ORIGINAL ARTICLE

Total Antioxidant Activity and Enzymatic Inhibition against Alpha-Amylase, Alpha-Glucosidase and Pancreatic Lipase of Irradiated *Archidendron bubalinum*

Nor Liyana Ja'afar[1], Chai Pui Teng[2], Noor Syafiqa Aqila Mohd Rosmi[2], Siti Nur Atiqah Rambli[2], Nurul Husna Shafie[2], Daryl J Arapoc[3], Hasnah Bahari[1]

[1] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3] Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.

ABSTRACT

Introduction: *Archidendron bubalinum* is an underutilised plant with numerous antioxidant properties and has a great potential to inhibit enzymes linked with diabetes and obesity. Food irradiation is an advanced technology to prolong the lifespan of plant, prevent physical spoilage and eradicate food borne disease. Present study was aimed to determine the total antioxidant activity, enzymatic inhibition against alpha-amylase, alpha-glucosidase and pancreatic lipase and the toxicity levels of non-irradiated and irradiated (3, 6, 9 & 12 kGy) hot aqueous extract of *A. bubalinum*. **Methods:** The antioxidant ability of the extract was determined by total phenolic content (TPC), total flavanoid content (TFC), Diphenyl-1-Picrylhydrazyl (DPPH), β-carotene assay and ferric reducing antioxidant potential (FRAP) assay. The inhibitory activities were evaluated using α-amylase, α-glucosidase, and pancreatic lipase inhibition assay. The toxicity levels of *A. bubalinum* extract were determined using Brine shrimp and Zebra-fish assays. **Results:** Results showed that irradiated *A. bubalinum* at 12 kGy demonstrated the highest TFC (448.99 ± 5.02 mg GAE/g), FRAP (2.55 ± 0.40 mmol Fe2+/g) and β-carotene bleaching activity (79.49%). Whereas, non-irradiated *A. bubalinum* samples expressed the highest TPC (2517.07 ± 15.81 mg GAE/g) and exhibited the lowest IC_{50} values of α-amylase (31.99 ± 3.15 µg/ml), α-glucosidase (23.40 ± 0.69µg/ml) and pancreatic lipase (32.81 ± 7.96 µg/ml) activity. The toxicity assays also showed no significant different between irradiated and non-irradiated samples. **Conclusion:** The study suggests that gamma irradiation has the prospective future to increase antioxidant properties and maintaining the enzyme inhibitory activities to preserve the sample of *A. bubalinum* for commercial purposes.

Keywords: *Archidendron bubalinum*, Antioxidant, Gamma irradiation, Enzymatic inhibition, Toxicity assay

Corresponding Author:
Hasnah Bahari, PhD
Email: haba@upm.edu.my
Tel: +603-89472769

INTRODUCTION

Food irradiation is defined as a process of physical, non-thermal approach of food preservation at ambient temperature (1). Irradiation treatment can be divided into ionizing or non-ionizing radiation as the main function to preserve the food. The sources of ionizing radiation could be gamma rays, high-energy electrons and X-rays, while the sources of non-ionizing radiation are such as ultraviolet rays, visible light, microwaves and infrared (2). Ionizing radiation involved electromagnetic radiation, generated from Cobalt-60 or Cesium-137 (3) to control the foodborne pathogens, eliminate the microbial loads and insect infestations. Additionally, gamma irradiation together with refrigeration had proved to prolong the shelf life of food products as well as maintain their nutritional and organoleptic quality (4). Nonetheless, food irradiation does not enhance the nutritional properties of the food products but sometimes will diminished the vitamins content but unlikely change the overall competence of dietary intake (5). Besides, a study reported that significant reduction of total ascorbate and significant drop of carotenoids content was detected in nine herb samples (basil, bird pepper, black pepper and others) (6).

Previous study, the irradiation selected local herbs including Hempedu Bumi, Mas Cotek, and Tongkat Ali has shown satisfactory results on microbiological sanitization, with no changes on the total chemical profiles. Moreover, this process did not cause any

significant changes in the content of basic biologically active components of investigated herbal plants (7). In fact, among other viable technologies for food preservation, irradiation of food is known as a safe and effective method for a range of specific applications. Through its use, chemical and physical stability was preserved, and foodborne pathogens can be eliminated, and wider trade of many food items can be expedited. Hence, food irradiation is practicable in our local food processing industries.

Archidendron bubalinum is an underutilised plant from *Fabaceae* family and broadly abundant in Southeast Asia, generally known as "Kerdas or Keredas" (Malaysia), "Nieng-Nok" (Thailand) and "Kabau" (Indonesia) (8). Often consumed as "Ulam" among locals, it is characterised by strong sulphurous odour but disappeared upon cooking (9). It is reported that matured *A. bubalinum* has great content of moisture (52.1%), fair content of crude protein (6-10%) and little fat content (0.23%) as compared to *Pithecellobium jeringa* (10) and loaded with high concentration of flavonoid and alkaloid compound (11). Flavonoid has potential to scavenge free radicals against oxidation and has potential in health management as an anti-inflammatory, anti-allergy, anti-viral and anti-carcinogenic values (12). The substituents from the *A. bubalinum*'s bark has potential as an antipyretic agent (8) whereas the husks contained different phytoconstituents such as hexadecanoic acids that contribute to the antioxidant and antimicrobial activity (13).

