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Radioprotective effect and antioxidant activities of Thai Gac fruit (*Momordica cochinchinensis* Spreng)

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ABSTRACT

Background: Radiation therapy or radiotherapy has been used to treat cancer which often causes significant adverse effects due to its cytotoxicity on both cancer and nearby intact cells. In addition, ionizing radiation generates free radicals which can damage cellular component leading to DNA damage and cell death. All cells and tissues have variable sensitivity and response to radiation injury. An antioxidant, a stable molecule, which can donate an electron to neutralize free radical. It delays or inhibits cellular damage mainly through its free radical scavenging property. Recently, there has been increasing interest in the prevention of disease mediated by free radicals. Gac fruit (*Momordica cochinchinensis* Spreng) is a major source of β-carotene and lycopene which can be found mainly in the red seed membrane. Both are well known antioxidant agents that can resist the negative effects of free radicals.

Objectives: The objective of this study was to determine the radioprotective effect, total phenolic content and antioxidant properties from various parts of Thai Gac fruit extracts.

Materials and methods: Each part of Thai Gac fruit including peel, pulp, aril, and seed were separately extracted using different solvents, 95% ethanol and ethyl acetate, for extraction. The total phenolic content and antioxidant activities were examined using DPPH radical scavenging assay and lipid peroxidation inhibition assay. Radioprotective effect of the extracts was evaluated by Dicentric Chromosome (DC) assay on TK6 lymphoblast cells after gamma irradiation.

Results: The ethanolic extraction of peel (EtP) showed the highest total phenolic content (140.43±0.63 mg GAE/gm), along with significant antioxidant activity which the values of SC_{50} were 3.72±0.12 mg/ml and 2.57±0.36 mg/ml for DPPH scavenging and lipid peroxidation inhibition, respectively. However, EtP and the ethyl acetate extract of pulp (EACN) exhibited higher LC_{50} values when compared to LC_{50} values of L-ascorbic acid (SC_{50}=0.25±0.04 mg/ml) and α-tocopherol (LC_{50}=0.85±0.14 mg/ml) as standard reagents. Cytotoxicity of Thai Gac fruit was studied on TK6 cells which revealed non-toxic at the concentrations up to 100 µg/ml and 25 µg/ml for EtP and EACN, respectively. Moreover, the DC assay showed that EtP exhibited radioprotective effect in a dose-dependent manner while EACN could reduce radiation-induced chromosomal abnormalities in TK6 lymphoblast cells.

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Conclusion: The ethanolic extract of peel (EtP) exhibited the highest level of antioxidant activities and radioprotective effect when compared to those of other solvent extractions, suggesting it might be used as a potential natural radioprotective agent. It was found that EtP could reduce the damage caused by radiation. Our findings suggested that the peel of Thai Gac fruit extracted by 95% ethanol exhibited a potent antioxidant activity and radioprotective effects in cell line.

Introduction

Radiotherapy is a fundamental approach in cancer treatment, used in around 60-80% of cases, due to its effectiveness in eradicating cancer cells.[1,2] Cancer is also known as salient "free radical" disease. The initiation and promotion of cancer have been associated with chromosomal defects and oncogene activation. Free radicals from those of endogenous reactions and initiated by ionizing radiation, may lead to tumor formation. However, ionizing radiation (IR) affects not only cancerous cells but also surrounding healthy tissues, resulting in various side effects. These adverse effects arise from both direct and indirect IR damage. Direct effects involve DNA strand breaks, while indirect effects are caused by reactive oxygen species (ROS) like hydroxyl radicals, which are produced from radiolysis of water within cells.[1] These ROS attack cellular structures, leading to inflammation, genomic instability, and long-term health risks such as fibrosis and secondary cancers.[2,3] The toxicity to normal tissues often limits the IR dose, highlighting the need for protective agents that can mitigate these negative impacts and optimize therapeutic outcomes.[4]

Amifostine, a widely studied synthetic radioprotector, has proven effective in minimizing radiation damage to healthy tissues. However, many other synthetic radioprotectors have been limited in use due to toxicity and short duration of effect.[4] This has led to a growing interest in natural products as safer, more sustainable alternatives. Natural compounds from medicinal plants have been reported to exhibit radioprotective effects through various mechanisms, including DNA repair enhancement, anti-inflammatory activity, upregulation of intracellular radioprotective enzymes, modulation of signaling and apoptotic pathways, immunomodulation, and free radical scavenging.[1,2,5,6] These agents, particularly antioxidants, neutralize reactive oxygen species (ROS) generated by radiation, reducing oxidative stress and protecting critical cellular components like DNA and lipids from damage.[7] All antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Examples of natural compounds with radioprotective effects include polyphenols like quercetin and curcumin, as well as resveratrol and genistein, which have shown antioxidant and anti-inflammatory benefits in studies.[2,3] Additionally, the flavonoid apigenin, administered post-irradiation in an *in vivo* study, showed strong radioprotective effects by reducing oxidative DNA damage in bone marrow cells and suppressing inflammatory responses in bone marrow-derived macrophages.[8] These findings collectively highlight the potential of natural products as viable radioprotective agents.

