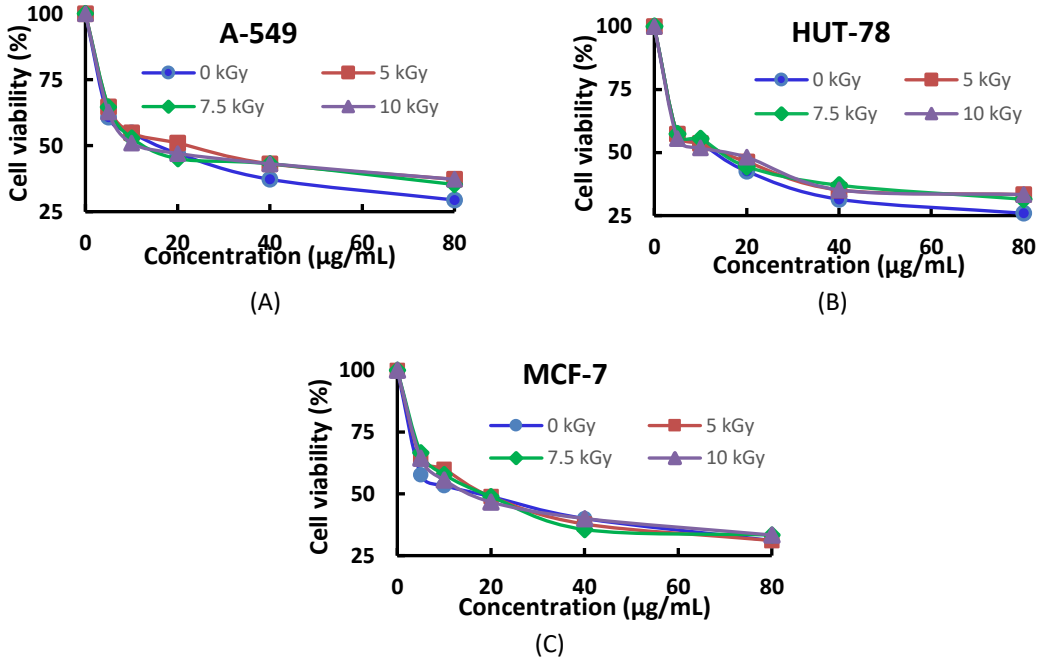
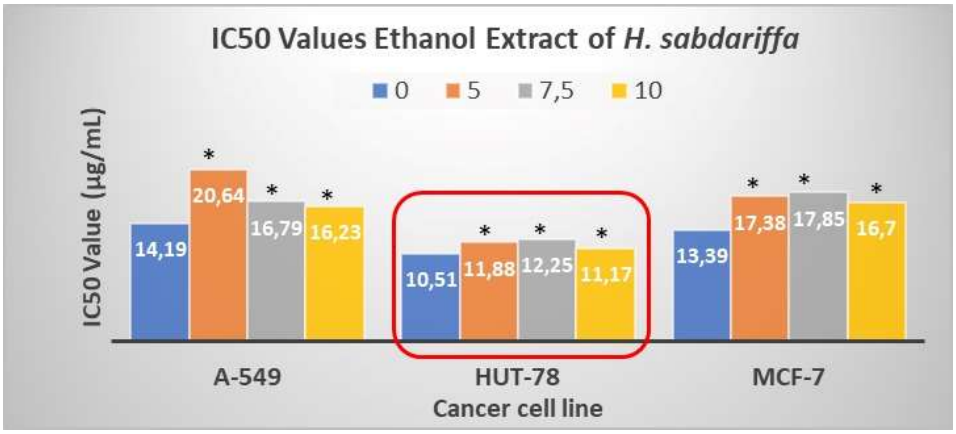


Figure 4. Cell viability of cancer cell line (A-549, HUT-78, MCF-7) of HS-EE under various irradiation dose

Table 4. The linier regression equation and R value of HS-EE under various irradiation dose