Alpha-amylase facilitate the hydrolysis of oligo-saccharide into maltose, maltotriose and small malto-oligosaccharide in stomach and intestine (14). Alpha-glucosidase is also one of the major hydrolysing enzymes helps in digestion and absorption by hydrolysing starch and disaccharides to glucose (15). Pancreatic lipase is produced by the pancreas which responsible for digestion and absorption of fat (16). Therefore, the central focus of this study is to determine the influence of irradiation on enzymatic inhibition of *A. bubalinum* against enzymes linked with diabetes and obesity. Ingestion of *A. bubalinum* was found to cause the formation of sharp needle-like crystals in kidney due to the presence of djenkolic acid in *A. bubalinum* seed (17). Additionally, no literature was found on the toxicity level of *A. bubalinum* up till now. Hence, present study aimed to determine the level of toxicity of non-irradiated and irradiated samples of *A. bubalinum* using Brine shrimp and Zebra fish lethality assays.

Brine shrimp and Zebra fish toxicity assays are the common in-vivo method that have been used for toxicity testing in crude plant samples. Brine shrimp assay required no aseptic techniques and no special equipment (18). Meanwhile, Zebrafish embryo are transparent which easier for the observation to occur in order to obtain lethality of the test plant extracts (19).

MATERIALS AND METHODS

Preparation of sample

The samples of *Archidendron bubalinum* were collected from Selangor, Malaysia. (Voucher No: SK 3155/17). The seeds were separated from its pods then oven-dried at 40°C for 3 days then grinded. The samples were irradiated with different dosages of gamma-ray (3, 6, 9, & 12 kGy) at Malaysian Nuclear Agency. Hot aqueous extraction method was used to extract the samples. Fifty grams of dried samples were added in 500 ml of distilled water then placed in 70°C water bath for 18 hours. The samples were filtered then freeze-dried and stored at -20°C (20).

Total Phenolic Content (TPC)

Twenty microliters of samples and 100 µl Folin-Ciocalteu reagent (diluted 1:10 with deionised water) were mixed together and incubated for 5 minutes. Then, 80 µg of 7 % sodium carbonate (Na_2CO_3) was added and placed in dark at 28°C for 30 minutes then measured at 770 nm. The standard used was gallic acid solutions (21). TPC was noted as mg of gallic acid (GAE) per g of extract samples.

Total Flavonoid Content (TFC)

Twenty-five microliters of extract (5 mg/ml), 100 µl of distilled water and 5% 7.5 µl of sodium nitrite were mixed and set for 5 minutes. Later, 10% 7.5 µl of aluminium chloride hexahydrate was added and incubated for 5 minutes then 50 µl of sodium hydroxide (1M NaOH) and 60 µl of distilled water were added before measured at 405 nm. The standard used was quercetin solutions. TFC was noted in mg of quarcetin equivalents (QE) per g of extract (22).

Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Activity

DPPH powder of 3.9 mg was added in 10 mL of methanol. Next, 10µl from different concentration of sample extracts were pipetted into 100 µl of DPPH solution in dark for 30 minutes them measured at 540 nm (23). The butylated hydroxytoluene (BHT) was used as standard and calculated as follows:

$$\text{Scavenging activity (\%)} = \left[1 - \frac{\text{Absorbance sample}}{\text{Absorbance control}}\right] \times 100$$

Ferric Reducing Antioxidant Potential (FRAP)

Three reagents of 300 mM acetate buffer (pH 3.6) with 1 volume of 10 mM 2, 4, 6 tripyridyl-s-triazine (TPTZ) and 1 volume of 20 mM $FeCl_3.6H_2O$ were prepared. Next, 30 ml of FRAP reagent was warmed at 37°C. Then, 20 µL of sample (62.5, 125, 250, 500 and 1000 µg/ml) was added into 180 µL of the FRAP reagent then warmed for 30 minutes at 37°C before measured at 570 nm. The standard used was Iron sulphate ($FeSO_4$). FRAP data were demonstrated as mmol Fe^{2+}/g dried extract (24).

ß-Carotene Bleaching Activity

One milliliter of beta-carotene, 10 ml of 0.2 mg/ml chloroform, 20µl of linoleic acid and 200µl of Tween 40 were added together then agitated at 40°C. Then, 50 ml of deionised water was put in and vigorously agitated before incubated in 50°C. Two-hundred microliters of aliquots was added into 20 µl of extracts then agitated and incubated in 50°C for 2 hour. Butylated hydroxytoluene (BHT) was used as standard and measured at 492 nm at 0 minute (t = 0) and 120 minutes (t = 120 min) (24).

Alpha-amylase inhibition assay

Half milligram of alpha-amylase and 1 mL of 20mM phosphate buffer (pH 6.9 with 6.7 mM NaCl) were mixed. Next, 50 µL of the α-amylase was added into 50 µL of sample extracts (62.5, 125, 250, 500 and 1000 µg/ml) before warmed at 25°C. After 30 minutes, 50 µL of starch solution (1% w/v) in 20mM sodium phosphate buffer was added then incubated at 25°C for 3 minutes. 100 µL of dinitrosalicylic acid (DNS) color reagents then was added. The mixture was placed in 85°C for 15 minutes and then cooled to room temperature and was read at 540 nm. Acarbose was used as a positive control. α-amylase inhibition was calculated based on the equation below (25):

Inhibition activity (%) = [1-(Δ sample)(Δ control)] x 100

Alpha-glucosidase inhibition assay

Alpha-glucosidase was dissolved in phosphate buffer (0.1 M, pH 6.9) to give a concentration of 1 unit/ml. Next, 50 ml of sample extract (62.5, 125, 250, 500 and 1000 µg/ml) was added into 25 ml of α-glucosidase (0.15 unit/ml) and 25 ml of glutathione (3 mM) then incubated at 37°C for 5 minutes before read at 405 nm. Fifty microliters of p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate solution (5 mM) in 0.1 M phosphate buffer (pH 6.9) was added at 5 seconds intervals. The mixture was then placed at 37°C for 30 minutes. Then, 100 µL of sodium carbonate solution (0.1 M) was added then measured at 405 nm. Acarbose was used as a positive control. The results were calculated as follow (26):

