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Article

Antioxidant Properties and Radioprotective Effects of Bioactive Extracts from *Pleurotus sajor-caju* on Yeast Cells against γ-rays

Rosnani Abdul Rashid[1], Youichirou Matuo[2] and Yoshinobu Izumi[3, †]

[1] Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, Malaysia,
[2] Graduate School of Engineering, University of Fukui, Japan,
[3] Research Institute of Nuclear Engineering, University of Fukui, Japan
[†] izumi@u-fukui.ac.jp

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Exposure to ionizing radiation results in the generation of free radical, such as anion radicals, cation radicals, and neutral radicals, that can induce biological damage. In this report, we studied the antioxidant properties and radioprotective effects of extracts from Pleurotus sajor-caju on wild-type yeast cells. Phenolics and flavonoids are bioactive compounds, known as antioxidants, that have gained interest among researchers due to their benefits for human health. The total phenolic content (TPC) of the extracts was determined by Folin–Ciocalteu assay and the total flavonoid content (TFC) was determined by aluminum chloride assay. The antioxidant activities were determined by performing 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The radioprotective effects of the extracts on yeast cells exposed to γ-rays were evaluated based on the survival rate (SR) and mutation frequency. The percentage of surviving yeast cells increased while those undergoing mutation decreased after irradiation in the presence of extracts, indicating that bioactive compounds from the extract of P. sajor-caju can reduce the damage induced by γ-rays. Thus, bioactive compounds from the extracts of P. sajor-caju have the potential to minimize or ameliorate the effects of ionizing radiation.

Key Words: ionizing radiation, antioxidant properties, radioprotective effects, extract, *Pleurotus sajor-caju*

1. Introduction

Mushrooms are widely cultivated and consumed in many countries due to their taste, low cost, and nutritional properties. Research on mushroom bioactive compounds intensified in the early 21st century.[1–3] The active compounds derived from the fruiting bodies and mycelia have various medicinal properties such as antioxidant, antitumor, antiviral, anti-inflammatory, anticoagulant, antibacterial, antifungal, antidiabetic, and immune-modulating effects.[4–6]

The bioactive compounds in mushrooms can be divided into four groups, namely i) polysaccharides, ii) terpenes, iii) phenolic compounds, and iv) peptides and proteins.[6] Many studies have reported that phenolic compounds exhibit antioxidant activity in biological systems and act as free radical inhibitors, peroxide decomposers, metal inactivators, or oxygen

scavengers. Studies have focused on the effects of heat treatment on the bioactive compounds, such as phenolics, flavonoids, and ergothioneines, of *Pleurotus ostreatus*, *Pleurotus sajor-caju*, and *Pleurotus djamor*.[7] However, there have been no reports on the effects of ionizing radiation on bioactive compounds in extracts obtained from the fruiting body of *P. sajor-caju*.

The key role of antioxidants is to reduce free radicals, which are reactive chemical compounds. Free radicals search out ways of pairing up their electrons, and thus, they often attack nearby chemical compounds.[5] The formation of free radicals can be triggered by ionizing radiation, such as γ- and X-rays, even at low absorbed doses. This process can cause damage of biomolecules in living cells, such as DNA.[8] Therefore, to protect the cells from damage and to support cell functions, antioxidants are needed to scavenge these free radicals.

Although radiation is used in various fields, it can also pose risks to humans. In order to reduce radiation risks, it is important to manage and keep the absorbed doses as low as reasonably achievable.[9] On the other hand, if effective radiation protection agents can be developed or discovered, they will have the possibility in reducing health risks. Human cells contain natural radioprotectors; however, the amount is insufficient to overcome the harmful effects of daily radiation received under normal conditions.

Notably, many mushrooms have high antioxidant contents, which enable them to scavenge free radicals and prevent cell damage.[10] Numerous studies have been conducted to investigate bioactive compounds in mushrooms, including studies on the effects of bioactive compounds from mushroom extracts on cancer patients. For example, the dietary intake of mushrooms was shown to minimize undesirable side effects after chemotherapy and radiation therapy.[11] Overall, consuming mushrooms is beneficial for supporting the immune system, in addition to mitigating the harmful effects of radiation.

