Manila Journal of Science ***16(1)*** *2023, pp. 1–12*

Radioprotective Potential of *Leea manillensis syn. guineensis* (Abang-abang) Leaf Extract on Gamma-Irradiated Human Blood Lymphocytes In Vitro

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ABSTRACT

The purpose of the study is to assess the radioprotective potential of *Leea manillensis syn. guineensis* (Abang-abang) extract on gamma-irradiated human lymphocytes through in vitro micronucleus assay. The unadulterated extract of *Leea manillensis syn. guineensis* (Abang-abang) was diluted into varying concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL, after which they were added to the irradiated blood samples. Based on the study and data gathered, the extract of *Leea manillensis syn. guineensis* (Abang-abang) was found to have significantly reduced the number of micronuclei in the 1,000 binucleated cells. From the four concentration levels, it was the 0.4 mg/mL concentration of *Leea* m*anillensis syn. guineensis* (Abang-abang) that exhibited the greatest radioprotective activity in terms of the reduced micronucleus formation from which it yielded a mean of 33 micronuclei. Furthermore, based on the correlational and regression analysis, there is a significant relationship between plant extract concentration and the number of micronuclei formation and it is apparent that the plant concentration level of *Leea manillensis syn. guineensis* has a strong inverse correlation with the number of micronuclei ($r = -0.90$, $p = 0.04$).

This study can benefit people who are excessively exposed to ionizing radiation, specifically cancer patients who have undergone radiotherapy. This study will provide a great innovation on how the effect of ionizing radiation in the public will lessen. This study provided evidence that the leaf extract of the plant *Leea manillensis syn. guineensis* (Abang-abang) gives a protection to minimize ill effects to patients who are inevitably exposed to ionizing radiation. This study provided a contribution to the utilization of *Leea manillensis syn. guineensis* (Abang-abang), which is endemic here in the Philippines.

Keywords: Abang-abang, radioprotective activity, micronucleus formation, gamma irradiation, cobalt-60, *Leea manillensis syn. guineensis* (Abang-abang)

INTRODUCTION

In everyday life, people are exposed to different kinds of radiation. Radiation is the energy released by unstable nuclei in the form of electromagnetic waves and high-energy particles that cause ionization. There are two main types of radiation: ionizing radiation and nonionizing radiation. According to the World Health Organization (WHO), ionizing radiation is a type of radiation with enough energy to remove electrons from atoms, causing them to become charged or ionized and form free radicals. Exposure to ionizing radiation can have serious health implications for humans.

Research and discoveries about radiation, which began in the 1890s, have brought benefits to humankind. Today, radiation is utilized in medicine, academia, industry, agriculture, archaeology (carbon dating), space exploration, law enforcement, geology, mining, electricity generation, and other fields (United States Nuclear Regulatory Commission, n.d.). There are three main types of ionizing radiation: alpha, beta, and gamma radiation. In recent years, technological advancements have continued to improve and flourish around the world, leading to increased exposure of humans to higher levels of ionizing radiation (Yan Na Li et al., 2016). Many precautionary measures have been studied to decrease human exposure to ionizing radiation, including the use of suitable radioprotectors derived from plants and herbs. These radioprotectors contain substances that can help prevent the harmful effects of ionizing radiation, which can have negative long-term effects on human health.

According to Jagetia (2007), plants and herbs can provide radioprotection if they contain antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and free-radical scavenging properties. Plants with these properties are likely to exhibit radioprotective activity. Flavonoids, which help reduce and protect humans from ionizing radiation, are significant in decreasing micronucleus formation in binucleated cells caused by ionizing radiation. Radioprotectors have great potential for pharmacological development, as the harmful effects of ionizing radiation can lead to damage or even death of normal tissue or cells (Weiss & Landauer, 2003).

Radioprotection refers to the use of certain agents or techniques to protect living organisms from the damaging effects of ionizing radiation (Ghosh et al., 2014). Exposure to high levels of radiation can cause damage to cellular components, including DNA, leading to mutations and even cancer. The use of chemical radioprotectors has been widely investigated to prevent or mitigate the effects of ionizing radiation on biological systems.