$\Delta A_{405} = (A_{405})$initial - (A_{405}) final Inhibition activity (%)
= [1- (Δ sample)(Δ control)] x 100

Pancreatic lipase inhibition assay

Crude porcine protein pancreatic lipase (PPL) (1 mg/ml) was mixed with Tris-HCL buffer (2.5 mM, pH 7 with 2.5 mM NaCl) and centrifuged at 1,500 rpm for 10 minutes. P-Nitrophenyl butyrate (PNPB) substrate was dissolved to give a concentration of 0.2 mM. Then, 50 µL of porcine pancreatic lipase solution was added into 100 µL sample (31.25, 62.5, 125, 250 and 500 µg/ml). The volume was then diluted to 1 ml using the Tris-HCL buffer then incubated at 37°C for 15 minutes. Later, the samples were read at 405 nm. One hundred microliters of p-nitrophenyl butyrate (PNPB) substrate was added. An aliquot of 250 µL was transferred to separated microplate wells and measured at 405 nm. Orlistat was used as a positive control. The results were calculated as follow (26):

$\Delta A_{405} = (A_{405})$initial - (A_{405}) final Inhibition activity (%)
= [1- (Δ sample)(Δ control)] x 100

Brine Shrimp Toxicity Assay

The eggs of Brine shrimp were hatched in artificial seawater for 36-48 hours. Ten to fifteen hatched brine shrimps was added into several concentrations (62.5, 125, 250, 500 and 1000 µg/ml) of the tested plant extracts for 24 hours at room temperature then the number of dead hatched brine shrimps were counted. The negative control was distilled water and the positive control paracetamol. The LC_{50} of the tested crude plant extracts was determined based on graph (27).

Zebrafish Embryo Toxicity Testing

Distilled water was used as negative control and Paracetamol as positive control. The zebrafish embryo toxicity testing was started right after 24 hours post-fertilisation of the eggs and ended after 72 hours post-fertilisation. The healthy zebrafish embryos were separated from coagulated embryo and were transferred into 96-well plates (1 embryo per 1 well plate). The zebrafish embryo was exposed to the different concentrations (62.5, 125, 250, 500 and 1000 µg/ml) of tested plant extracts with semi-static condition at 28 °C. The two apical observation were recorded every 24 hours until it reaches 72 hours post-fertilisation (48 hours of exposure to the tested plant extracts). The number of coagulated embryos were determined after 24 and 48 hours of exposure. The absence of heartbeat were recorded within 15 seconds after 24 and 48 of exposure (28) under inverted microscope.

Statistical Analysis

The findings were expressed as mean ± standard deviation and analysed using IBM SPSS 22. One-way ANOVA (Tukey's test) was applied to identify the significant difference between each of samples ($p < 0.05$).

RESULTS

Total Phenolic Content (TPC)

The total phenolic content of non-irradiated extract was the highest (2517.07 ± 15.81 mg GAE / g extract) and significantly different ($p < 0.05$) in comparison to all irradiated hot aqueous extracts of *A. bubalinum* (Table I). In comparison between irradiated samples, TPC of hot aqueous extract of *A. bubalinum* at 6 kGy (1142.73 ± 3.44 mg GAE / g extract) was the highest followed by 3 kGy (570.53 ± 41.45 mg GAE / g extract), 9 kGy (539.80 ± 20.78 mg GAE / g extract) and is at 12 kGy (462.87 ± 34.87 mg GAE /g extract). TPC of all irradiated samples were different significantly ($p < 0.05$) when compared to

Table I: Total phenolic content (TPC) and total flavonoid content (TFC) of *A. bubalinum* extracts

Phytochemicals	Hot aqueous extracts of *A. bubalinum*				
	Non- Irradiated	**3 kGy**	**6 kGy**	**9 kGy**	**12 kGy**
TPC (mg GAE / g extract)	2517.07 ± 15.81[a]	570.53 ± 41.45[b]	1142.73 ± 3.44[c]	539.80 ± 20.78[b]	462.87 ± 34.87[e]
TFC (mg QE / g extract)	167.82 ± 0.01[a]	83.77 ± 5.02[b]	112.75 ± 5.02[c]	83.77 ± 5.02[b]	448.99 ± 5.02[e]

Values are expressed as mean ± standard deviation, SD (n=3). Mean values with different letters were significantly different at ($p < 0.05$) between all samples, non-irradiated and irradiated *A. bubalinum* (3 kGy, 6 kGy, 9 kGy & 12 kGy). The concentration used was 1000 µg/ml of crude dried extract.

each other except for 3 kGy and 9 kGy of irradiated *A. bubalinum*.

Total Flavonoid Content (TFC)

There was a significant different ($p < 0.05$) between non-irradiated and irradiated of hot aqueous extracts of *A. bubalinum* (Table I). Besides, in comparison among irradiated extracts of *A. bubalinum*, TFC also significantly different ($p < 0.05$) except for 3 kGy and 9 kGy ($p > 0.05$). The irradiated *A. bubalinum* at 12 kGy (448.99 ± 5.02 mg QE / g extract) expressed the highest TFC followed by 6 kGy (112.75 ± 5.02 mg QE / g extract), 9 kGy (83.77 ± 5.02 mg QE / g extract) and 3 kGy (83.77 ± 5.02 mg QE / g extract).