Irradiation dose (kGy)	A-549	HUT-78	MCF-7
Control (-)	y = 0.671x + 4.227 R = 0.9969	y = 0.744x + 4.239 R = 0.9841	y = 0.575x + 4.382 R = 0.9769
5	y = 0.581x + 4.235 R = 0.9925	y = 0.575x + 4.382 R = 0.9769	y = 0.757x + 4.060 R = 0.9917
7.5	y = 0.604x + 4.259 R = 0.9737	y = 0.575x + 4.382 R = 0.9769	y = 0.770x + 4.035 R = 0,9888
10	y = 0.508x + 4.387 R = 0.9736	y = 0.611x + 4.335 R = 0.9810	y = 0.664x + 4.187 R = 0.9986



*Significantly different (p<0.05) compared to 0 kGy

Figure 5. The IC_{50} values of HS-EE under various irradiation dose against cancer cell lines

The in vitro bioassay against human cancer cell lines were in accordance with the several studies obtained by Beltagi et al, Winarno et al, and Susanto et al who revealed that gamma irradiation could decrease the anticancer activity (18,40,41). Based on Figure 5 and the categories from the NCI, the IC_{50} value of each sample of cancer cells (A-549, HUT-78, and MCF-7) has an IC_{50} value ≤ 21 µg/mL (high cytotoxic activity). The IC_{50} of HUT-78 had the lowest IC_{50} value followed by MCF-7, and A-549. It can be concluded which HS-EE after gamma irradiation still has anticancer activity against cancer cell lines (A-549, HUT-78, and MCF-7). Statistically, at a 95% confidence level ($p<0.05$) the IC_{50} value of irradiated HS-EE had a significant different compared to the unirradiated HS-EE (control) with the decrease its antiproliferative activity was range 13-45%.

The anticancer properties of HS comes from its quercetin content. The quercetin compound in the HS can inhibit the proliferative of cancer cells but it will not damage to normal cells (9), as well as can inhibit several enzymes that are carcinogenic by 36% (10). HS has high antioxidant properties that can increase apoptosis or programmed cell death of cancer cells by reducing cell proliferation, inducing apoptosis, and mitochondrial depolarization through upregulation of Bax and p53. Therefore, it will down-regulate BCl-2 by activating several caspase enzymes and cytochrome-c (5,42). Caspase activity is responsible both directly and indirectly for protein breakdown by proteolysis during the process of apoptosis (43).

The quercetin content, TPC, TFC, and antioxidant activity in HS-EE each irradiation dose correlated positively with in vitro analysis on cancer cell lines with IC50 values greater than unirradiated HS-EE. In other words, the anticancer properties of HS are influenced by the concentration of the phenolic compounds and secondary metabolites, i.e. quercetin. Gamma irradiation treatment of HS resulted in degradation of the quercetin content which caused decreased anticancer and antioxidants properties.

CONCLUSION

Gamma irradiation at a dose of 5 kGy could reduce microbiological contamination of both bacteria and mold in H. sabdariffa ethanolic

extract. However, total phenolic, flavonoid, antioxidant, and quercetin levels in its ethanolic extract decreased after gamma irradiation compared to the unirradiated ethanolic extract. The cytotoxic effect of its ethanolic extract on human cancer cell lines (A-549, HUT-78, and MCF-7) was still very strong to inhibit cancer cells with the IC50 value were 10.51, 13.39, and 14.19 µg/mL for HUT78, MCF-7, and A- 549, respectively.