Radioprotective agents have been reported to minimize the effects of radiation such as hypotension, vomiting, nausea, sneezing, and hot flashes.[12] The discovery and development of antioxidant radioprotectors with less toxicity is also essential. For example, the radioprotective effect of the *Cordyceps militaris* mushroom has been reported.[13]

The present study aimed to clarify the antioxidant properties of bioactive extracts obtained from *P. sajor-caju* and elucidate the protective effects on yeast cells exposed to gamma radiation, based on their survival rate (SR) and mutation frequency.

2. Materials and methods

2·1 Chemicals and reagents

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) kit, Folin–Ciocalteu reagent, gallic acid, quercetin, aluminum chloride, and other reagents were purchased from Sigma-Aldrich, Germany. DPPH can simulate and estimate hydroxyl radical donation, while FRAP can simulate and estimate reduction activity.

2·2 Mutation detection in microsatellites of *P. sajor-caju*

For DNA extraction, the fruiting bodies of *P. sajor-caju* from irradiated mycelium by gamma rays and some fruiting bodies were non-irradiated as a control are selected. The irradiation for the mycelium *P. sajor-caju* was carried out at the Biobeam GM 800 radiation facility at Malaysian Nuclear Agency using ^{137}Cs as a source at dose LD_{50} 2.2 kGy with dose rate 0.227 Gy/s.[14] Fruiting bodies with fast-growing mycelia and high yield were selected. The DNA of irradiated and non-irradiated *P. sajor-caju* was extracted using the cetyltrimethyl ammonium bromide method described by Rosnani *et al.*,[15] with minor modification. Furthermore, inter-simple sequence

repeat (ISSR) primer IS 46 was selected for this study based on the number of amplification bands. ISSR markers are DNA sequences of 100–3000 bp amplified by PCR using a ISSR primer. ISSR primer IS 46 has the following sequence: TGTGTGTGT GTGTGTG RC (18 mer), where R can be an A or G base. The images of DNA bands were obtained using an automatic imaging system.

Among the repetitive sequences present in the genome, those with repeating unit sequences of several bases are called microsatellites. Since microsatellites are ubiquitous in genomes, ISSRs are used as a means to detect mutations such as duplications and deletions. In particular, ISSR primers have two adjacent microsatellite sequences, and by using them as primers, it is possible to amplify the sandwiched region and detect mutations.

2·3 Preparation of extract from *P. sajor-caju*

The bioactive compounds in non-irradiated and irradiated *P. sajor-caju* were extracted from the dried powder of fruiting bodies using either water, ethanol, or azeotropic solvents (80% ethanol : 20% water) separately. Each extract was then filtered using a vacuum pump and filter paper (Whatman No. 1). Following filtration, the solvents were evaporated using a rotary evaporator to concentrate the extracts. Each concentrated extract was freeze-dried for 24 h and the product was weighed and stored at 4°C in a tight container. The extraction yield was calculated using Eq. (1):

$$\text{Extraction yield}\,(\%)=\frac{\text{Mass of extract}}{\text{Mass of sample}}\times 100 \quad (1)$$

The extract used in the study of radioprotective effects must be sterilized using a filter that does not allow fungi to pass through. After sterilization, the toxicity of the extract on the yeast cells was evaluated using the colony assay method ($n=3$ for each type of extraction solvent). It was confirmed that there is no cytotoxicity (data not shown), before conducting further experiments.

2·4 Determination of total phenolic content (TPC) by Folin–Ciocalteu assay

The TPC was determined using the method described by Egra et al.,[16] with minor modification. First, 1 mg of extract was dissolved in 1 mL of pure water. The mixture was vortexed and then shaken and incubated for 30 min at room temperature. Next, 100 μL of the extract solution was pipetted into a new tube, and 0.75 mL of Folin–Ciocalteu reagent was added. The Folin–Ciocalteu reagent was first diluted 10 times in ionized water. The final mixture was shaken and incubated for 5 min at room temperature under dark conditions. Later, 0.75 mL of 7.5% sodium carbonate was added, vortexed, and incubated for 1.5 h. The absorbance was measured at a wavelength of 725 nm using a UV/VIS spectrophotometer (Hitachi UH5300, Japan). Gallic acid was used as a standard.

