

## Proximate composition, minerals contents, functional properties of Mastura variety jackfruit (*Artocarpus heterophyllus*) seeds and lethal effects of its crude extract on zebrafish (*Danio rerio*) embryos

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### Abstract

Jackfruit (*Artocarpus heterophyllus*) is a popular and valuable fruit in Malaysia. The present study aims to determine the proximate composition, mineral contents and functional properties of jackfruit seed powder (JSP) of Mastura cultivar and assess the toxicity of the jackfruit seed crude extract using embryonic zebrafish model. The proximate analysis results obtained showed that the JSP had 69.39% carbohydrate, 13.67% protein, 10.78% moisture, 2.41% ash, 0.75% fat and 3.00% crude fiber. The energy value reported was 345 kcal/100 g. Most abundant mineral found in the JSP was potassium (7.69 mg/g) followed by phosphorus (1.29 mg/g), magnesium (1.03 mg/g), calcium (0.41 mg/g) and sodium (0.05 mg/g). Water absorption capacity (2.35 g/g), oil absorption capacity (1.14 g/g) and bulk density (0.67 g/cm<sup>3</sup>) were recorded for the JSP. The values for swelling power and solubility were 4.12 and 9.98, respectively. Furthermore, the various concentrations of jackfruit seed crude extract showed lethal developmental effects against zebrafish embryos during 96 hrs of exposure duration. Increased mortality was observed in embryos after exposure to concentrations above 15.625 µg/mL in dose and time-dependent manner. Based on the results, it can be concluded that JSP has great potential to be utilized in the formulations of food and other functional products. Additionally, the presence of toxic effects in the crude extract of JSP indicates the future studies required in isolating and identifying the compounds that might be responsible for the toxicity.

## 1. Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) tree is a monoecious evergreen tree possibly indigenous to the rain forests of the Western Ghats in the Southwestern of India (Baliga *et al.*, 2011). Locally known as "Nangka", jackfruit is a tropical fruit belongs to the family of Moraceae and is a close relative of cempedak (*Artocarpus champedon*), breadfruit (*Artocarpus altilis*) and tarap (*Artocarpus adoratissimus*) (Subhadrabandhu, 2001). The tree grows well in warm and moist regions and can be found extensively cultivated in many parts of Asia including Malaysia, Indonesia, Thailand, Philippines, Sri Lanka and Bangladesh (Baliga *et al.*, 2011). Jackfruit tree is a non-seasonal crop which produces the largest oblong-cylindrical in shape edible

fruit in the world. In Malaysia, jackfruit is important in cultivation as commercial edible fruit. There has been growing interest in exploiting jackfruit as a commercial crop since the plants grow well under the Malaysian climate. In Malaysia, there are more than 30 jackfruit clones registered by the Department of Agriculture (DOA). Among all of them, Tekam Yellow (J33) and Mastura are exceptionally popular between consumers and recommended for planting (Azeez *et al.*, 2015).

Jackfruit is composed of rind, edible bulbs of yellow flesh and seeds. A ripe jackfruit contains well succulent, aromatic and flavorful yellow sweet bulbs and about 100 to 500 seeds, which represent around 8 to 15% of the total fruit weight (Madruga *et al.*, 2014). Each of the yellow bulbs encloses a smooth, oval, oblong or oblong-

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ellipsoid shape, 2-3 cm long and 1-2 cm in diameter of the light brown outer layer coated a thin and whitish cotyledon (Menaka *et al.*, 2011). The ripe fruits bulbs are widely consumed; the seeds, however, are far less utilized with occasional uses as dessert, minor ingredients in culinary recipes or eaten as a snack after boiling, steaming or roasting. Most of the time, the seeds are left untreated or discarded as wastes due to the bland taste and texture of the seeds (Kooch *et al.*, 2016).

In recent years, much attention has been focused on jackfruit seed to be exploited commercially, owing to the considerable amounts of carbohydrates and protein in the seed (Hettiaratchi *et al.*, 2011). Jackfruit seed is also reported to contain two different lectins, namely jacalin and artocarpin which possess several biological activities, including antibacterial, antifungal and anticarcinogenic properties (Kabir and Daar, 1994; Kabir, 1995; Swami *et al.*, 2012; Nair *et al.*, 2013). Hence, the toxicity assessments of the jackfruit seed are vital since these can serve as baseline information and an indication of bioactive molecules to explore the potential of the seed in the formulation of food or medicinal products. Today, the Zebrafish (*Danio rerio*) has emerged as one of the most versatile biological models that have been widely used to investigate the toxicity of chemical compounds on the human and aquatic environment (Dai *et al.*, 2014). The popularity is probably due to their easy and cheap maintenance in the laboratory, short life cycle, high fertility, fast *ex utero* development and transparent embryos (Kimmel *et al.*, 1995; Stephens *et al.*, 2016).

