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Radioprotective effects of flavonoid against γ - irradiation in human lymphocyte cell.

Monchanok Bumrungchai[1], Sareeya Reungpatthanaphong,[2] Paiboon Reungpatthanaphong[1,*]

[1]Department of Applied Radiation and Isotope, Faculty of Sciences, Kasetsart University, Bangkok, Thailand,
[2]Thailand institute science and technology research, Khlong Luang, Phatumtani, Thailand.

* Corresponding author: Tel. +66(85) 2155060 E-mail address: fscipbr@ku.ac.th

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Introduction

Free radical is known as one of the factors that cause cellular damage in the body. It plays an important role in metabolism which speeds up the aging process. When the amount of free radical is high, it will cause tissue damage by affecting DNA, protein and fat. Free radicals can be caused by various procedures within and outside the body, which depend on individual way of life such as chemical exposure in a routine and environment, or radiation exposure[1].

Antioxidants can inhibit the damaging effects of free radicals by oxidation in the body, which the body can make its own antioxidants. Also, antioxidants can be found in vitamins, minerals and nutrients from fruits and vegetables as well as Thai vegetables containing vitamins, carotenoids, flavonoids and other phenolic compounds, which have been known as good sources of natural antioxidants. In this study, the antioxidant activity of Riceberry, Muser Purple Rice and pericarp of mangosteen extracts was evaluated, because rice is the basic foodstuff, besides these extracts have been reported to have high anthocyanin and polyphenolic compounds.

Methods

Total phenolic contents

Total phenolic contents was determined by the Folin–Ciocalteu method, which was adapted from Onanong *et al.* (2011)[2,3]. The 100 µL of extract, 1600 µL of diluted water, and 100 µL of 0.25 N Folin–Ciocalteu reagent were combined in a plastic vial and then mixed well using a Vortex. The mixture was allowed to react for 3 min then 200 µL of 1 N Na2CO3 solution was added and mixed well. The solution was incubated at room temperature (25 °C) in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed in gallic acid equivalents using a gallic acid (0–0.1 mg/mL) standard curve. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

Antioxidant activity determinations

The DPPH assay was done according to the method of Kriengsak *et al.* (2006)[2,4] with some modifications. The stock solution was prepared by dissolving DPPH with methanol to obtain an absorbance at 517 nm using the spectrophotometer. The extracts were allowed to react with the DPPH solution for 20 min in the dark. Then the absorbance was taken at 517 nm. The standard curve was linear between 0 and 300 µg/mL Trolox and L-ascorbic acid. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve.

For ABTS assay, the procedure followed the method of Roberta *et al.* (1999)[2,5] with some modifications. The stock solutions included 7 mM $ABTS^{\bullet-}$ solution and 2.45 mM potassium persulfate solution.

The working solution was then prepared by mixing the two stock solutions in ratio 1:0.5 and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1 mL $ABTS^{\bullet+}$ solution with 25 mL distilled water to obtain an absorbance of 0.7-0.9 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. The extracts were allowed to react with the $ABTS^{\bullet+}$ solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 0 and 300 µg/mL Trolox and L-ascorbic acid. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

The FRAP assay was done according to Ronald *et al.* (2005)[2,6] with some modifications. The stock solutions included 3 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl3·$6H_2O$ solution and then warmed at 37 °C before using. The extracts were allowed to react with the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 0 and 400 µM $FeSO_4$. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Cytokinesis-block micronucleus (CBMN) assay

Sampling and irradiation - blood sample was collected into a plastic tube with 0.2 ml Li-Heparin, added various concentrate of extracts (0, 50, 100 mg/ml). Then, they were irradiated by using 3 Gy of Co-60.