2·5 Determination of total flavonoid content (TFC) by aluminum chloride assay

The TFC was determined using the method described by Egra et al.,[16] with minor modification. First, 5 mg of the sample was dissolved in 1 mL of ethanol. The mixture was vortexed, and then shaken and incubated for 30 min at room temperature. Then, 250 μL of the extract solution was pipetted into a new tube and 75 μL of 5% sodium nitrite and 1.25 mL of pure water were added. This mixture was incubated at room temperature for 5 min. Next, 150 μL of 10% $AlCl_3$ was added and incubated at room temperature for 6 min. Then, 500 μL of 1 M NaOH and 275 μL of pure water were added and incubated at room temperature for 20 min. The absorbance was measured at 510 nm using a UV/VIS spectrophotometer (Hitachi UH5300, Japan). Quercetin was used as a standard because it is a well-known flavonoid commonly

found in plant extracts.

2·6 Determination of antioxidant activity by DPPH analysis

The antioxidant activity of the *P. sajor-caju* extracts was determined by DPPH analysis as described by Makpol et al.,[17] with minor modification. The irradiated and non-irradiated extracts were used at a concentration of 1 mg/mL (w/v). Then, 0.1 mg/mL of DPPH was prepared in ethanol, and various concentrations of the *P. sajor-caju* extracts were prepared using distilled water and then added into a Falcon tube containing 1.5 mL of DPPH reagent. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 10 min. The absorbance was measured at 517 nm using a UV/VIS spectrophotometer (Hitachi UH5300, Japan) and compared with a blank sample holder. The percentage of DPPH scavenging activity was calculated using Eq. (2), where Ab is the absorbance.

$$\text{Free radical scavenging activity}(\%) = \frac{\text{Ab}_{\text{blank sample}} - \text{Ab}_{\text{sample}}}{\text{Ab}_{\text{blank sample}}} \times 100 \quad (2)$$

whereas; $\text{Ab}_{\text{blank sample}}$ is the measurement taken from sample without extract. $\text{Ab}_{\text{sample}}$ is the measurement taken from sample with extract.

2·7 FRAP analysis

The antioxidant activity by FRAP analysis in *P. sajor-caju* extracts was measured based on the reduction of the ferric (Fe^{3+}) complex to ferrous (Fe^{2+}), which has an intense blue color. First, 1 mg/mL (w/v) of the extract was prepared using distilled water. The FRAP kit from Sigma-Aldrich (catalog number MAK369) was used, and the standards and samples were prepared using the method provided by Sigma-Aldrich, with slight modification. The absorbance of each solution was measured at 593 nm using a UV/VIS spectrophotometer (Hitachi UH5300, Japan). The standard curves of $FeSO_4 \cdot 7H_2O$ with concentrations of 0, 4, 8, 12, 16, and 20 nmol were plotted. The mean FRAP value for each sample was determined from the standard curves, and the ferrous equivalent was calculated using Eq. (3):

$$\text{mM Ferrous equivalent} = \frac{\text{Ferrous ammonium sulfate amount from a standard curve (nmol)} \times \text{Sample dilution factor}}{\text{Sample volume added into the reaction } (\mu\text{L})} \quad (3)$$

2·8 Preparation of yeast strain S288c

Budding yeast cells (wild-type strain 288c) were grown in yeast extract peptone dextrose (YPD) liquid media without additives as a control and with the extracts of non-irradiated or irradiated *P. sajor-caju*. After 2 h of incubation at 30°C, the yeast cells in the YPD liquid media were diluted and filtered through nitrocellulose membrane filters (Milipore®, Billerica, MA) using a vacuum filtration unit (Sterifil aseptic system, Millipore®, Billerica, MA). For determining the SR, each membrane filter contained 200 yeast cells ($n=5$ for each dose radiation for each treatment), and for determining the mutation frequency, each membrane filter contained 2.0×10^7 yeast cells ($n=3$ for each dose radiation for each dose treatment). Each membrane was then placed in a 5-cm petri dish. Samples for each treatment used extracts from the same extraction batch.

2·9 Radiation of wild-type yeast strain S288c

Yeast cells without additives and with additives were irradiated with ^{60}Co γ-rays at the Radiation Laboratory in the Institute of Scientific and Industrial Research, Osaka University, using doses of up to 150 Gy and dose rates of 0.83–3.30 Gy/min.

2·10 Analysis of the SR

To obtain the SR of the yeast cells, irradiated cells on membranes were transferred to solid YPD media

to grow for 3 d at a temperature of 30°C. The number of colonies was counted, and the SR was determined using Eq. (4):

$$\text{SR}(\%) = \frac{\text{Number of colonies}_{\text{irradiated with X Gy}}}{\text{Number of colonies}_{\text{non-irradiated}}} \times 100 \quad (4)$$

whereas; X Gy is absorbed dose in gray unit

Using the data from the SR, the radiation protection ratio (PR) of the extracts was calculated using Eq. (5):

$$\text{Protection ratio}(\%) = \frac{[\text{SR}_{\text{with additives at X Gy}} - \text{SR}_{\text{without additives at X Gy}}]}{\text{SR}_{\text{without additives at X Gy}}} \times 100 \quad (5)$$

whereas; SR is the survival rate.