Total Antioxidant Activity

Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

Irradiated *A. bubalinum* at 12 kGy (76.00%) demonstrated the highest DPPH scavenging activity followed by 6 kGy (74.79%), 3 kGy (74.61%) and 9 kGy (74.17%) (Table II). The EC_{50} values between non-irradiated and irradiated extracts of *A. bubalinum* showed no significant different ($p > 0.05$). Besides, in comparison among irradiated *A. bubalinum*, EC_{50} values were found did not significant ($p > 0.05$) each other. The EC_{50} among irradiated extracts showed that 6 kGy has the lowest EC_{50} values (97.83%) which indicated high scavenging activity. However, EC_{50} values was significantly different ($p < 0.05$) as compared to positive control (BHT).

β-Carotene bleaching activity

The ß-Carotene bleaching activity of hot aqueous extracts of *A. bubalinum* increased as the time interval increase up to 120 minutes. Results showed that there were no significant different ($p > 0.05$) in beta-carotene bleaching activity between non-irradiated and irradiated extracts of *A. bubalinum* (Table II). There were also no significant different ($p > 0.05$) in beta-carotene bleaching activity in all irradiated *A. bubalinum*.

Ferric Reducing Antioxidant Potential (FRAP)

There was no significant different ($p > 0.05$) between irradiated samples and non-irradiated extracts of *A. bubalinum* (Table II). Besides, reducing power among irradiated extracts were found significantly different ($p < 0.05$) between 12 kGy and other dosages of irradiation (3 kGy, 6 kGy & 9 kGy). The irradiated *A. bubalinum* at 12 kGy possessed the highest antioxidant activity followed by 3 kGy (1.84 ± 0.19 mmol Fe2+/ g dry weight), 6 kGy (1.82 ± 0.17 mmol Fe2+/ g dry weight) and 9 kGy (1.81 ± 0.20 mmol Fe2+/ g dry weight).

Alpha-amylase Inhibitory Activity

Non-irradiated *A. bubalinum* exhibited the strongest inhibitory activities with the lowest IC_{50} value (31.99 ± 3.15 µg/ml) (Table III). Irradiated *A. bubalinum* at 9 kGy expressed the highest IC_{50} value (43.81 ± 3.21 µg/ml) followed by 6 kGy (43.26 ± 2.96 µg/ml), 12 kGy (39.38 ± 0.87 µg/ml) and 3 kGy (36.02 ± 5.76 µg/ml) but no significant difference ($p > 0.05$) was observed. There were significant differences ($p < 0.05$) between positive control (acarbose) and both of irradiated and non-irradiated *A. bubalinum*.

Alpha-glucosidase inhibitory activity

Non-irradiated *A. bubalinum* had the lowest IC_{50} value (23.40 ± 0.69 µg/ml). Irradiated *A. bubalinum* at 12

Table II: EC_{50} values of DPPH, ß-carotene and FRAP assay of *A. bubalinum* hot extracts.

Anti-oxidant activity	Hot aqueous extracts of *A. bubalinum*					
	Non- Irradiated	**3 kGy**	**6 kGy**	**9 kGy**	**12 kGy**	**BHT**
DPPH EC_{50} (µg/ml)	99.50 ± 4.33[a]	98.67 ± 4.07[a]	97.83 ± 3.33[a]	99.00 ± 3.97[a]	99.17 ± 4.91[a]	5.13 ± 0.13[b]
ß-Carotene Bleaching assay (%)	58.96± 4.47[a]	76.92± 0.00[a]	76.92± 0.00[a]	67.47± 10.45[a]	79.49± 4.45[a]	92.30± 0.01[b]
FRAP (mmol Fe^{2+}/ g dry weight)	2.19 ± 0.27[a,b]	1.84 ± 0.19[b]	1.82 ± 0.17[b]	1.81 ± 0.20[b]	2.55 ± 0.40[a]	

Results are expressed as mean ± SD (n=3). Mean with different letters indicate significant at $p < 0.05$, EC_{50} values, efficient concentration of extract (µg/ml) represent 50% of the radicals scavenged by the samples. A lower EC_{50} indicates the higher antioxidant activity, Butylated hydroxytoluene (BHT) is used as positive control.

Table III: IC_{50} of hot aqueous extract of irradiated and non-irradiated *Archidendron bubalinum* against alpha-amylase, alpha-glucosidase and pancreatic lipase enzyme inhibition.

Enzyme inhibition	Hot aqueous extracts of *A. bubalinum*					Positive control
	Non-irradiated	3 kGy	6 kGy	9 kGy	12 kGy	
IC_{50} of α-amylase inhibition (μg/ml)	31.99 ± 3.15[b]	36.02 ± 5.76[ab]	43.26 ± 2.96[ab]	43.81 ± 3.21[ab]	39.38 ± 0.87[ab]	5.41 ± 0.26[c]
IC_{50} of α-glucosidase inhibition (μg/ml)	23.40 ± 0.69[a]	23.50 ± 4.34[a]	25.12 ± 3.05[a]	26.71 ± 1.97[a]	27.50 ± 1.29[a]	5.19 ± 1.00[b]
IC_{50} of pancreatic lipase inhibition (μg/ml)	32.81 ± 7.96[a]	37.71 ± 1.48[a]	47.32 ± 0.92[a]	46.41 ± 2.81[a]	42.47 ±7.02[a]	8.36 ± 5.27[b]

Data are expressed as mean ± standard deviation of two independent experiments and each were performed in triplicates. Values with difference letters are significantly different at $p < 0.05$. IC_{50} is referred to the concentration of extract required to inhibit 50% of the enzyme activity and obtained by non-linear regression using GraphPad. The lower values of IC_{50} indicate stronger inhibitory activity.

kGy had the highest IC_{50} value (27.50 ± 1.29 μg/ml) followed by 9 kGy (26.71 ± 1.97 μg/ml), 6 kGy (25.12 ± 3.05 μg/ml) and 3 kGy (23.50 ± 4.34 μg/ml) (Table III). Significant difference ($p > 0.05$) was not presented across all samples. Acarbose showed significant difference ($p < 0.05$) when compared with non-irradiated and irradiated *A. bubalinum*.