Gac fruit (*Momordica cochinchinensis* Spreng.), native to Southeast Asia-particularly Thailand and Vietnam-is a dioecious vine traditionally valued for its medicinal properties and nutrient profile.[9,10] The aril and peel of Gac are rich in carotenoids, including β-carotene and lycopene, which are known for their antioxidant, anti-inflammatory, and anticancer properties.[9,11,12] Lycopene, in particular, has shown radioprotective effects by scavenging free radicals, enhancing cellular antioxidant defenses, and reducing radiation-induced DNA and lipid damage.[13] Additionally, several studies have identified phenolic compounds and flavonoids in Gac, further enhancing its antioxidant potential.[9] Considering the bioactive compounds and biological activity, Gac shows strong potential as a natural radioprotective agent. However, research on its specific radioprotective effects remains limited.

Therefore, this study aimed to address this gap by analyzing the total phenolic content and antioxidant activities of various Thai Gac extracts and assessing their radioprotective effects.

Materials and methods

Preparation and extraction

Thai Gac fruits (*M. cochinchinensis* Spreng.) were collected from Sai Noi district, Nonthaburi province, Thailand. The fruits were thoroughly washed and divided into 4 parts: peel, pulp, aril, and seeds (Figure 1). Each part was dried in a hot air oven at 60 °C and ground into a fine powder.[12] For extraction, 200 grams of each powdered sample was separately macerated in 1000 ml of ethyl acetate or 95% ethanol, in a ratio 1:5, at room temperature with occasional shaking. After 5 days, the extracts were filtered through cotton wool and then through filter paper using a Buchner funnel. The filtrates were pooled, and the solvent was removed using a rotary evaporator. The crude extracts were stored at 4 °C for future use, and the extraction yield was calculated based on the dry weight of each sample.

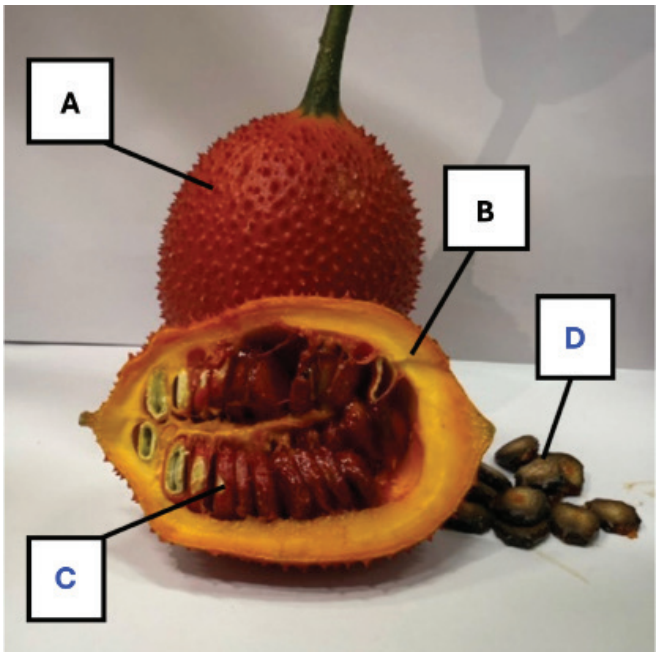


Figure 1. *Parts of Thai Gac fruit (Momordica cochinchinensis Spreng). A: peel, B: pulp, C: aril, D: seed*

Total phenolic content using Folin-Ciocalteu assay

The total phenolic content (TPC) was evaluated using the Folin-Ciocalteu assay.[14] A 50 µL aliquot of the extract (1 mg/mL) and 50 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) at 0.5 mg/mL in ethanol were added to the wells of a 96-well microplate. Then, 75 µL of 7.5% sodium carbonate solution (Loba Chemie, India) was added to each well. Distilled water was used as the control, and different concentrations of gallic acid (Sigma-Aldrich, USA) served as the standard. The reaction was allowed to proceed in the dark at 25 °C for 90 minutes. Absorbance was measured at 725 nm using a microplate reader. The total phenolic content was calculated based on a standard curve of gallic acid and reported as mg of gallic acid equivalents (GAE)/gm of extract. Each extract was tested in triplicate.