Although the chemical components, amino acid profile and anti-nutritional factors in seed of three Malaysian jackfruit cultivars (J29, J31 and J33) can be found in literature (Azeez *et al.*, 2015), no study has been explored on the proximate composition, chemical components and functional properties of the jackfruit seed powder of Mastura cultivar. Besides, recent studies on the toxicity and cytotoxicity of jackfruit seed extracts only involved the use of various cancer cell lines and brine shrimp assay (Mohd Ali *et al.*, 2014; Burci *et al.*, 2018). To our knowledge, there are no studies that illustrate the effect of jackfruit seed crude extract using a zebrafish model. Moreover, studies on both chemical composition and toxicity of the crude extracts of plant seeds are quite scarce. Thus, the objectives of this study were: (1) to determine the proximate composition, chemical components as well the functional properties in the Malaysian jackfruit seed powder of Mastura cultivar and (2) to evaluate the toxicity of the jackfruit seeds crude extract using the zebrafish embryo model.

## 2. Materials and methods

### 2.1 Plant material

A cultivar of whole jackfruit (*Artocarpus heterophyllus*), locally known as Mastura were purchased from a fruit supplier located in Gambang, Kuantan, Pahang. The fruits selected were ripe, uniform in size and without defects.

### 2.2 Treatment of jackfruit seed

The jackfruits were cut open and the seeds were separated from the flesh. The fresh seeds were manually decorticated, cleaned and their white arils were peeled off. The seeds were soaked in water overnight to easily remove the thin whitish membrane coating the seeds (Dasaesamoh and Seechamnaturakit, 2014). The outer layer (brown coat) covering the cotyledons were also removed by rubbing the seeds between the hands and thoroughly washed with tap water. The seeds were packed in plastic pouches and stored in a freezer (-20°C) until further use.

### 2.3 Preparation of jackfruit seed powder

Jackfruit seed powder (JSP) was prepared according to the procedure described by Tulyathan *et al.* (2002) with slight modifications. The frozen fleshy white cotyledons were thawed, sliced into tiny pieces and then dried at 37°C for 24 hrs to reduce moisture content. The dried chips were ground into fine powder by a mixer-grinder and passed through an 80-mesh sieve to remove big chunks. The obtained seed flour was sealed in a plastic bag and stored in a refrigerator (below 10°C) for further use and analysis.

### 2.4 Preparation of jackfruit seed crude extract

The crude extract of jackfruit seeds was prepared by soaking 10 g of JSP with 100 ml of phosphate buffered saline (10 mM, pH 7.4) at 4°C for 6 hrs. The mixture was then incubated for another 5 hrs at 25°C with constant shaking at 150 rpm in a water bath (Memmert). After incubation, the mixture was centrifuged for 20 min at 10000 rpm (Eppendorf 5810R). The supernatant obtained was filtered through a 0.45µm syringe filter and kept at 4°C for further uses.

### 2.5 Proximate analysis

Moisture content was determined according to the procedure by Malaysian Standard (MS 1191:2013), where 5 g of JSP sample were weighed into porcelain dish and dried in an air oven maintained at 105°C until constant weight achieved. Ash content was determined using AOAC method 920.87 (AOAC, 2000) by dry-bashing in a furnace at 550°C for 24 hrs. The crude fiber was determined by hydrolysis with acid and alkali

followed by the determination of ash contents of the residue adapted from the AOAC method 962.09 (AOAC, 2000). The protein content was determined using the Kjeldahl method (Kjeltec 2300). In the Kjeldahl method, JSP was digested with concentrated sulphuric acid, potassium sulphate followed by titration with potassium hydroxide and sodium thiosulphate solution. The nitrogen value obtained was converted to protein by multiplying with a factor of 6.25. The fat was extracted in a Soxhlet apparatus (Soxtec Avanti 2055). The total carbohydrate was determined using the difference method by subtracting the values for ash, crude protein, fat, crude fiber and moisture from 100. The energy value (kcal/100 g) was estimated by multiplying the crude protein, carbohydrate, fat and crude fiber by the factors of 4, 4, 9 and 2, respectively.

## 2.6 Mineral analysis

Microwave digestion was used for sample decomposition to determine magnesium and iron using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES), method no. 984.27 (AOAC, 2000). The acid solution dissolved from ash residue was used for calcium, sodium and potassium analyses by Atomic Absorption Spectrophotometer (AAS), method no. 975.03 (AOAC, 2000).

## 2.7 Functional properties

### 2.7.1 Bulk density

Bulk density was determined according to the procedure described by Narayana and Narasinga (1984). 3.0 g of JSP samples were placed in a 15 mL graduated cylinder. The cylinder was gently packed by tapping the cylinder on the workbench until a constant volume was obtained. The bulk density was calculated as the weight of JSP (g) divided by the seed powder volume (ml).