Plants have been identified as a potential source of radioprotective agents due to their natural ability to withstand environmental stress, including exposure to ionizing radiation (Jagetia, 2007). Several plant-derived compounds such as flavonoids, polyphenols, and alkaloids have been shown to have radioprotective properties through various mechanisms, including scavenging of free radicals, modulation of cellular signaling pathways, and DNA repair enhancement (Hosseinimehr, 2017). Some examples of such radioprotectors include apigenin, quercetin, resveratrol, curcumin, and catechins (Hosseinimehr, 2014).

Despite the potential of plant-derived radioprotectors, further research is needed to optimize their efficacy, safety, and mode of action (Hosseinimehr, 2017). One promising approach is the use of nanotechnology to improve the delivery and bioavailability of these compounds. For example, nanoparticle-mediated delivery of curcumin

has been shown to enhance its radioprotective effects in vitro and in vivo (Biswas et al., 2017).

The researcher's approach to this study is to investigate the potential of a novel plant-derived compound as a radioprotector. This compound has not been previously investigated for its radioprotective properties and therefore represents a novel approach to the development of radioprotective agents. The approach involved in vitro studies to evaluate the radioprotective potential as well as its mechanisms of action.

MATERIALS AND METHODS

Plant Collection and Verification

Leea manillensis syn. guineensis leaves were obtained from Mt. Maculot, Cuenca, Batangas City. It was then brought to the National Museum of the Philippines, Padre Burgos Ave, Ermita, Manila, for verification processes.

Phytochemical Analysis

After collection and verification, *Leea manillensis syn. guineensis* leaves have undergone qualitative phytochemical analysis.

Preparation of Plant Extraction

One kilogram of *Leea manillensis syn. guineensis* leaves were extracted at the "Chemicals and Minerals Division," Industrial Technology Development Institute of the Department of Science and Technology (DOST), located in Bicutan, Taguig, Metro Manila. The extraction method involved pulverizing 1.0 kg of dried *abang-abang* leaves using a Wiley mill. The resulting 745 g of plant material was then soaked in 3.0 L of 95% ethyl alcohol for 48 hours to extract the desired compounds. The mixture was filtered to remove solid plant material, and the filtrate was concentrated using a rotary evaporator at 60°C under vacuum for 3 hours. Subsequently, the concentrated extract underwent further evaporation using a water bath at 60°C to obtain a semisolid extract. The extraction of 745.0 g of dried *abang-abang* leaves resulted in a total of 2.8 L of ethanolic extract.

Ethanol has demonstrated effectiveness in extracting specific classes of plant compounds, such as flavonoids, phenolic acids, alkaloids, terpenoids, and other secondary metabolites. In this research, the concentration of the extract was varied from 0.1 mg/mL to 0.4 mg/mL based on the study conducted by Joksic et al. (2008). Their study showed that the selection of the 0.1- to 0.4-mg/mL concentration range for the extract of the flowering plant *Gentiana dinarica* had a dose-dependent radioprotective effect on lymphocytes exposed to ionizing radiation, with the highest radioprotective effect observed at a concentration of 0.4 mg/mL.

Blood Extraction

The blood sample was drawn from a male individual with an accomplished donor consent form (*see attached document*). The extraction was performed by a licensed medical technologist from DOST-Philippine Nuclear Research Institute (PNRI). The blood sample was transferred to aseptically sterile glass tubes treated with lithium heparin as an anticoagulant. Male donors are typically preferred for micronucleus assays because they do not have the confounding factor of the menstrual cycle, which may affect the results of the assay (Hayashi et al., 1990).

Application of Ethanolic Leaf Extract

Leea manillensis syn. guineensis extract was added to the blood extract at concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL. This addition was done 30 minutes prior to irradiation using cobalt-60 isotope, based on previous findings indicating that a concentration range of 0.1–0.4 mg/mL of the extract from the flowering plant *Gentiana dinarica* had a dose-dependent radioprotective effect on lymphocytes exposed to ionizing radiation.