Antioxidant activity

DPPH free radical scavenging activity

The antioxidant activity of the Gac extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, following the method described by.[14] Each extract was prepared at varying concentrations and added (50 µL) into the wells of a 96-well microplate. Then, 50 µL of DPPH solution (Sigma-Aldrich, USA) at 0.5 mg/mL was added to each well. The reaction was allowed to proceed in the dark at 25 °C for 30 minutes. L-ascorbic acid (Sigma-Aldrich, USA) was used as the standard. Absorbance was measured at 515 nm using a microplate reader. The percentage of DPPH radical scavenging activity was calculated using the following formula (1):

$$\%Scavenging = [(A_0-A_1)/A_0]\times 100 \text{ — (1)}$$

Where:

A_0 represented the absorbance of the control,

A_1 represented the absorbance of the samples.

The percentage of DPPH radical scavenging activity was plotted against the extract concentrations, and the concentration required to achieve 50% antioxidant activity (SC_{50}) was calculated from the graph and reported. Experiments for each extract were performed in triplicate.

Lipid peroxidation inhibition

Lipid peroxidation inhibition was assessed using the Ferric-thiocyanate method as described elsewhere.[14] Aqueous solutions of each extract were prepared at varying concentrations (0.001-10 mg/mL). A 50 µL aliquot of each sample solution was mixed with 50 µL of linoleic acid dissolved in 50% DMSO (Sigma-Aldrich, USA) in a microplate. To initiate the reaction, 50 µL of 5 mM NH_4SCN and 50 µL of 2 mM $FeCl_2$ were added. The reaction mixture was incubated at 37 °C for 60 minutes in the dark. The α-tocopherol (Sigma-Aldrich, USA) was used as the standard. Absorbance was measured at 490 nm using a microplate reader, and the percentage of lipid peroxidation inhibition was calculated using the following formula.

$$\%lipid\ peroxidation\ inhibition = [(A_0-A_1)/A_0]\times 100 \text{ — (2)}$$

In this calculation, A_0 represented the absorbance of the control, and A_1 represented the absorbance of the sample. The graph showing the relationship between the percentage of lipid peroxidation inhibition and the extract concentration was plotted. The concentration required to achieve 50% inhibition of lipid peroxidation (LC_{50}) was then reported. Triplicate testing was conducted for each extract.

Cytotoxicity assay

Cell viability was evaluated to determine the effect of the extracts on cell survival using the MTT assay, measuring the number of live cells. This assay is based on the principle that living cells produce enzymes capable of reducing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into formazan which absorbs light at a wavelength of 570 nm, allowing quantification of live cells. In this experiment, 10,000 TK6 cells/well (50 µL) were cultured in 96-well plates with different concentrations of the extracts. The plates were incubated in a cell culture chamber at 37 °C with 5% CO_2 for 24 hours. Following incubation, MTT solution was added, and the microplates were incubated for an additional 3 hours. After that, 200 µL of DMSO was added, and absorbance was measured at 570 nm. The percentage of cell viability was then calculated in comparison to the control group. The IC_{20} values (the concentration that inhibits 20% of cell growth) were determined using nonlinear regression analysis (log(inhibitor) vs response). These concentrations were further confirmed to be non-toxic (p>0.05) using One-way ANOVA followed by Dunnett's post-hoc test compared to the untreated control group. Triplicate testing was conducted for each extract.

Radioprotective activity using the Dicentric assay

Radioprotective effect of the selected extracts was assessed based on the principle that radiation can cause DNA strand breaks, particularly double-strand breaks, leading to errors in the cell's repair process. As a result, dicentric chromosomes, having two centromeres indicating chromosomal abnormalities are formed. In this study, the Dicentric Assay was used to evaluate the radioprotective effects of the extracts. TK6 lymphocyte cells were treated with varying concentrations of the test extracts, as indicated by the cytotoxicity assay, and then exposed to gamma radiation (^{137}Cs) at a dose of 1 Gy with a dose rate of 3.13 Gy/min at the Nuclear Technology Research Center (NTRC). Amifostine served as the standard. A total of six experimental tubes were used for this assay as follows.[13,15]

Tube 1: TK6 lymphocyte cells control (no extract and radiation)

Tube 2: TK6 lymphocyte cells+1 Gy; (no extract)