Pancreatic Lipase Inhibitory Activity

Non-irradiated *A. bubalinum* exhibited the strongest inhibitory activity with IC_{50} (32.81 ± 7.96 μg/ml) (Table III). Among irradiated *A. bubalinum*, 3 kGy had the lowest IC_{50} value (37.71 ± 1.48 μg/ml) followed by 12 kGy (42.47 ± 7.02 μg/ml), 9 kGy (42.47 ± 7.02 μg/ml) and 6 kGy (47.32 ± 0.92 μg/ml) of irradiated samples and no significant difference ($p > 0.05$) was observed. The orlistat was significantly different between irradiated and non-irradiated *A. bubalinum*.

Brine shrimp lethality assay

All samples were observed with no significant different ($p > 0.05$) in term of LC_{50} values and percentage of mortality (%) (Table IV). Paracetamol has the highest LC_{50} (575.44 μg/ml) followed by irradiated *A. bubalinum* at 6 kGy (85.11 μg/ml), 12 kGy (43.45 μg/ml), non-irradiated (38.9 μg/ml), 9 kGy (37.15 μg/ml) and 3 kGy (33.11 μg/ml) expressed the lowest LC_{50}. There were significant different in the % of mortality between Paracetamol and all of *A. bubalinum* samples.

Zebra fish lethality assay

There was no significant different ($p > 0.05$) between all samples in term of LC_{50} at 48 hours, 72 hours and percentage of mortality at both time (Table IV). Irradiated *A. bubalinum* at 6 kGy expressed the highest LC_{50} (512.86 μg/ml) followed by 12 kGy (446.68 μg/ml), 9 kGy (416.87 μg/ml), 3 kGy (407.38 μg/ml) and non-irradiated samples expressed the lowest LC_{50} (398. 11 μg/ml). Meanwhile, at 72 hours, irradiated *A. bubalinum* at 9 kGy expressed the highest LC_{50} (100.00 μg/ml) followed by 6 kGy (97.72 μg/ml), 3 kGy (95.50 μg/ml), 12 kGy (16.98 μg/ml) and non-irradiated samples expressed the lowest LC_{50} (15.14 μg/ml).

Table IV: Brine shrimp and Zebra fish toxicity assay

Toxicity Assay	Hot aqueous extracts of *A. bubalinum*					Paracetamol
	Non-irradiated	3 kGy	6 kGy	9 kGy	12 kGy	
Brine shrimp						
LC_{50} (μg/ml)	38.90	33.11	85.11	37.15	43.65	575.44
Percentage of Mortality (%)	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	94.12 ± 5.88[a]	98.53 ± 1.45[a]	63.02 ± 11.82[b]
Zebra fish						
LC_{50} (μg/ml) (48 hpf)	398.11	407.38	512.86	416.87	446.68	ND
LC_{50} (μg/ml) (72 hpf)	15.14	95.50	97.72	100.00	16.98	ND
Percentage of Mortality (%) after 48 hpf	100 ± 0.00[a]	100 ± 0.00[a]	83.34 ± 16.67[a]	100 ± 0.00[a]	83.34 ± 0.00[a]	0.00 ± 0.00[b]
Percentage of Mortality (%) after 72 hpf	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]	83.34 ± 16.67[a]	0.00 ± 0.00[b]

Concentration of tested plant extracts used were 62.5 to 1000 μg/ml. The results were obtained from two independent experiments. The higher the LC_{50} the lower the toxicity. The LC_{50} of the extracts were obtained from linear correlation between probits and log_{10} concentration.

DISCUSSION

Ionizing radiation is capable to promote quantitative and qualitative changes in plant material and secondary substances. Phytochemicals screening revealed that *Archidendron bubalinum* husks expressed the presence of alkaloid, flavonoid, tannin, saponin, phenols and terpenoid (11,13). Present study showed that there was reduction in total flavonoid content (TFC) among lower dosages of irradiation. The declining of TFC might be due to the radiolytic chemical reaction that occurred when the catechol group was disturbed by the attacked of free radicals from radiation thus affecting the flavonoids level (29). In agreement to a study by Shahzad and others that irradiated C. tubercullata extracts was demonstrated with the decrease of flavonoid content as compared to non-irradiated (30).

The occurrence of reduction in phytochemicals might be instigated by the accumulation of radiation or free radicals in plant materials (31). Another study also reported that gamma-irradiated Sainfoin extracts exhibited the highest flavonoids content as compared to the non-irradiated extracts (32). However, gamma irradiation interacted with atoms and molecules that generated free radicals in cells and consequently altered the vital component in plant cells that affect the morphological and biochemical of plants (33).

Total phenolic content in non-irradiated extracts of *A. bubalinum* exhibited the increased value in comparison with all irradiated extracts. Irradiation at high dose could result higher extractability of phenolic compounds triggered by cellular compounds modification at high temperature or caused by the disintegration of insoluble phenolic compounds (34). Study on olive leaves extract reported the lowest phenolic contents when the irradiated with higher radiation (35). The condition happened because the impact of irradiation that eventually diminished the protection against glycoside wall leading to the loss of bioactive compounds (36). Ghadi and others stated that the type of plant, topographical and ecological settings are the factors that linked with the reduced phenolic content in irradiated vegetables (37). Study indicated that the greater exposure of gamma rays act as preventive effects whereby the lower exposure occasionally more stimulating effects for radicals production (32).