Irradiation of Blood Samples and Cell Culture of Human Blood Lymphocytes

The extracted blood was exposed to a dose of 2 Gray (Gy) of ionizing radiation from a cobalt-60 radioisotope and was subsequently incubated at a temperature of 37°C at the gamma irradiation facility of the PNRI. The duration of irradiation was 1 hour. There are other irradiation parameters provided by PNRI including a dosimeter, specifically a Gafchromic® dosimeter, used to measure the absorbed dose of radiation in the blood sample. The PNRI uses a dose mapping system called the Radiological Assessment and Dosimetry Laboratory (RADL) to determine the distribution of the radiation dose throughout the sample. The gamma irradiator at PNRI is equipped with a rotating platform that ensures uniform exposure to radiation. Prior to analysis, the sample was incubated at 37°C for a certain period to allow for any biological changes to occur.

Isolation of Lymphocytes and Counting of Micronuclei for Micronucleus Assay

The irradiated blood sample was added to the culture medium, which is PB-Max Karyotyping; then, it was incubated in a 5% carbon dioxide atmosphere for 24 hours. Cytochalasin-B was then added to inhibit the division of cytoplasm, and it was terminated for 72 hours after the addition of PB-Max Karyotyping Medium (Gibco). After the incubation, the blood sample underwent an isolation process through centrifugation of lymphocytes at 1200 rpm for 10 minutes. The supernatant was removed and was treated with KCl to lyse red blood cells. Afterward, the culture was centrifuged again to 1200 rpm for 10 minutes. The supernatant was once more removed and replaced by freshly made fixatives including methanol: acetic acid and was diluted with Ringer's solution and was centrifuged again and washed with a fixative without the Ringer's solution until the cell suspension was clear. After that, the supernatant was removed and the pellet was suspended. The suspension was dropped into the clean slides and allowed to dry using a slide warmer. Cells were stained by Giemsa dye. Used slides were rinsed quickly in distilled water and allowed to air dry. Culture lymphocytes were grouped into five (5) consisting of four (4) experimental groups treated with plant concentration (0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL) and one (1) negative control without plant extract.

Examination of Slides and Assessment of Micronucleus Frequency

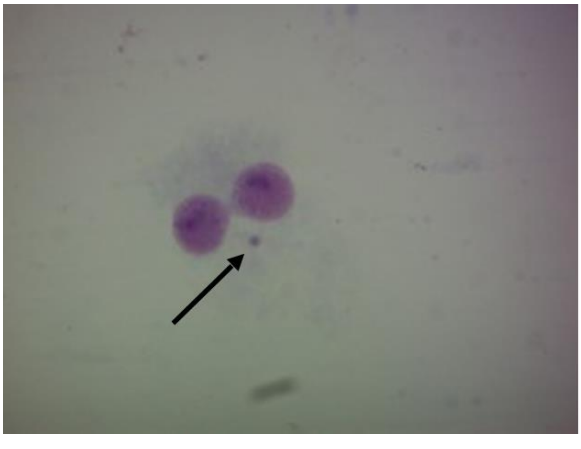
Every 1,000 binucleated cells from each slide of the varied concentration of 0.1 mg/mL to 0.4 mg/mL were examined along with the counting of micronuclei formation. To assess micronuclei in binucleated cells, the researcher looks for the presence of one or more micronuclei in each binucleated cell, evaluates the intensity and relative size of the suspected micronuclei compared to the binuclear material, and distinguishes micronuclei from artifacts or other structures. The cytokinesis-block micronucleus assay (CBMN assay) is a widely used technique for assessing micronuclei. Several scoring criteria and guidelines, such as those recommended by the International Collaborative Project on Micronucleus Frequency in Human Populations (ICPM), have been proposed to standardize the assessment of micronuclei in different cell types (Fenech, 2007).