Tube 3-5: TK6 lymphocyte cells+extract at 3 different concentration + 1 Gy

Tube 6: TK6 lymphocyte cells+Amifostine+1 Gy

Following irradiation, the cells were supplemented with RPMI-1640 culture medium with 10% Fetal bovine serum, then incubated at 37 °C with 5% carbon dioxide for 24 hours. After incubation, 100 µL of Colcemid solution was added to arrest cell division at metaphase by inhibiting spindle fiber formation, followed by an additional 24-hour incubation. Cells were then fixed and harvested by centrifugation, with the supernatant removed and replaced by 0.075 M potassium chloride (KCl) solution. The fixative of methanol and acetic acid (3:1) was gradually added with gentle mixing to prevent clumping. After three cycles of centrifugation and resuspension in fresh fixative, slides were prepared by placing the cell suspension onto slides and staining with 2% Giemsa in phosphate buffer (pH 6.8). After washing and drying, coverslips were mounted with a mounting medium. Dicentric chromosome counts were analyzed under a microscope, comparing irradiated and extract-treated samples with non-irradiated, extract-free tube as controls and Amifostine as the positive standard. Triplicate testing was conducted for each extract.

Statistical analysis

The results of total phenolic content and antioxidant activity were presented as the mean ± standard deviation (SD). For the cytotoxicity study, the IC_{20} values were determined using nonlinear regression analysis (log (inhibitor) vs. response). Statistical significance between the control and extract-treated groups was evaluated using one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc test. A p<0.05 was considered statistically significant, while concentrations with p>0.05 compared to the control were selected as non-toxic doses for the radioprotective assay.

Results

Plant extraction

Extracts from different parts of the Gac fruit were obtained through maceration using ethyl acetate or 95% ethanol as solvents, at a material: solvent ratio of 1:5. After filtration, solvent removal, crude extracts were produced, with yields and extract characteristics summarized in Table 1.

Table 1. The percentage yield (%Yield) from different parts of Gac extracts.

Parts of Gac fruit	Solvent	Dried plant (gm)	Crude extract (gm)	Yield (%)
Peel	Ethyl acetate	200.21	0.86	0.43
	95% Ethanol	200.28	2.11	1.05
Pulp	Ethyl acetate	144.10	0.93	0.64
	95% Ethanol	154.63	5.14	3.32
Aril	Ethyl acetate	204.00	38.52	18.88
	95% Ethanol	204.87	26.78	13.07
Seed	Ethyl acetate	200.09	8.09	4.04
	95% Ethanol	201.12	11.09	5.51

The percentage yield (%Yield) from different parts of Gac fruit extracts was obtained, using ethyl acetate or 95% ethanol as solvents. The results indicated that the aril with ethyl acetate extract showed the highest %yield (18.88%), while the ethanolic extract showed 13.07%. Moreover, the ethyl acetate extract of the peel and pulp showed the lowest yield, at 0.43 and 0.64, respectively.

Total phenolic content using Folin-Ciocalteu assay

The total phenolic content (TPC) was analyzed using the Folin-Ciocalteu reagent method, based on the redox reaction of molybdotungstate ions. The total phenolic content was determined using a standard curve of gallic acid and expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/gm). The results showed in Table 2, the highest TPC was found in the ethanolic extract of peel (140.43±0.63 mg GAE/gm), followed by the ethyl acetate extract of peel (136.03±0.91 mg GAE/gm). The ethyl acetate extract of seed showed a substantial level (110.23±0.89 mg GAE/gm), while the lowest TPC was observed in the ethanolic extract of seed (6.18±0.24 mg GAE/gm).

Table 2. Total Phenolic Content (TPC) and antioxidant activity from different parts of Gac fruit's crude extracts

Parts of Gac fruit	Solvent	Total phenolic content	Antioxidant activity (mg/mL)	
		(mg GEA/gm)	DPPH assay (SC_{50})	Lipid peroxidation inhibition (LC_{50})
Peel	Ethyl acetate	136.03±0.91	10.15±1.55*	3.09±0.62*
	95% Ethanol	140.43±0.63	3.72±0.12*	2.57±0.36*
Pulp	Ethyl acetate	82.34±0.75*	3.79±0.14*	3.17±0.78*
	95% Ethanol	91.46±0.56*	6.10±0.10*	4.22±0.99*
Aril	Ethyl acetate	58.33±0.68*	10.21±0.85*	5.43±0.45*
	95% Ethanol	20.99±0.83*	5.76±0.15*	3.74±0.15*
Seed	Ethyl acetate	110.23±0.89*	36.70±1.36*	5.41±0.42*
	95% Ethanol	6.18±0.24*	13.88±0.94*	10.60±1.7*
L-ascorbic acid	-	ND	0.25±0.04*	ND
α-tocopherol	-	ND	ND	0.85±0.14*