2·11 Analysis of the mutation frequency

To determine the mutation frequency, the irradiated cells on the membrane were transferred to 5-Fluoroorotic Acid (5-FOA) media to grow for 10 d at 30°C. The surviving colonies were counted, and the mutation frequency was determined as a function of the absorbed dose. 5-FOA is converted by the gene involved in uracil metabolism (*URA3*) to 5-fluorouracil, which is toxic. The *URA3* mutant cell can grow in 5-FOA if uracil is provided, while cells containing *URA3* cannot grow. The number of colonies was counted, and the mutation frequency was determined using Eq. (6):

$$\text{Mutation freqeuncy}(\%) = \frac{\text{Number of mutated colonies}_{\text{irradiated X Gy}}}{\text{Number of colonies}_{\text{non-irradiated}}} \times 100 \quad (6)$$

whereas; X Gy is absorbed dose in gray unit.

3. Results

3·1 Mutation detection in microsatellites

Figure 1 shows the agarose gel electrophoresis results for PCR amplified microsatellites with ISSR primer IS 46 in non-irradiated and irradiated *P. sajor-caju*. The concentration of the agarose gel was 1.5%, and the number of the DNA bands were identified from the image. The non-irradiated *P. sajor-caju* has one band, whereas irradiated *P. sajor-caju* has two bands. The number of bands is the genetic information of non-irradiated and irradiated *P. sajor-caju* used in this study. The irradiated *P. sajor-caju* used in this study a new variety obtained by gamma-rays. ISSR amplification is a reproducible, therefore it can be accessed genetic diversity among closely related varieties.[18] A change in the number of bands indicates that a sequence with a new microsatellite has been inserted between adjacent microsatellites.

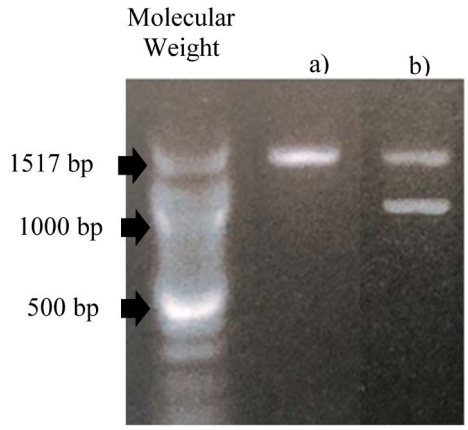

Fig. 1 Agarose gel electrophoresis for PCR amplified microsatellites with ISSR primer IS 46 in non-irradiated (a) and irradiated (b) *P. sajor-caju*.

3·2 Yield of extraction

Three types of solvents were used for extracting the bioactive compounds from *P. sajor-caju*, as shown in Table 1. The highest yield was obtained using the azeotropic solvent, and the lowest yield was obtained using ethanol. Water was used to dissolve water-soluble compounds, and ethanol was used to dissolve alcohol-soluble compounds.

Table 1 Yields of *P. sajor-caju* extracts obtained using different solvents

Sample	Extraction solvent	Mass of sample (g)	Extract (%)
P. sajor-caju (non-irradiated)	water	100	1.46
	ethanol	100	0.22
	azeotropic (water:ethanol)	100	2.46
P. sajor-caju (irradiated)	water	100	2.12
	ethanol	100	0.69
	azeotropic (water:ethanol)	100	2.72

Table 2 Total phenolic and flavonoid contents of *P. sajor-caju* extracts obtained using different solvents

Sample	Extraction solvent	Phenolics (mg GAE/g)	Flavonoids (mg KE/g)
P. sajor-caju (non-irradiated)	water	23.49	10.75
	ethanol	13.02	2.52
	azeotropic (water:ethanol)	20.14	5.58
P. sajor-caju (irradiated)	water	25.73	15.31
	ethanol	12.88	5.26
	azeotropic (water:ethanol)	23.35	5.40

3·3 Total phenolic and flavonoid contents

Table 2 shows the TPC and TFC of non-irradiated and irradiated *P. sajor-caju* extracts obtained using different solvents. The TPC was detected using the Folin–Ciocalteu method, which is based on electron transfer from phenolic compounds in the extracts to the reagent. Meanwhile, the TFC was detected using the aluminum chloride method, which is based on the hydroxyl groups of flavonoids forming a yellow complex with aluminum chloride.