Statistical Treatment

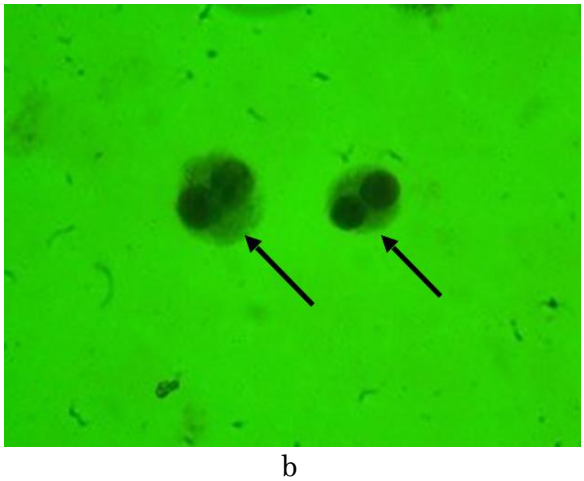
Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess the differences among varying concentrations of the ethanolic leaf extract of *Leea manillensis syn. guineensis*. Correlation and regression analysis were also used to determine the relationship and association between the plant extract concentration and the number of nuclei. A p value of less than 0.05 was considered significant. Values are presented as the mean ± standard deviation for each group. Post hoc tests were utilized to determine if there is a significant difference among the varying concentrations of 0.1 mg/mL, 0.2 mg/mL, 0.3 mg/mL, and 0.4 mg/mL and the negative control. Additionally, Tukey's honestly significant difference test (Tukey's HSD) was performed to assess the homogeneity of variances and to locate specific differences between the experimental and negative group.

RESULTS AND DISCUSSION

This research study evaluated the radioprotective potential of *Leea manillensis syn. guineensis* (Abang-abang) extract on gamma-irradiated human lymphocytes in vitro using the micronucleus assay. According to Fenech (2000), the frequency of micronuclei per 1,000 binucleated cells served as an indicator of the degree of ionizing radiation received by the blood samples. Thus, the formation of micronuclei in binucleated cells denotes the degree of degradative action of radiation exposure. **Figure 1** showed a binucleated cell under the magnification of oil immersion objective with the formation of a micronucleus. Upon the application of *Leea manillensis syn. guineensis* (Abang-abang) leaves extract, the formation of micronuclei within a binucleated cell was minimal.



a



b

Figure 1. (a) Micronucleus formation in the binucleated cell of irradiated human lymphocytes (1000×) and (b) binucleated cells of irradiated human lymphocytes after the application of the *Leea manillensis syn. guineensis* (Abang-abang) extract (1000×).

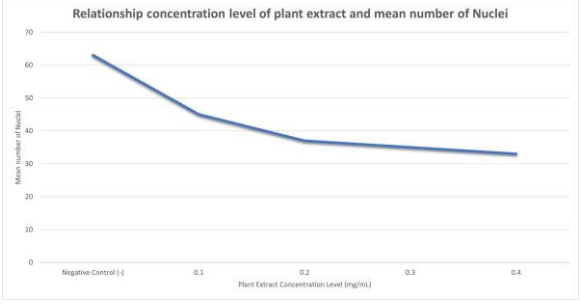
Table 1. Phytochemical Analysis of *Abang-Abang* Leaf Extract (Sample Code: OCS-2016-1266) in DOST-ITDI Standard and Testing Division

Phytochemical	**Results**
Sterols	(-)
Triterpenes	(++)
Flavonoids	(+)
Alkaloids	(+)
Saponins	(+++)
Glycosides	(+)
Tannins	(+++)

Note. (+) = traces, (++) = moderate, (+++) = abundant, (-) = absence of constituents.

Table 1 shows that the extract contains the presence of flavonoids, alkaloids, and glycosides in trace amounts. Triterpenes were detected in moderate traces, while saponins and tannins were present in abundant traces. However, sterols were found to be absent in the extract.

The formation of micronuclei in the negative control (-) in **Figure 1** was discernible in 1,000 binucleated cells compared to the treated concentrations.



Graph 1. The graph shows the relationship of the average means of the number of micronuclei of four different concentrations and the negative control group.