Pancreatic lipase holds a significant role in the hydrolysis of triglycerides and total dietary fats up to 70% (38). Thus, prospective plant extracts that targeting this enzyme could be a new approach in obesity management. Irradiated and non-irradiated *A. bubalinum* were not significantly different ($p > 0.05$) in all alpha-amylase, alpha-glucosidase and pancreatic lipase inhibition. This explained that the radiation did not exert any effect on the phytochemical properties of *A. bubalinum* samples. However, the positive control (Acarbose and Orlistat) demonstrated significantly lowered IC_{50} in all three enzyme inhibitions assays as compared to both irradiated and non-irradiated *A. bubalinum* samples. This further explained that the both non-irradiated and irradiated *A. bubalinum* samples did not possessed inhibitory ability on the enzymes-linked obesity as opposed to the currently available drug treatment. This encouraged further study on different pathways of obesity therapy.

On the other hand, gradual increase in scavenging activity in non-irradiated and 12 kGy of *A. bubalinum* extracts mainly due to the interruption of nutritional contents such as glucose or amino acid that triggered the induction of Maillard Reaction Product (MRP) (39). The MRP is often defined as nonenzymatic browning reaction is the result of heating the foods at high temperature. Different types of flavours and brown colour were generated from the chemical reaction between amino acids and reducing sugars (40). The reaction was taken into advantages by food industry for giving food different flavour, colour, and aroma.

The finding also in accordance with previous study that explored the free radical scavenging activity of Boletus edulis that had highest antioxidant capacity in non-irradiated extracts, however, the contradictory effects was observed in irradiated extracts of Russula delica that increased in scavenging activity of gamma treatment (41). Pereira et al. described that the methanol extracts of irradiated Arenaria montana increased in scavenging activity in comparison with non-irradiated extracts (31). These different antioxidant activities was showed due to degradation of vital molecules in plant component of *A. bubalinum* into phenolic compound or the alteration in conformational molecules that contribute to scavenging activity.

Past study found that the scavenging abilities of non-irradiated and irradiated samples of soybeans were not significantly different which also followed the similar effect of gamma irradiation in this study (42). Kumari and colleagues emphasized that irradiation can break the complex form of cellulosic fibre to facilitate the release of active compounds (phenolics and tannins) that embedded in cellulosic matrix (43). However, gamma irradiation via DPPH assay for these samples probably did not form these active chemicals (polyphenols). All phenolic compound may not reduce DPPH scavenging activity because the stearic hindrance occurred during irradiation. Previous study reported that irradiated various plant extracts presented non-significant increase of beta-carotene bleaching level (31). A different study also stressed that beta-carotene oxidation process had disadvantages that it would partly collapse when different antioxidant exist and interfering the process by deactivating the linoleate free radicals and other element that are in the systems (44). Moreover, as the irradiation doses at 12 kGy had the highest reducing power, it proved that particular doses had strong electron donors

and undergone reduction in oxidation immediately in lipid peroxidation process that eventually may alter them as primary or secondary metabolites in antioxidant activity (45). Irradiation induced the antioxidant activity in plant extracts as it easily adapted to rough conditions by stimulating the endogenous. Corresponcence to current findings, Pйrez and others reported that the oregano extracts expressed higher ferric reducing power when exposed with gamma irradiation (46).

Despite no significant different between non-irradiated and irradiated samples of *A. bubalinum* were observed in several assay, irradiation was demonstrated to extend the shelf life of food in past study. According to Singh and colleagues, irradiation is capable to improve the shelf life of tomatoes with no noticeable antagonistic effects on quality and sensory aspects (47). Fresh fruits and vegetables have shorter shelf life without any preservation. Without appropriate packaging, *A. bubalinum* can stay fresh for about a week at ambient temperature. High content of moisture makes *A. bubalinum* perishable food item (10). It can be infested by pathogens and microorganisms which exhilarated the spoilage leading to the main economic loss in agriculture industry. In our study, food irradiation is the focus as it is as safe as other conventional food preservation techniques. Several regulatory agencies like World Health Organisation and Food and Drug Act made an assurance that food irradiation is a safe method with respect to food processing for humans (48).

A. bubalinum has shown high profile of cytotoxicity at a concentration of 200 µg/m. *A. bubalinum* is one of eighteen tested plants that showed "strong active" with 50 % greater cell viability due to significant cytotoxicity effects when tested at lower concentration (49). The LC_{50} of both hot aqueous extracts of non-irradiated and irradiated *A. bubalinum* were found to be lesser than 1000 µg/ml. Meyer et al. mentioned that the LC_{50} greater than 1000 µg/ml is classified as having no potential toxicity, however, extracts with LC_{50} not more than 1000 µg/ml are considered as having potential toxicity (27). On the other hand, Clarkson's toxicity standard for the evaluation of toxicity of plant extracts stated that LC_{50} of more than 1000 µg/ml are categorized as non-toxic, meanwhile, extracts with LC_{50} in the range of 500 to 1000 µg/ml are low toxic, extracts with LC_{50} of 100 – 500 µg/ml are categorized as moderately toxic, and the extracts with LC_{50} of less than 0 – 100 µg/ml are categorized as greatly toxic (50). In brief, both non-irradiated and irradiated *A. bubalinum* extract were considered toxic for consumption. Therefore, extensive study has to be conducted to determine the safety level of *A. bubalinum*.

CONCLUSION

The application of gamma irradiation treatment was revealed to have potential in enhancing the antioxidant properties. Irradiated *Archidendron bubalinum* exhibited the highest total flavonoid content at 12 kGy. The non-irradiated and irradiated samples expressed no significant different in all enzymatic inhibition assays of alpha-amylase, alpha-glucosidase and pancreatic lipase. In a nutshell, gamma irradiation has the potential to be used as one of the approaches to maintain enzymatic inhibition activities and to preserve the samples for commercial purposes in the future. The food irradiation treatments were not giving any effects to the toxicity of the plant extracts. However, extensive study must be conducted to further confirm the safety level of *A. bubalinum* consumption.