The results showed that the water extract contained the highest TPCs and TFCs among the extracts obtained from non-irradiated *P. sajor-caju*. This finding is consistent with the results reported by Boonsong et al.,[19] who compared water extracts with extracts obtained using 50% ethanol and diethyl ether. Phenolics and flavonoids can be found in the cell walls and high polarity compounds. Thus, these compounds were easily dissolved using water, which is more polar than ethanol. The high TPC and TFC values were indicative of higher antioxidant activity.

3·4 Antioxidant properties by DPPH and FRAP analysis

The value of the free radical scavenging activity is presented as the percentage of the inhibition, where the color of DPPH changes from violet to yellow due to the hydrogen transfer from the antioxidant to the DPPH as in Fig. 2. The results showed that antioxidant activity from non-irradiated and irradiated *P. sajor-caju* extracts was highest from the water extract, followed by the azeotropic solvent and ethanol. The percentage changes in the antioxidant activity between non-irradiated and irradiated *P. sajor-caju*

were 14.25% for water extracts, 21.99% for azeotropic extracts, and 0.68% for ethanol extracts.

Similar results were observed for the FRAP analysis, as shown in Fig. 3, demonstrating that the antioxidant activity of extracts from non-irradiated and irradiated *P. sajor-caju* was highest from the water extract, followed by the azeotropic solvent and ethanol. The FRAP is based on the rapid reduction of the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) by anti-oxidants present in the extract at low pH. The color of the FRAP solution changes from colorless to blue due to the reduction of ferric to ferrous ions, induced by electron transfer from the antioxidant to the FRAP radical. The antioxidant activity of non-irradiated *P. sajor caju* was 14.3 mM for water extracts, 12.12 mM for azeotropic extracts, and 11.05 mM for ethanol extracts, and for irradiated *P. sajor-caju*, the antioxidant activity was 16.52 mM for water extracts, 12.51 mM for azeotropic extracts, and 15.22 mM for ethanol extracts.

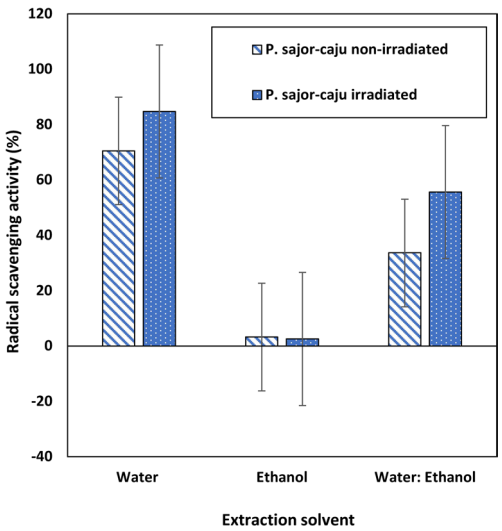

Fig. 2 DPPH radical scavenging activities of *P. sajor-caju* extracts obtained from different solvents. Data are reported as the mean±S.E. (n=3).

3·5 SR of yeast cells

The SR of yeast cells with the extract was higher than that of yeast cells without the extract as shown in Fig. 4. Moreover, the SR of yeast cells with the extract from irradiated *P. sajor-caju* was higher than that of yeast cells with an extract from non-irradiated *P. sajor-caju*. This was due to the higher antioxidant properties of the extracts obtained from irradiated *P. sajor-caju*.

The SR for yeast cells with the extract from *P. sajor-caju* irradiated at a dose of 50 Gy was approximately two times higher (90.4%) than that of yeast cells without extract (59.2%). Based on