The results of this study revealed an inverse correlation between plant extract concentrations and the number of micronuclei, as demonstrated in Graph 1. The negative control group exhibited the highest number of micronuclei, with an average of 63, while the number of micronuclei decreased with increasing concentrations of plant extract treatment, indicating a concentration-dependent response. The concentration of 0.4 mg/mL showed the least number of micronuclei, indicating the highest ionizing radiation protective activity in terms of the number of micronuclei. The 0.3-mg/mL and 0.2-mg/mL concentrations followed with 35 and 37 micronuclei, respectively, and the 0.1-mg/mL concentration exhibited 45 micronuclei in 1,000 binucleated cells.

Despite the observed ionizing radiation protective ability of the plant extract, the number of micronuclei observed in the study ranged only from 33 to 63 in 1,000 binucleated cells, indicating that other factors, particularly the dose of ionizing radiation, may also influence the formation of micronuclei. These findings suggest that further studies are needed to fully understand the factors that affect micronucleus formation and the potential of plant extracts as a protective agent against ionizing radiation.

Furthermore, the graph depicts an inverse relationship between the concentrations of *Leea manillensis syn. guineensis* (Abang-abang) plant extract and the number of micronuclei formed in blood irradiated with 2 Gy. The results showed that the application of varied concentrations of the plant extract (0.1–0.4 mg/mL) led to a decrease in the number of micronuclei formed in 1,000 binucleated cells. Notably, all the concentrations tested showed a significant reduction in micronuclei formation compared to the negative control.

Similar to the findings of this study, previous research on *Origanum vulgare* demonstrated that increasing concentrations of plant extract led to a reduction in micronuclei formation in human lymphocytes exposed to ionizing radiation. At a concentration of 100 g/mL, there was a significant 70% reduction in micronuclei formation ($p < 0.0001$; Arami et al., 2013).

Tukey's Honest Significant Difference

Table 2. The Detailed Triplicates Comparisons of the Varied Plant Concentrations

Tukey Post-Hoc Test – Frequency

		Positive	0.1	0.2	0.3	0.4	negative
Positive	Mean difference	—	−10.67 *	−2.67	−1.00	1.33	−29.00 ***
	p-value	—	0.037	0.943	0.999	0.997	< .001
0.1	Mean difference		—	8.00	9.67	12.00 *	−18.33 ***
	p-value		—	0.158	0.065	0.018	< .001
0.2	Mean difference			—	1.67	4.00	−26.33 ***
	p-value			—	0.992	0.767	< .001
0.3	Mean difference				—	2.33	−28.00 ***
	p-value				—	0.967	< .001
0.4	Mean difference					—	−30.33 ***
	p-value					—	< .001
negative	Mean difference						—
	p-value						—

Note. * p < .05, ** p < .01, *** p < .001

Based on **Table 2**, the comparison with negative control has a significant relationship between the amount of concentration of plant extract, with p value less than 0.001. Also **Table 2** results can be interpreted that the varied concentrations of 0.2 mg/ mL, 0.3 mg/mL, and 0.4 mg/mL do not demonstrate a notable difference. Hence, all varied concentrations exhibited ionizing radiation protection activity effectively.

Table 3. Correlation Analysis of Concentration Level and Mean Number of Nuclei

Correlation Matrix

		Concentration Level	Mean Number of Nuclei
Concentration Level	Pearson's r	—	
	p-value	—	
Mean Number of Nuclei	Pearson's r	−0.90 *	—
	p-value	0.037	—

Note. * p < .05, ** p < .01, *** p < .001

Table 4. Regression Analysis of Concentration Level and Mean Number of Nuclei

Regression Statistics	
Multiple R	0.90
R Square	0.81
Adjusted R Square	0.75
Standard Error	6.14
Observations	5

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	490	490	12.99	0.04
Residual	3	113.2	37.73		
Total	4	603.2			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	56.6	4.76	11.90	0.00	41.46	71.74	41.46	71.74
Concentration	-70	19.43	-3.60	0.04	-131.82	-8.18	-131.82	-8.18

Based on the correlation and regression analysis, Table 3 and Table 4, respectively, the p value = 0.04, which is less than the alpha = 0.05, which indicates that there is a significant relationship between the concentration level of plant extract and the number of micronuclei. The Pearson's correlation coefficient is equal to −0.90, which implies the inverse correlation or negative correlation of the concentration level and the number of micronuclei. Another study published in the *Journal of Radiation Research and Applied Sciences* also reported similar results regarding the effect of plant extracts on micronuclei formation. The study investigated the radioprotective potential of black cumin (*Nigella sativa*) seed extract on human lymphocytes exposed to gamma radiation.