Note: **significant differences at p<0.05, ND: not determined.*

Antioxidant activity

The study of antioxidant activity using the DPPH method, the ethanolic extract of peel's Gac fruit exhibited the highest free radical scavenging activity (SC_{50}=3.72±0.12 mg/mL), followed by the ethyl acetate extract of pulp (SC_{50}=3.79±0.14 mg/mL, respectively. As for the extraction of seed in ethyl acetate and 95% ethanol showed the lowest activity (SC_{50}=36.70±1.36 mg/mL and 13.88±0.94 mg/mL, respectively) when compared to other parts. All extracts were significantly less effective than the L-ascorbic acid standard (SC_{50}=0.25±0.04 mg/mL) at p<0.05. Similarly, in the lipid peroxidation inhibition assay, the ethanolic extract of peel's Gac fruit exhibited the highest activity (LC_{50}=2.57±0.36 mg/mL), followed by ethyl acetate extracts of peel and pulp (LC_{50}=3.09±0.62 mg/mL and 3.17±0.78 mg/mL, respectively). In addition, the ethanolic extract of seed showed the lowest lipid peroxidation inhibition (LC_{50}=10.60±1.71 mg/ml) when compared to other extracts. The activity of all extracts was significantly lower than α-tocopherol standard (LC_{50}=0.85±0.14 mg/mL) at p<0.05). The results of total Phenolic Content (TPC) and antioxidant activity from different parts of Gac fruit's crude extracts were shown in Table 2.

Cytotoxicity assay

Based on the results of total phenolic content and antioxidant activity using DPPH assay and Lipid peroxidation inhibition, the ethanolic extract of peel (EtP) and the ethyl acetate extract of pulp (EACN) from Gac fruit were identified as promising candidates for further study on radioprotective effects. Consequently, these two extracts were selected for additional evaluation of their protective effects against radiation-induced cellular damage. The investigation would begin with cytotoxicity study on TK6 cells, a lymphoblast cell line, at various extract concentrations to determine the optimal non-toxic concentration range for subsequent radioprotective studies.

The cytotoxicity assay showed that after a 24-hour incubation of TK6 cells with EtP extract at concentrations ranging from 6.25 to 200 µg/mL, control cells exhibited 100% viability (Figure 2). Cells treated with 0.1%DMSO as a solvent and EtP at concentrations between 6.25 and 100 µg/mL-maintained above 80% cell viability with values ranging from 80.32% to 86.61%. However, at 200 µg/mL, EtP reduced cell viability to 63.37%. Therefore, the non-toxic concentration range (IC_{20}) for EtP was identified as 6.25 to 100 µg/mL, which will be selected for radioprotective assay. Similarly, TK6 cells were treated with EACN extract (6.25 to 200 µg/mL), where control cells also exhibited 100% cell viability. Cells treated with DMSO and EACN concentrations between 6.25 and 25 µg/mL maintained over 80% cell viability, ranging from 81.70% to 90.11%. In contrast, EACN concentrations ranging from 50 to 200 µg/mL resulted in viabilities below 80%, with values ranging from 43.26% to 70.31%. Consequently, the non-toxic range (IC_{20}) for EACN was established at 6.25 to 25 µg/mL, which was selected for further radioprotective studies.

Radioprotective activity using the Dicentric assay

In the evaluation of radioprotective activity using the Dicentric assay, TK6 cells were incubated with extracts or Amifostine at non-toxic concentrations for 24 hours before exposing to 1 Gy of gamma radiation (^{137}Cs). Chromosomal abnormalities were assessed at the metaphase stage, with results recorded as the number of cells exhibiting dicentric chromosomes out of 50 analyzed metaphase cells. Untreated but irradiated cells served as the positive control, while untreated and unirradiated cells served as the negative control.