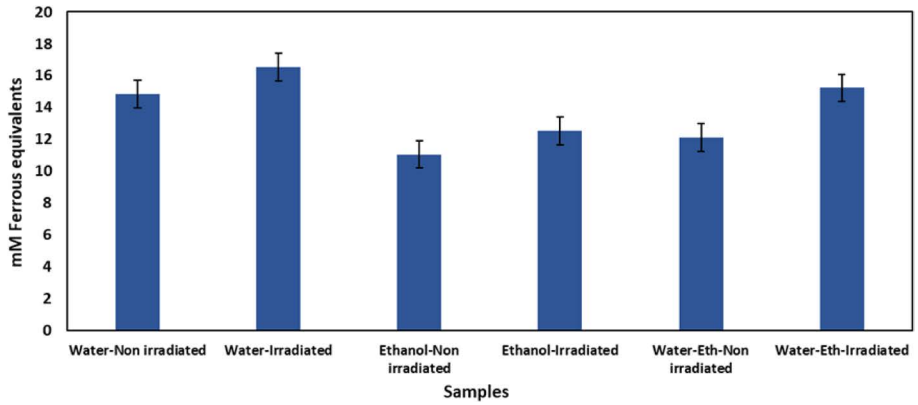

Fig. 3 Reducing of the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) for non-irradiated and irradiated *P. sajor-caju* extracts obtained from water, ethanol, and azeotrophic solvent. Data are reported as the mean±S.E. (n=3).

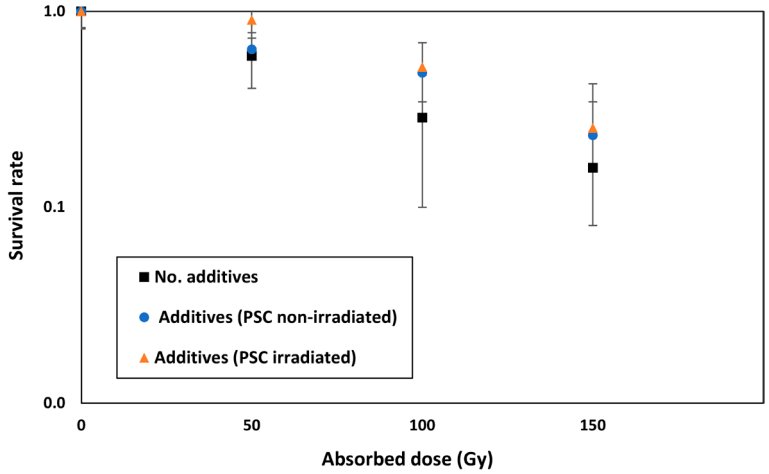

Fig. 4 The SR of yeast cells irradiated with gamma rays and the effects of adding *P. sajor-caju* extracts. Values are shown as the mean±S.E. (n=5).

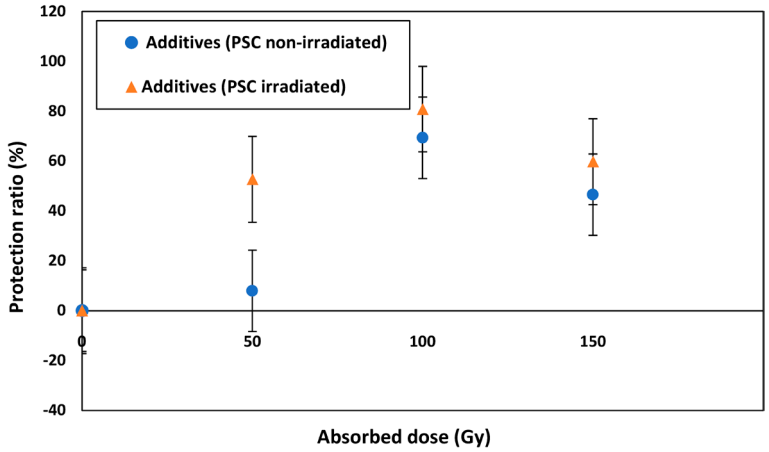

Fig. 5 PR calculated for extracts obtained from non-irradiated and irradiated *P. sajor-caju*. Values are shown as the mean±S.E. (n=5).

these findings, the extract protected the yeast cells against gamma radiation. From the SR, the PR was calculated using Eq. 5. The results show that the protection provided by the extracts from irradiated *P. sajor-caju* was higher than that from non-irradiated *P. sajor-caju*, as shown in Fig. 5. The highest difference in PR between the extracts from irradiated and non-irradiated *P. sajor-caju* was observed at an absorbed dose of 50 Gy.