ACKNOWLEDGEMENTS

The authors would like to thank Ministry of Higher Education (MoHE) (FRGS/1/2016/SKK08/UPM/02/14), and Graduate Research Fellowship, Universiti Putra Malaysia for the funding, and Malaysian Nuclear Agency for the assistance.

REFERENCES

1. Bhat R, Sridhar KR. Nutritional quality evaluation of electron beam irradiated (Nelumbo nucifera) seeds. Food Chem. 2008;107(1):174-84.
2. Alothman M, Karim AA. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Sci Tech. 2009;20(5):201-12.
3. FDA (Food and Drug Administration). Food Irradiation: What You Need to Know. 2018. https://www.fda.gov/food/resourcesforyou/consumers/ucm261680.htm. Accessed 10 June 2018.
4. Jiang T, Luo S, Chen Q, Shen, L, Ying. T. Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (Lentinus edodes). Food Chem. 2010;122(3):761-67.
5. Aziz NH, Souzan RM, Azza AS. Effect of γ-irradiation on the occurrence of pathogenic microorganisms and nutritive value of four principal cereal grains. App Radiation and Isotopes. 2006;64(12):1555-62.
6. Calucci L, Pinzino C, Zandomeneghi M, Capocchi A, Ghiringhelli S, Saviozzi F, et al. Effects of γ-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. J Agricul Food Chem. 2003;51(4):927-34.
7. Moosa S, Mohd Nasir MA. Effect of irradiation on total chemical profiles of ten selected local herbs. Malaysian Sci Techn Congress 2008 (COSTAM). 2008;1-7.
8. Lim TK. *Archidendron bubalinum*. Edible Medicinal and Non-Medicinal Plants. Dordrecht: Springer; 2012.
9. Ong HC, Azliza MA. Medicinal plants for diabetes by the orang asli in Selangor, Malaysia. Studies on

Ethno-Med. 2015;9(1):77-84.
10. Mohamed S, Rahman MS, Sulaiman S, Abdullah F. Some nutritional and anti-nutritional components in jering Pithecellobium-jeringa, keredas Pithecellobium-microcarpum and petai Parkia-speciosa. Pertanika. 1987;10(1):61-8.
11. Liliwirianis N, Musa NLW, Zain WZ, Kassim J, Karim SA. Premilinary studies on phytochemical screening of ulam and fruit from Malaysia. E-Journal Chem. 8(1), 285-88.
12. Middleton E, Kandaswami C. Effects of flavonoids on immune and inflammatory cell functions. Biochemical Pharmacology. 1992;43(6):1167-79.
13. Irawan C, Sulistiawaty L, Sukiman M, Sulistiawaty L. Volatile compound analysis using GC-MS, phytochemical screening and antioxidant activities of the husk of "Julang-Jaling" (*Archidendron bubalinum* Jack) I. C Nielsen) from Lampung, Indonesia. Pharmacog J. 2018;10(1): 92-8.
14. Scheen AJ. Is there a role for alpha-glucosidase inhibitors in the prevention of type 2 diabetes mellitus? Drugs. 2003;63(10):933-51.
15. Sindhu SN, Vaibhavi K, Anshu M. In vitro studies on alpha amylase and alpha glucosidase inhibitory activity of selected plant extracts. Eu J Exp Biol. 2013;3(1):128-32.
16. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. Drug Discovery Today. 2007;12(19):879-89.
17. Bunawan H, Dusik L, Bunawan SN, Amin NM. Botany, traditional uses, phytochemistry and pharmacology of Archidendron jiringa: A review. Global J Pharmaco. 2013;7(4):474-478.
18. Hamidi MR, Jovanova B, Panovska TK. Toxicological evaluation of the plant products using brine shrimp model. Macedonian Pharm Bulletin. 2014;60(1):9–18.
19. Mohd Akram MN, Mahanem MN, Wan Mohd Aizat WK, Azwam ML, Shazrul F. Toxicity test of xanthone from mangosteen on zebrafish embryos. Am Inst Physics. 2016; 1784:1-6.
20. Cheurfa M, Allem R. Study of hypocholesterolemic activity of Algerian Pistacia lentiscus leaves extracts in vivo. Revista Brasileira de Farmacognosia. 2015; 25(2):142-44.
21. Vijendren K, Syahida A, Marziah M. Antioxidant potential in different parts and callus of gynura procumbens and different parts of Gynura bicolor. BioMed Res Int. 2015; 15:1-7.
22. Samatha T, Shyamsundarachary R, Srinivas P, Swamy NR. Quantification of total phenolic and total flavonoid contents in extracts of Oroxylum Indicum L. Kurz. Asian J Pharma Cli Res. 2012; 5:177–79.
23. Nur Huda F, Zulaikha AR, Maryam MR, Faujan HI. Comparative analysis of phenolic content and antioxidative activities of eight Malaysian traditional vegetables. Malaysian J Anal Sci. 2015;19(3):611-24.
24. Prasad N, Bao Y, Kin WK, Hock EK, Jian S, Azrina A, et al. Phytochemicals and Antioxidant Capacity from Nypa fruticans Wurmb. Fruit. Evidence-Based Complement Alt Med. 2013; 2013:1-9.
25. Alakolanga AGAW, Kumar NS, Jayasinghe L, Fujimoto Y. Antioxidant property and α-glucosidase, α-amylase and lipase inhibiting activities of Flacourtia inermis fruits: characterization of malic acid as an inhibitor of the enzymes. J Food Sci Tech. 2015;52(12):8383-88.
26. Taukoorah U, Mahomoodally MF. Crude aloe vera gel shows antioxidant propersities and inhibits pancreatic lipase and glucose movement in vitro. Adv Pharmacol Sci. 2016; 2016:1-9.
27. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. J Med Plant Res. 1982; 45:31-4.
28. Busquet F, Strecker R, Rawlings JM, Belanger SE, Braunbeck T, Carr G, et al. OECD Validation study to assess intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test for Acute Aquatic Toxicity Testing. Reg Toxicol Pharmacol. 2016; 69:496-511.
29. El-Batal AI, Hashem AAM, Abdelbaky NM. Gamma radiation mediated green synthesis of gold nanoparticles using fermented soybean-garlic aqueous extract and their antimicrobial activity. SpringerPlus. 2013;2(1):1-10.
30. Shahzad N, Elahi R, Ali S. Effect of gamma irradiation on phytochemical content and antimicrobial activities of selected herbs. Austin J of Nutr Food Sci. 2017;5(3):1-15.
31. Pereira E, Barros L, Antonio AL, Verde SC, Santos-Buelga C, Ferreira ICFR, et al. Is gamma radiation suitable to preserve phenolic compounds and to decontaminate mycotoxins in aromatic plants? A case-study with aloysia citrodora pal6u. Molecules. 2017;22(3):1-13.
32. Mohajer S, Mat-Taha R, Lay MM, Esmaeili AK, Khalili M. Stimulatory Effects of Gamma Irradiation on Phytochemical Properties, Mitotic Behaviour, and Nutritional Composition of Sainfoin (Onobrychis viciifolia Scop.). Sci World J. 2014; 2014:1-9.
33. Wi SG, Chung BY, Kim JH, Baek MH, Yang DH, Lee JW, et al. Ultrastructural changes of cell organelles in Arabidopsis stems after gamma irradation. J Plant Biol. 2005;48(2):195-200.
34. Villavicencio ALCH, Mancini-Fiho J, Delince'e H, Greniner R. Effect of irradiation on anti-nutrients (total phenolics, tannins and phytate) in Brazilian beans. Radiation Physics and Chemistry. 2000;57(3):289-93.
35. Aouidi F, Ayari S, Ferhi H, Roussos S, Hamdi M. Gamma irradiation of air-dried olive leaves: Effective decontamination and impact on the antioxidative properties and on phenolic compounds. Food Chem. 2011;127(3):1105-13.