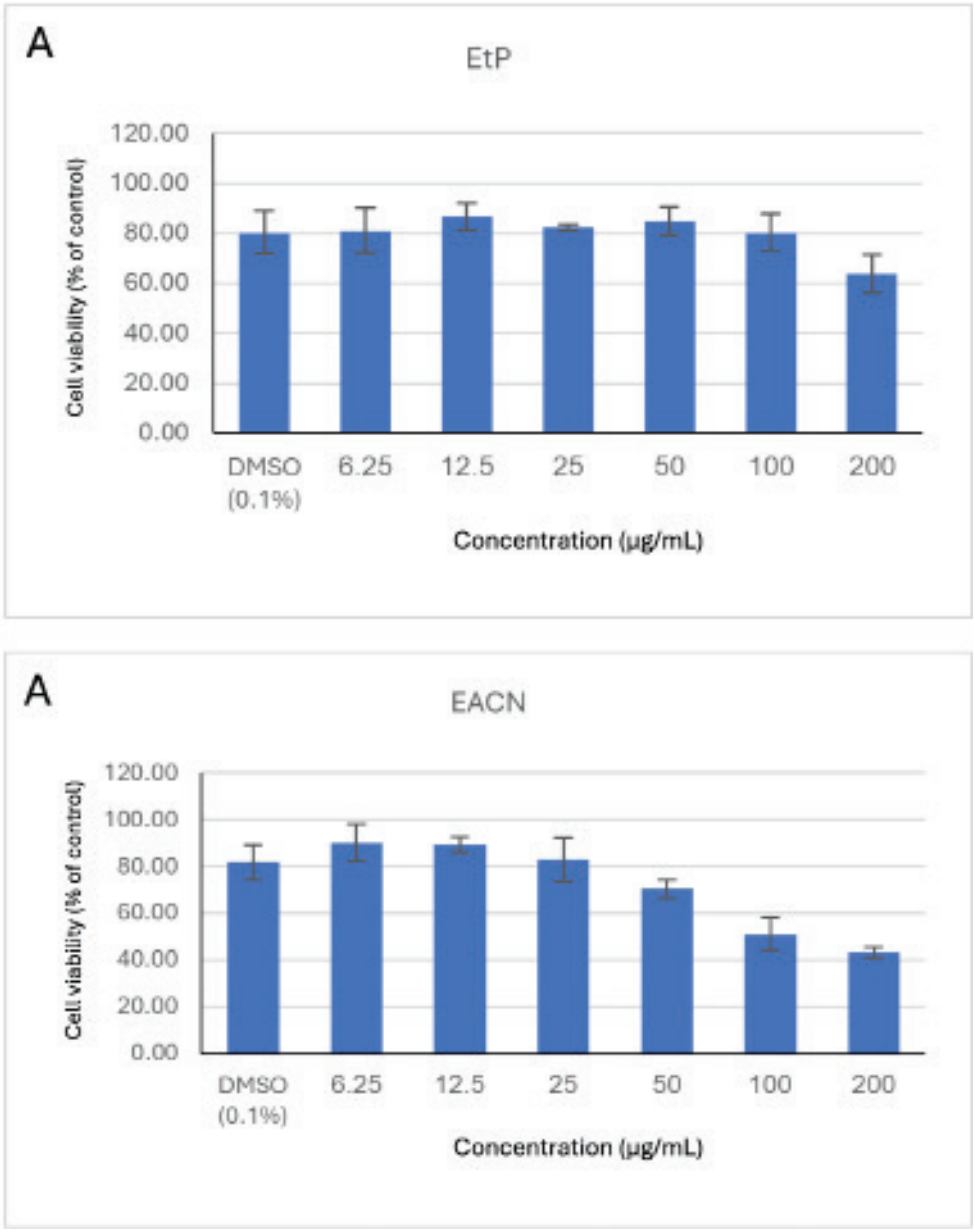


Figure 2. *Percentage viability of TK6 cells incubated with EtP (A) and EACN (A).*

Table 3 presents the percentage of TK6 cells displaying dicentric chromosomes (DC) across various treatments and concentrations. The ethanolic extract of the peel (EtP) demonstrated a promising radioprotective effect, with a dose-dependent decrease in DCs. The percentage of DCs was reduced to 31.3%, 50.7%, and 76.0% at EtP concentrations of 100, 50, and 25 µg/mL, respectively. The ethyl acetate extract of pulp (EACN) showed limited radioprotective effects, with high DC levels of 79.3%, 86.0%, and 96.0% at concentrations of 25, 12.5, and 6.25 µg/mL, respectively. As the standard, Amifostine demonstrated the strongest radioprotective effect, with DC levels ranging from 15.3% to 64.7% across different concentrations. The positive control (radiation treatment only) showed 96.7% DCs, while the negative control (no radiation treatment) showed no DCs, validating the experimental setup.

Discussion

Gac fruit (*Momordica cochinchinensis* Spreng.) is known for its abundance of bioactive compounds, including β-carotene, lycopene, and various phenolic compounds, which contribute to its antioxidant and health-promoting properties. Studies have shown that these bioactive compounds in Gac fruit possess antioxidant, anti-inflammatory, and anticancer activities.[9,11,12] Considering their protective effects against oxidative stress, these compounds have significant potential for radioprotective applications. To date, no studies have investigated the radioprotective activity of Gac fruit, making this research the first to explore this potential aspect.