3·6 Mutation frequency of yeast cells

The mutation frequency of yeast cells exposed to γ-rays with and without *P. sajor-caju* extracts are shown in Fig. 6. The interaction of γ-rays with biological materials forms free radicals that can harm or damage the yeast cells, directly or indirectly. In the present study, *P. sajor-caju* extracts were added to protect and minimize the damage of ionizing radiation, especially damage caused by reactive intermediate species. The results showed that the mutation frequency in yeast cells with no *P. sajor-caju* extract

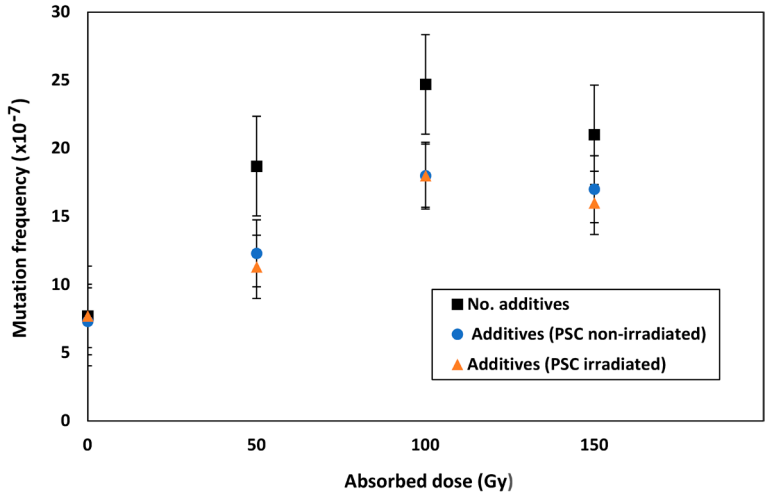

Fig. 6 The mutation frequency of yeast cells exposed to gamma rays and the effects of adding *P. sajor-caju* extracts. Values are shown as the mean±S.E. (n=3).

was higher for all doses of radiation compared with that in cells given extracts. The extract from irradiated *P. sajor-caju* was likely more effective because of the enhanced antioxidant properties.

Yeast cells with and without *P. sajor-caju* extracts showed peak mutation frequencies at an irradiation dose of 100 Gy, and exhibited decreased frequencies at 150 Gy. Cells have mechanisms for repairing the damage from radiation, but at the higher dose, the repairing mechanism cell may be unsuccessful, leading to cell death, inactivity, or mutation. From these results, mutation frequency was related to two outcomes, mutagenesis and cell death. The results from the current study are consistent with the findings reported by Matuo et al.,[20] where yeast cells with additives showed a low mutation frequency compared with those without additives. Overall, the extract from *P. sajor-caju* can reduce the incidence of mutations.

4. Discussion

The present study aimed to evaluate the effects of radiation on the bioactive compounds of *P. sajor-caju*, and the protective effects of the bioactive compounds were determined by evaluating the yield of extraction and antioxidant properties. The DNA profile of *P. sajor-caju* was obtained after ionizing radiation using ISSR primer, and the effects of radiation on bioactive compounds were identified for non-irradiated and irradiated *P. sajor-caju*. Microsatellites means that a mutation has occurred in the genome of *P. sajor-caju*. It is presumed that the components of the extract have changed due to this mutation.

As shown in Table 1, the yield of bioactive extracts from fruiting bodies of non-irradiated and irradiated *P. sajor-caju* was highest from the water extract, followed by the azeotropic solvent and ethanol. According to Nawaz et al.,[21] the combination of polar and nonpolar solvents increased the extraction efficiency while maintaining good antioxidant activity. We also observed that the yield of extracts from the azeotropic solvent was higher than that from either water or ethanol alone. However, the azeotropic extract contained more water-soluble compounds than the ethanol extract, mainly because the overall yield of the water extract was higher than that of the ethanol extract. Moreover, *P. sajor-caju* extracts were not toxic to yeast cells grown in a YPD liquid medium.

Table 2 shows that irradiation may have enhanced the TPC and TFC. Some studies have reported that ionizing radiation can increase the TPC content in food products.[22] Phenolics and flavonoids are natural antioxidants that scavenge free radicals by donating electrons and neutralizing the radicals.[23, 24]

The antioxidant activities were determined by performing DPPH and FRAP analysis, as shown in Figs. 2 and 3. The DPPH radical scavenging activity and FRAP were highest in extracts that contained high TPCs and TFCs because these extracts had a high hydrogen-donating capacity, thereby scavenging DPPH and FRAP radicals. These trends have also been observed by Boonsong et al.[19] According to Luciana et al.,[25] radiation can increase the total antioxidant activity, and the antioxidant activities have a high correlation with TPCs in fruits. Phenolic compounds are secondary metabolites produced in plants and contain an aromatic ring with at least one hydroxyl group, which can neutralize reactive species and help protect against oxidative stress.[26]