36. Kortei NK, Odamtten GT, Appiah V, Obodai M, Narh Mensah DL, Akonor PT, et al. Preliminary shelf life studies of In-vitro antioxidant potential of gamma irradiated dried mushrooms (Pleurotus ostreatus Ex. Fries) Kummer in Ghana. British J Pharm Res. 2016;9(2):1-13.
37. Ghadi FE, Ghara AR, Ghanbari T. Effect of gamma irradiation on the total phenolic content and free radical -scavenging activity of iranian date palm mazafati (Phoenix Dactylifera L.). Int J Latest Res Sci Tech. 2015;4(5):149-53.
38. Yoshikawa M, Shimoda H, Nishida N, Takada M, Matsuda H. Salacia reticulata and its polyphenolic constituents with lipase inhibitory and lipolyticactivities have mild antiobesity effects in rats. J Nut. 2002;132(7):1819-24.
39. Kim JH, Shin MH, Hwang YJ, Srinivasan P, Kim JK, Park HJ, et al. Role of gamma irradiation on the natural antioxidants in cumin seeds. Radiation Physics and Chemistry. 2009;78(2):153-57.
40. Bastos DM, Monaro Й, Siguemoto Й, Sйfora M. Maillard reaction products in processed food: pros and cons. In Food Industrial Processes-methods and Equipment. 2012;1-23.
41. Fernandes B, Barreira JCM, Antonio AL, Oliveira MBPP, Martins A, Ferreira ICFR. Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. Innov Food Sci and Emerging Tech. 2014; 22:158-66.
42. Byun MW, Son JH, Yook HS, Jo C, Kim DH. Effect of gamma irradiation on the physiological activity of Korean soybean fermented foods, Chungkookjang and Doenjang. Radiation Physics and Chemistry. 2002;64(3):245-48.
43. Kumari N, Kumar P, Mitra D, Prasad B, Tiwary BN, Varshney L. Effects of ionizing radiation on microbial decontamination, phenolic contents, and antioxidant properties of triphala. J Food Sci. 2009;74(3):109-13.
44. Nurul SR, Asmah R. Evaluation of antioxidant properties in fresh and pickled papaya. Int Food Res J. 2012;19(3):1117-24.
45. Bag GC, Devi PG, Bhaigyabati T. Research article assessment of total flavonoid content and antioxidant activity of methanolic rhizome extract of three hedychium species of manipur valley. Int J Pharm Sci Rev Res. 2015;30(28):154-59.
46. Pйrez MB, Calderyn NL, Croci CA. Radiation-induced enhancement of antioxidant activity in extracts of rosemary (Rosmarinus officinalis L.). Food Chem. 2007;104(2):585-92.
47. Singh A, Singh D, Singh R. Shelf life extension of tomatoes by gamma radiation. Rad Sci Technol. 2016;2(2):17-24.
48. Thayer DW, Josephson ES, Brynjolfsson A, Giddings GG. Radiation pasteurization of food. Council for Agricultural Science and Technology. 1996;7(1):1-10.
49. Murakami A, Ali AM, Mat-Salleh K, Koshimizu K, Ohigashi H. Screening for the in vitro anti-tumor-promoting activities of edible plants from Malaysia. Biosci, Biotech, Biochem. 2000;64(1):9–16.
50. Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, et al. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J Ethnopharm. 2004;92(2–3):177-91.