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REFERENCES

1. Purba H, Simanjuntak HA, Situmorang R. Phytochemical screening of bunga rosella (Hibiscus sabdariffa L) and antimicrobial activity test. J Pendidik Kim. 2020;12(2):70–8.
2. Laskar YB, Mazumder PB. Insight into the molecular evidence supporting the remarkable chemotherapeutic potential of Hibiscus sabdariffa L. Biomed Pharmacother [Internet]. 2020;127(November 2019):110153. Available from: https://doi.org/10.1016/j.biopha.2020.110153
3. Bayani GF El, Marpaung NLE, Simorangkir DAS, Sianipar IR, Ibrahim N, Kartinah NT, et al. Anti-inflammatory Effects of Hibiscus Sabdariffa Linn. on the IL-1β/IL-1ra Ratio in Plasma and Hippocampus of Overtrained Rats and Correlation with Spatial Memory. Kobe J Med Sci. 2018;64(2):E73–83.
4. Sari F, Klau MHC. Profil Kromatografi Fraksi dari Ekstrak Etanol Kelopak Rosella (Hibiscus sabdariffa L.). Pros Semin Has Penelit 2020. 2020;45–50.
5. Sobantu MP, Okeleye BI, Okudoh VI, Meyer M, Aboua YG. In Vitro Antioxidant Mechanism of Action of Hibiscus Sabdariffa in the Induction of Apoptosis against Breast Cancer. J Herbs, Spices Med Plants [Internet]. 2022;00(00):1–16. Available from: https://doi.org/10.1080/10496475.2022.2135661
6. Fithrotunnisa Q, Arsianti A, Kurniawan G, Qorina F, Tejaputri NA, Azizah NN. In vitro cytotoxicity of Hibiscus sabdariffa Linn extracts on A549 lung cancer cell line. Pharmacogn J. 2020;12(1):14–9.
7. Malacrida A, Cavalloro V, Martino E, Cassetti A, Nicolini G, Rigolio R, et al. Anti-multiple myeloma potential of secondary metabolites from hibiscus sabdariffa. Molecules. 2019;24(13):1–15.
8. Huang DY, Dai ZR, Li WM, Wang RG, Yang SM. Inhibition of EGF expression and NF-κB activity by treatment with quercetin leads to suppression of angiogenesis in nasopharyngeal carcinoma. Saudi J Biol Sci [Internet]. 2018;25(4):826–31. Available from: https://doi.org/10.1016/j.sjbs.2016.11.011
9. Vafadar A, Shabaninejad Z, Movahedpour A, Fallahi F, Taghavipour M, Ghasemi Y, et al. Quercetin and cancer: New insights into its therapeutic effects on ovarian cancer cells. Cell Biosci [Internet]. 2020;10(1):1–17. Available from: https://doi.org/10.1186/s13578-020-00397-0
10. Salehi B, Machin L, Monzote L, Sharifi-Rad J, Ezzat SM, Salem MA, et al. Therapeutic Potential of Quercetin: New Insights and Perspectives for Human Health. ACS Omega. 2020;5(20):11849–72.
11. BPOM. Peraturan Badan pengawas Obat dan Makanan No.3 tahun 2018. Vol. 151, Peraturan Menteri Kesehatan Republik Indonesia Nomor 3 Tahun 2018. 2018. p. 10–7.
12. Luo DQ, Zhao SS, Tang YR, Wang QJ, Liu HJ, Ma SC. Analysis of the effect of 60Co-γ irradiation sterilization technology on the chemical composition of saffron using UPLC and UPLC/Q-TOF-MS. J Anal Methods Chem. 2018;2018.
13. Singleton E V., David SC, Davies JB, Hirst TR, Paton JC, Beard MR, et al. Sterility of gamma-irradiated pathogens: A new mathematical formula to calculate sterilizing doses. J Radiat Res. 2020;61(6):886–94.
14. Kemenkes RI. Farmakope Indonesia edisi VI. Departemen Kesehatan Republik Indonesia. 2020. 2371 p.
15. Pine TAD, Alam G, Attamimi F. Standarisasi Mutu Ekstrak Daun Gedi (Abelmoschus manihot (L.) Medik) dan