In this study, the total phenolic content and antioxidant activity of Gac fruit demonstrated significant potential, particularly in the peel extract

with 95% ethanol, which exhibited the highest phenolic content. This aligns with findings from Abdulqader *et al*. which showed that aril and peel extracts of Gac possess high levels of carotenoids, phenolics, and flavonoids.[16] The total phenolic content in Gac fruit varies across different parts at different age, as reported by multiple studies.[16] Notably, phenolic compounds such as rutin, cinnamic acid, and 2-(3,4-dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid are particularly prevalent in the peel, while hydroxybenzoic acids (e.g., gallic acid, protocatechuic acid, p-hydroxybenzoic acid) and hydroxycinnamic acids (e.g., chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid) are found throughout all parts of the fruit.[16,17] These phenolic compounds are potent antioxidants that act in a structure-dependent manner, with radical-scavenging and metal-chelating abilities being key mechanisms in oxidative stress protection. Multiple studies have demonstrated a strong correlation between total phenolic content and the antioxidant activity of plant extracts, supporting Gac's potential for health-promoting and therapeutic applications.[9,10,16,17]

Table 3. Percentage of cells with dicentric chromosomes (DC) following 1 Gy gamma radiation exposure (^{137Cs})

Samples	**Concentration** (µg/mL)	**Cells in metaphase**	**Dicentric cells (DC)** Mean±SD	**% Dicentric cells**
Ethanol extract of peel (EtP)	100	50	15.7±3.9	31.3
	50	50	25.3±1.7	50.7
	25	50	38.0±3.7	76.0
Ethyl acetate extract of pulp (EACN)	25	50	39.7±4.0	79.3
	12.5	50	43.0±3.7	86.0
	6.25	50	48.0±1.4	96.0
Amifostine	100	50	7.7±1.2	15.3
	50	50	11.7±2.6	23.3
	25	50	19.3±3.1	38.7
	12.5	50	24.0±4.3	48.0
	6.25	50	32.3±4.1	64.7
Positive control (radiation treatment only)		50	48.3±0.5	96.7
Negative control (no radiation treatment)		50	0	0

The antioxidant activity was confirmed by DPPH radical scavenging and lipid peroxidation inhibition assays, where ethanolic extract of peel (EtP) exhibited the strongest DPPH radical scavenging activity, followed by the ethyl acetate extract of pulp (EACN), similar to findings by Reungpatthanaphong *et al,*.[10,12] which showed that peel extracts from Thai Gac fruit displayed the highest overall antioxidant activities, particularly from FRAP assay. The antioxidant activity of EtP was positively correlated with its total phenolic content which was the highest among the extracts tested. This result is consistent with previous studies that report a strong correlation between the high phenolic content of Gac fruit and its potent antioxidant activity.[18] Additionally, the lipid peroxidation inhibition assay further supported these findings, with both the ethanolic (EtP) and ethyl acetate (EACP) extracts of peel, ranking as the most effective in inhibiting lipid peroxidation, a key mechanism for preventing oxidative degradation of cellular membranes and protecting against oxidative damage.[13] These extracts were found to contain the highest level of phenolic compounds which likely contribute to their protective effects against oxidative DNA damage.

Regarding the extraction yield, our results showed that while the aril exhibited the highest percentage yield (18.88%), it did not correlate with the highest antioxidant activity. This discrepancy suggests that a high yield in the aril is primarily composed of bulk lipids and carotenoids, whereas the ethanolic extract of the peel (EtP), despite its lower yield (1.05%), contains a more concentrated profile of polar phenolic compounds. These specific phenolics are more effective in DPPH radical scavenging and protecting against radiation-induced damage, indicating that the quality and concentration of bioactive phytochemicals are more critical than the total extraction yield.[20]

Previous studies have reported that phenolic compounds such as rutin and quercetin acted as a metal ion chelator inhibiting iron ion-dependent lipid peroxidation.[6,19,20] Interestingly, the ethyl acetate extract of pulp (EACN) also demonstrated notable lipid peroxidation inhibition despite its lower phenolic content. This may be attributed to the presence of

high levels of other bioactive compounds, particularly β-carotene and lycopene, which are known to enhance lipid protection.[16,17] While EtP demonstrated superior activity due to its high phenolic concentration, the notable effectiveness of EACN suggests a synergistic effect between its phenolic constituents and rich carotenoid content, such as β-carotene and lycopene. This explains why the aril, despite having the highest yield and carotenoid levels, did not exhibit the strongest antioxidant activity, as the specific combination and concentration of bioactive compounds in the peel and pulp may provide more potent protective mechanisms against oxidative stress. These consistent findings across studies emphasize the potential of Gac fruit, particularly its peel and pulp, as rich sources of antioxidants, which may be valuable for radioprotective applications, demonstrating the capacity to mitigate radiation-induced cellular damage via oxidative stress reduction.