The SR was higher, whereas mutation frequency was lower, for yeast cells with the addition of *P. sajor-caju* extracts than those without extracts. The mutation frequency shows a maximum at an absorbed dose of 100 Gy and tends to decrease at a dose of 150 Gy due to cell injury and leak for repair. This is consistent with the work reported by Matuo et al.,[20] whereby the SR of *rad52* yeast cells with epigallocatechin gallate (EGCg) was higher than those without EGCg and decreased when the radiation dose was increased. The *P. sajor-caju* extracts contain a natural radioprotector, comprising a mixture of active compounds, which is different from other studies that used single compounds, such as EGCg, vitamin C, and epicatechin. In the present study, the specific compounds imparting the highest effects of radioprotection were not identified. However, the phenolic and flavonoid contents of the extracts from non-irradiated and irradiated *P. sajor-caju* were obtained. Notably, flavonoids have a protective effect on cell damage induced by ·OH.[27]

According to Matuo et al.,[20] for yeast cells without radioprotective additives, the cells survived due to their repair mechanism. They used yeast strain *rad52*$^{-}$ because the cells do not have repair genes for double-strand breaks (DSB) in the DNA, and strain S288c was used as a control. When the repair gene was removed from the yeast cells (strain *rad52*$^{-}$), higher cell death was observed compared with the control (strain S288c). This showed that yeast cells survived due to their efficient repair mechanism, where most of the DSB damage induced by gamma radiation was successfully repaired. Furthermore, the results suggested that the main cause of cell death by radiation was DSBs in the DNA.

Owing to the repair mechanism, the yeast cells with the addition of *P. sajor-caju* extract survived due to the presence of radioprotectors that scavenged free radicals. Gamma radiation is a type of radiation with low linear energy transfer, and thus the cell damage is predominantly caused by indirect effects such as water radiolysis. However, a radioprotector may protect the cells by scavenging free radicals created from the indirect effects of radiation before they damage and kill the yeast cells.

5. Conclusions

We studied the effects of radiation on the bioactive compounds of *P. sajor-caju* and evaluated their ability to protect yeast cells from radiation damage. The results suggested that the possibility of gamma radiation increased the bioactive contents of *P. sajor-caju* as well as their antioxidant activities. Furthermore, these bioactive compounds protected yeast cells against damage from ionizing radiation. Overall, the extracts containing bioactive compounds from *P. sajor-caju* have radioprotective properties, as indicated by the increased SR and decreased mutation frequency.

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要　旨

Pleurotus sajor-caju の生理活性抽出物による酵母細胞へのγ線照射に対する抗酸化特性および放射線防護効果

Rosnani Abdul Rashid[1]，松尾陽一郎[2]，泉　佳伸[3,†]

[1]マレーシア原子力庁農業技術・生物科学部門，[2]福井大学大学院工学研究科，
[3]福井大学附属国際原子力工学研究所
[†]y_izumi@u-fukui.ac.jp

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電離放射線の照射により生じたアニオンラジカル，カチオンラジカルおよび中性フリーラジカル等の中間活性種により，生物学的損傷が誘発される可能性がある。この論文では，出芽酵母の野生株に対する *Pleurotus sajor-caju* 抽出物の抗酸化特性および放射線防護効果について研究した。フェノール類とフラボノイドは，抗酸化物質として知られる生理活性化合物であり，健康に有益であるとして注目されている。*Pleurotus sajor-caju* 抽出物の総フェノール含量についてはフォーリン–チオカルト法によって，総フラボノイド含量については塩化アルミニウム法によって決定した。抗酸化活性については1,1-ジフェニル-2-ピクリヒドラジルに対するフリーラジカル消去能および，鉄イオンに対する還元反応を利用した抗酸化能分析によって評価した。γ線を照射した酵母細胞に対する，抽出物の放射線防護効果について，生存率および突然変異誘発頻度に基づいて評価した。抽出物を添加した場合，照射による酵母細胞の生存率は未添加の場合と比較して上昇し，突然変異誘発頻度は低下した。この結果は *P. sajor-caju* 抽出物に含まれる生理活性化合物がγ線によって誘発される損傷を低減したことを示している。以上より，*P. sajor-caju* 抽出物に含まれる生理活性化合物は，電離放射線の影響を軽減する可能性がある。