UUji Efek Antioksidan dengan Metode DPPH. Jf Fik Uinam. 2015;3(3).
16. Zuraida, Sulistiyani, Sajuthi D, Et al. Fenol, Flavonoid, dan Aktivitas Antioksidan Pada Ekstrak Batang Pulai (Alstonia acholaris R.Br). J Penelit Has Hutan. 2017;35(3):211–9.
17. Molole GJ, Gure A, Abdissa N. Determination of total phenolic content and antioxidant activity of Commiphora mollis (Oliv.) Engl. resin. BMC Chem [Internet]. 2022;16(1):1–11. Available from: https://doi.org/10.1186/s13065-022-00841-x
18. Susanto, Winarno EK, Winarno H. The effect of Gamma irradiation on herbal medicine (Annona muricata, morinda citrifolia, and Typhonium flagelliforme) against MCF-7 breast cancer. AIP Conf Proc. 2022;2391(March).
19. BPOM RI. Peraturan Badan Pengawas Obat dan Makanan No.32 Tahun 2019. In: BPOM Ri. 2019. p. 1–16.
20. Surjanto, Batubara R, Hanum TI, Julianti E. Potency of Fresh and Rotten Leaves of Gaharu (Wikstroemia tenuiramis Miq) Sumatera Endemic as Raw Material of Antioxidant Rich Tea. IOP Conf Ser Earth Environ Sci. 2019;305(1).
21. Jeong RD, Chu EH, Lee GW, Cho C, Park HJ. Inhibitory effect of gamma irradiation and its application for control of postharvest green mold decay of Satsuma mandarins. Int J Food Microbiol [Internet]. 2016;234:1–8. Available from: http://dx.doi.org/10.1016/j.ijfoodmicro.2016.06.026
22. Dharmarha V, Guron G, Boyer RR, Niemira BA, Pruden A, Strawn LK, et al. Gamma irradiation influences the survival and regrowth of antibiotic-resistant bacteria and antibiotic-resistance genes on romaine lettuce. Front Microbiol. 2019;10(APR):1–16.
23. Adhitia AM, Octaviani AN, Rissyelly, Basah K, Mun'im A. Effect of gamma irradiation on angiotensin converting enzyme inhibition, antioxidant activity, total phenolic compound and total flavonoid of peperomia pellucida herbs extract. Pharmacogn J. 2017;9(2):244–8.
24. Syahdi RR, Sakti AS, Kristiyanto A, Redmawati R, Munim A. Effect of gamma irradiation on some pharmacological properties and microbial activities of melinjo (Gnetum gnemon Linn.) Seeds. Pharmacogn J. 2019;11(1):177–82.
25. Ashtari M, Khademi O, Soufbaf M, Afsharmanesh H, Askari Sarcheshmeh MA. Effect of gamma irradiation on antioxidants, microbiological properties and shelf life of pomegranate arils cv. 'Malas Saveh'. Sci Hortic (Amsterdam) [Internet]. 2019;244(July 2018):365–71. Available from: https://doi.org/10.1016/j.scienta.2018.09.067
26. Alijaniha F, Emadi F, Naseri M, Kamalinejad M, Motevaseli E, soodi M, et al. Effect of gamma irradiation on cytotoxicity, phenolics content and acute toxicity of Cuscuta chinensis L. extract. Radiat Phys Chem [Internet]. 2021;185(1471):109508. Available from: https://doi.org/10.1016/j.radphyschem.2021.109508
27. Khawory MH, Amat Sain A, Rosli MAA, Ishak MS, Noordin MI, Wahab HA. Effects of gamma radiation treatment on three different medicinal plants: Microbial limit test, total phenolic content, in vitro cytotoxicity effect and antioxidant assay. Appl Radiat Isot [Internet]. 2020;157:109013. Available from: https://doi.org/10.1016/j.apradiso.2019.109013
28. Yusuf MS, Wulandari I, Amelia L, Et al. Effect of gamma irradiation on suruhan (Peperomia pellucida (L.) Kunth) herb powder. Pharmacogn J. 2017;9(2):239–43.
29. Abdul M, Ramadhani F, Chaerani K, Amelia L, Arrahman A. Effects of Gamma Irradiation on Microbiological, Phytochemical Content, Antioxidant Activity and Inhibition of Angiotensin Con- verting Enzyme (ACE) Activity of Peperomia pellucida (L.) Kunth. J Young Pharm. 2017;9(1):230–3.
30. Ernawati, Suryadi H, Mun'im A. Effect of gamma irradiation on the caffeoylquinic acid derivatives content, antioxidant activity, and microbial contamination of Pluchea indica leaves. Heliyon [Internet]. 2021;7(8):e07825. Available from: https://doi.org/10.1016/j.heliyon.2021.e07825
31. El-Beltagi HS, Aly AA, El-Desouky W. Effect of Gamma irradiation on some biochemical properties, antioxidant and antimicrobial activities of Sakouti and Bondoky dry dates fruits genotypes. J