Based on the total phenolic content analysis and antioxidant assays, the ethanolic extract of peel (EtP) and the ethyl acetate extract of pulp (EACN) were selected as candidates for evaluating radioprotective activity using the dicentric chromosome (DC) assay, a widely recognized method for assessing chromosomal damage and repair following radiation exposure.[16] The DC assay results indicated that EtP exhibited a dose-dependent reduction in dicentric chromosomes in a lymphoblast cell line (TK6 cells), showing greater effectiveness than EACN in reducing radiation-induced cellular damage. The safety profile of the extracts was confirmed using the TK6 human lymphoblast cell line, which is a standard model for genetic toxicology and radiation research due to its stable karyotype and p53 competency.[15] The IC_{20} values identified in this study (up to 100 µg/mL for EtP) demonstrate that the radioprotective effects occur at non-toxic concentrations. While this study focused on TK6 cells to maintain consistency with the Dicentric Chromosome Assay, further studies on a broader range of normal human cell lines would be beneficial to fully establish the clinical safety of Thai Gac fruit extracts. This finding aligned with Arora *et al.* who demonstrated that natural compounds with high phenolic content enhance DNA repair mechanisms and act as antioxidants, counteracting radiation-induced reactive oxygen species (ROS) damage.[6]

Beyond direct free radical scavenging, the radioprotective mechanisms of EtP may involve the upregulation of endogenous antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which reinforce the cellular defense system against oxidative stress. Furthermore, bioactive compounds in Gac peel, particularly phenolic acids and flavonoids, have been reported to enhance DNA repair pathways and modulate inflammatory signaling, such as inhibiting pro-inflammatory cytokines that contribute to secondary radiation injury.[13,21] Supporting studies by Srinivasan *et al.* and Cavusoglu and Yalcin demonstrated lycopene's ability to reduce radiation-induced chromosomal damage, such as dicentrics and micronuclei, in human lymphocyte by reducing lipid peroxidation and enhancing antioxidant enzyme activities like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[13,21]

Limitations

This study is primarily limited by its focus on the TK6 human lymphoblast cell line, requiring further research on a broader range of normal human cell lines to fully establish clinical safety.

Conclusion

This study demonstrated the potent antioxidant and radioprotective activities of Thai Gac fruit (*Momordica cochinchinensis* Spreng.), particularly from the ethanolic extract of peel (EtP). Among the different parts tested, EtP exhibited the highest total phenolic content and showed superior performance in both DPPH radical scavenging and lipid peroxidation inhibition assays, which correlated well with its radioprotective efficacy. The results of Dicentric Chromosome (DC) assay revealed that EtP effectively reduced radiation-induced chromosomal damage, suggesting its potential role in radiation protection. Notably, the peel, often considered a byproduct, was identified as the most promising source of bioactive compounds, thus offering added economic and functional value.

Our findings suggest that Gac fruit peel extract had the potential to be developed as a natural radioprotective agent, providing an alternative to synthetic agents like amifostine, with the advantages of reduced side effects and broader clinical applicability. Comparison with the standard radioprotector, Amifostine, revealed that while Amifostine remains the more potent agent, EtP at its highest non-toxic concentration (100 µg/mL) showed a comparable trend in reducing dicentric chromosome formation. The clinical significance of EtP lies in its potential to overcome the limitations of Amifostine, which is often associated with systemic toxicity and adverse side effects such as hypotension. As a natural product derived from agricultural byproducts, EtP offers a safer, cost-effective, and more sustainable approach for radiation protection, particularly in scenarios involving repeated low-dose exposure or as a supportive dietary strategy during radiotherapy. Future studies focusing on the safety and mechanism of action of peel from Gac fruit as bioactive compounds are recommended to further explore its potential in clinical and therapeutic application.

Ethical approval

No study in human or animals was performed by any authors.

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Conflict of interest

The authors declare that there is no conflict of interest regarding in this research, including financial, personal, authorship, or other factors which could affect this research, and all results presented in this article.

CRediT authorship contribution statement

Anont Laorngnual and **Sareeya Reungpatthanaphong**: experimental design, experimental work, results analysis, investigation, writing: draft manuscript; **Paiboon Reungpatthanaphong**: experimental design, experimental work, results analysis, investigation, resources, writing: draft and final preparation.

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