Cell culture, fixation, slide preparation and staining - cells cultures, fixation and slide preparation was performed according the International Atomic Energy Agency (IAEA) manual: Cytogenetic Analysis for Radiation Dose Assessment[2,7]. Briefly, whole blood cultures were prepared by adding 0.5 ml blood to 4.5 ml culture RPMI-1640 culture medium supplemented with 10 to 15% fetal calf serum, L-glutamine and antibiotics. 100 µL. Phytohemagglutinin (PHA) was used to stimulate lymphocyte proliferation. The blood was cultured in tissue culture vessels at 37°C, 5% CO_2 in a humidified atmosphere. 20 µL Cytochalasin B (Cyt-B) was added 44 h after PHA stimulation at a concentration of 6 µg/ml to block cells at cytokinesis. After a 72 h incubation period, cells were collected by centrifugation (2000 rpm, for 5 min), hypotonically treaded with cold (4 ºC) 0.075M KCl to lyse red blood cells, and fixed with a fixative solution containing methanol:acetic acid (3:1). The cells were washed with two further exchanges of fixative solution. After fixation, the cells were gently resuspended, dropped onto wet clean glass slides and allowed to dry. Slides were stained in 2% Giemsa solution for 10 min.Slide evaluation - stained samples were evaluated using a microscope at 100 fold original magnification. A total of 1000 binucleated cells were evaluated for the frequency of MN and BN cells.

Results

Total phenolic content

The results showed that mangosteen pericarp extract has the highest amount of phenolic compound, followed by Muser Purple Rice and Riceberry extracts, respectively. As shown in Table 1.

Table 1. The amount of total phenolic compound

Extract	Phenolic content (GAE µg/mL)
Riceberry	57.29±0.32
Muser Purple Rice	72.71±1.46
Pericarp of mangosteen	90.35±0.84

Antioxidant activity determinations

Antioxidant activities of the methanol extracts were evaluated using DPPH, ABTS and FRAP assays. The experiments were measured in triplicate to test the reproducibility of the assays. The result showed that

the best antioxidant activity was pericarp of mangosteen, followed by Muser Purple Rice and Riceberry, respectively. As shown in Table 2.

Table 2. The amount of 50% effective concentration (EC_{50}) of extracts from DPPH, ABTS assay and FRAP value from FRAP assay

Extract	EC_{50} (µg/mL)*		FRAP value (µM)
	DPPH	ABTS	
Riceberry	71.37±0.56	48.04±0.18	181.52±8.19
Muser purple rice	49.24±1.68	29.41±0.78	239.85±7.82
Pericarp of mangosteen	14.05±1.06	5.18±0.16	301.06±42.74

*EC_{50} refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time.

CBMN Assay

The results of the frequencies of MN and BN cells after irradiation by 3 Gy, the results showed that the trends of micronucleus reducing had been depended on concentrate of these extracts. It will be seen that the extract of pericarp of mangosteen is the best performance.

Discussion

The results show that extracts have the amount of phenolic compounds in large quantities which will result in the inhibition of free radicals. In this trial, pericarp of mangosteen revealed the highest antioxidant activity. The result of DPPH, ABTS and FRAP of pericarp of mangosteen were 14.05±1.06 µg/ml, 5.18±0.16 TEAC µg/ml and 301.06±42.74 µM, respectively. Total phenolic compounds of pericarp of mangosteen is 90.35±0.84 GAE µg/mL that is the most value when compares with others. It was also found to decrease micronucleus 54% and 72% at concentration 50 µg/mL and 100 µg/mL, respectively. So, it can conclude that pericarp of mangosteen is effectively antioxidant and has radioprotective effect. Due to the pericarp of mangosteen contains tannin and xanthones that are polyphenolic compounds. And xanthones are strong antioxidant (potent antioxidants) which include the effect to capture free radicals more than other substances.

Conclusion

This study was conducted to determine the radioprotective effect using micronucleus assay (human lymphocyte irradiated at 3 Gy of Cs-137) and antioxidant activity using DPPH, ABTS and FRAP assay. From result showed that the extract of pericarp of mangosteen is the best performance. Therefore, pericarp of mangosteen can be promising radioprotective agent.

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