The results showed that the extract significantly reduced the frequency of micronuclei formation in a dose-dependent manner. Pearson's correlation coefficient was calculated to be −0.943, which indicates a strong negative correlation between the concentration of the extract and the number of micronuclei formed. The p value was found to be 0.017, which is less than the alpha value of 0.05, indicating a significant relationship between the concentration of the extract and the number of micronuclei formed.

These findings provide further evidence that plant extracts can provide radioprotection by reducing the formation of micronuclei in irradiated cells. The negative correlation observed between the concentration of the extract and the number of micronuclei formed suggests that higher concentrations of the extract may provide greater radioprotective effects. However, further studies are needed to determine the optimal concentration of the extract and to fully understand the underlying mechanisms of its radioprotective effects.

CONCLUSION

Based on the analysis conveyed, it can be concluded that the leaves of the plant *Leea manillensis syn. guineensis* (Abang-abang) contains an ionizing radiation protective effect. The study has identified that 0.4 mg/mL is the most effective level of concentration to reduce the number of micronuclei with a mean of 33. However, at 0.2 mg/mL concentration level, it produces an average of 37 micronuclei with the lowest standard deviation of 2.0 and lowest standard error of mean of 1.155 among the concentration levels. The study also provides that there is an inverse relationship between the amount of concentration level of *Leea manillensis syn. guineensis* plant extract and the number of micronuclei based on the result of the correlational analysis with $r = -0.90$ and p value = 0.04. The regression analysis also affirmed the significant relation of the amount of plant extract concentration and number of nuclei with p value = 0.40.

The study can be further improved through identification of the toxicity level concentration of *Leea manillensis syn. guineensis* or at which concentration level the plant extract can be toxic. Also, identify

other factors that can influence the decrease in micronuclei or improve the ionizing radiation protective effect of *Leea manillensis syn. guineensis* including the utilization of different parts of *Leea manillensis syn. guineensis* (Abang-abang), isolation of the phenolic constituents of *Leea manillensis syn. guineensis,* exposure to higher doses of gamma radiation in blood, and higher plant extract. Furthermore, the utilization of the dicentrics assay, which is specifically designed to detect chromosome damage resulting from radiation, is noteworthy.

The phytochemical analysis of *abang-abang* leaf extract conducted at the DOST-ITDI Standard and Testing Division using sample code OCS-2016-1266 showed the presence of several bioactive compounds, including flavonoids, alkaloids, glycosides, triterpenes, saponins, and tannins. These compounds are known to have various pharmacological properties, including anti-inflammatory, antioxidant, anticancer, and radioprotective activities.

Flavonoids are known to have potent antioxidant properties and can scavenge free radicals, which can cause DNA damage and contribute to the development of cancer and other chronic diseases. Alkaloids have been shown to possess a wide range of biological activities, including anticancer, anti-inflammatory, and analgesic properties. Glycosides have been reported to have a positive effect on cardiovascular health and can also exhibit antioxidant activity.

Triterpenes are known to have a radioprotective effect and have been shown to mitigate radiation-induced damage to the body's tissues. Saponins and tannins also have radioprotective properties and have been shown to protect against radiation-induced oxidative stress and DNA damage. Sterols, which were found to be absent in the extract, have been reported to have potent anticancer and anti-inflammatory properties.

Overall, the phytochemical analysis of *abang-abang* leaf extract suggests that it may have radioprotective potential, primarily due to the presence of triterpenes, saponins, and tannins. However, further studies are needed to confirm this potential and to determine the optimal dosage and administration of the extract.

In conclusion, the *abang-abang* leaf extract has several bioactive compounds with potential pharmacological properties, including radioprotective potential. The results of this study provide a basis for further research to investigate the radioprotective potential of this plant extract and explore its potential use in radiation therapy and other applications.

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