Radiat Res Appl Sci. 2019;12(1):437–46.
32. Moosavi KS, Hosseini S, Dehghan G, Jahanban-Esfahlan A. The effect of gamma irradiation on phytochemical content and antioxidant activity of stored and none stored almond (amygdalus communis L.) hull. Pharm Sci. 2014;20(3):102–6.
33. Douar-Latreche S, Benchabane O, Sahraoui N, Hazzit M, Mouhouche F, Baaliouamer A. Effect of Gamma Irradiation on the Chemical Composition and Antioxidant Activity of Thymus algeriensis Extracts. J Essent Oil-Bearing Plants. 2018;21(2):449–61.
34. Glevitzky I, Dumitrel GA, Glevitzky M, Pasca B, Otrisal P, Bungau S, et al. Statistical analysis of the relationship between antioxidant activity and the structure of flavonoid compounds. Rev Chim. 2019;70(9):3103–7.
35. Zhen J, Villani TS, Guo Y, Qi Y, Chin K, Pan MH, et al. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of Hibiscus sabdariffa leaves. Food Chem [Internet]. 2016;190:673–80. Available from: http://dx.doi.org/10.1016/j.foodchem.2015.06.006
36. Marfak A, Trouillas P, Allais DP, Calliste CA, Duroux JL. Redox reactions obtained by γ irradiation of Quercetin methanol solution are similar to in vivo metabolism. Radiat Res. 2003;159(2):218–27.
37. Maciel LG, do Carmo MAV, Azevedo L, Daguer H, Molognoni L, de Almeida MM, et al. Hibiscus sabdariffa anthocyanins-rich extract: Chemical stability, in vitro antioxidant and antiproliferative activities. Food Chem Toxicol [Internet]. 2018;113(January):187–97. Available from: https://doi.org/10.1016/j.fct.2018.01.053
38. Kusuma C, Caroline C, Sukarti E. Antioxidant analysis manggo juice fresh brand A and hand made. J Farm Indones. 2019;11(2):16–23.
39. Susanto, Winarno EK, Winarno H. Effect of gamma irradiation on jati belanda (Guazuma ulmifolia L) against human cancer cell lines. AIP Conf Proc. 2020;2296(November).
40. Hossam El-Beltagi S, Dhawi F, Amina Aly A, Abeer El-Ansary E. Chemical compositions and biological activities of the essential oils from gamma irradiated celery (Apium graveolens L.) seeds. Not Bot Horti Agrobot Cluj-Napoca. 2020;48(4):2114–33.
41. Winarno EK, Winarno H, Susanto. Antiproliferative Activity of Extracts and Fractions from Irradiated Curcuma zanthorrhiza Rhizomes Against Mouse Leukemia and Human Cancer Cell Lines. Atom Indones. 2019;45(3):159–64.
42. Yuliastri WO, Diantini A, Ghozali M, Sahidin I, Isrul M. Phytochemical Constituent and In-vitro Cytotoxic Activity of Hibiscus sabdariffa L. Calyx Fraction on Human Breast Cancer Cell Line MDA-MB-231. Rasayan J Chem. 2022;15(3):1619–25.
43. Shafabakhsh R, Asemi Z. Quercetin: A natural compound for ovarian cancer treatment. J Ovarian Res. 2019;12(1):1–9.