### 2.7.2 Swelling power and solubility

The swelling power was performed based on the method described by Leach *et al.* (1959) with modifications for small samples as adapted by Ocloo *et al.* (2014). About 1.0 g of JSP sample was weighed into a previously weighed 50 mL centrifuge tube with 40 ml of distilled water added. The mixture was gently stirred to avoid excess force that would rupture the granules and then heated in a thermostatically controlled water bath at 85°C for 30 mins, with constant stirring. The tubes were removed from the water bath, wiped and allowed to cool to room temperature. The tubes were centrifuged at 2200 rpm for 15 mins. Supernatants obtained were poured into a previously weighed crucible and evaporated to dryness in an oven at 105°C. The dried supernatant was weighed after cooling and the weight was used to calculate solubility. The precipitate paste was weighed, and the

value was used in the swelling power calculation.

### 2.7.3 Water absorption capacity

Water absorption capacity of the JSP samples was carried out using the method described by Ocloo *et al.* (2014). 1.0 g of JSP was added to 10 mL distilled water and magnetically stirred for 5 mins. The mixture was then centrifuged at 3500 rpm for 30 mins and the volume of supernatant obtained was recorded. The water absorption capacity (%) was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant expressed in percentage.

### 2.7.4 Oil absorption capacity

The oil absorption capacity of the JSP samples was performed with the method described by Sosulski *et al.* (1976) as modified by Ocloo *et al.* (2014). 1.0 g of JSP sample was placed in a pre-weighed centrifuge tube containing 10 mL of refined vegetable oil. The suspension was magnetically stirred at room temperature for 5 mins and then centrifuged at 3500 rpm for 30 mins. The volume of the supernatant was recorded before discarded, while the tube and content were re-weighed after draining for 10 mins. The oil absorption capacity was expressed as a percentage of oil absorbed based on the original weight.

## 2.8 Zebrafish embryo toxicity test

### 2.8.1 Collection and Exposure of zebrafish embryos

Zebrafish embryos were obtained from the Central Research and Animal Facility (CREAM), Kuliyyah of Science, IIUM Kuantan, Pahang. Fertilized zebrafish embryos were collected, wash extensively using tap water and then incubated at 28°C in E3 medium consist of 5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl<sub>2</sub>, 0.33 mM MgSO<sub>4</sub> and 0.05% (w/v) methylene blue as fungicide. Toxicity test was carried out according to the Fish Embryo Toxicity (FET) test guideline 236 developed by the Organization for Economic Co-operation and Development (OECD, 2013). The collected fertilized embryos were inspected under an inverted microscope to ensure that only alive and normally developed zebrafish embryos were used for the test. The fertilized embryos were then distributed in 96-well plates using a transfer pipette by adding one healthy embryo to each well. The embryos were immersed in 200 µL of freshly prepared E3 medium containing several concentrations of each sample of jackfruit seed crude extract (7.813 – 500 µg/mL). The well plates were covered using Parafilm to avoid evaporation of test solutions and incubated in a room-controlled temperature at 28±2°C and 12 dark:12 light cycle period throughout the experiments. The E3 medium was used as negative control. Zebrafish embryos

were exposed in duplicates to either a control or different concentrations of jackfruit seed crude extracts. Exposure was static, which means that the sample test solutions were not renewed throughout the experimental period to guarantee minimum manipulation of the embryos (Guarienti *et al.*, 2014; Basnet *et al.*, 2017). Twelve embryos were used for each concentration, so a total of 84 embryos for each replicate were analyzed. A replicate is considered valid when untreated embryos mortality and malformation is equal or lower than 10% at the end of the 96 hours exposure.

### 2.8.2 Evaluation of zebrafish embryos

Plates were inspected at 48 hpf, 72 hpf, 96 hpf and 120 hpf using an inverted microscope (Nikon Eclipse TS100) attached to a camera control unit (Nikon DS-L3). Dead embryos were counted and discarded daily. The zebrafish embryos were scored as dead when the embryos were coagulated, lack of somite formation, non-detachment of the tail and lack of heartbeat, while dead larvae were judged via the appearance of blood circulation, heartbeat and body color changes (OECD, 2013). The median lethal concentration (LC<sub>50</sub>) was determined based on cumulative mortality obtained from duplicate independent experiments at 48, 72, 96 and 120 hpf. The LC<sub>50</sub> values of zebrafish embryos were derived through Probit analysis using GraphPad Prism software (Version 7.00).

## 3. Results and discussion

### 3.1 Proximate and mineral evaluation of jackfruit seeds

Table 1 presents the proximate analysis results of the jackfruit seed powder used in this study. Ash, fat, crude protein, crude fiber, carbohydrate and moisture contents were recorded in grams per 100 g of dry material samples. From Table 1, the JSP was found to contain the highest amount of carbohydrates (69.39%). The result is comparable to 70.76% reported by Eke-Ejiofor *et al.* (2014). Moreover, the carbohydrate content in Mastura cultivar seed flour reported here is higher than the value reported for J33 Malaysia jackfruit clones (66.20%) (Azeez *et al.*, 2015). The carbohydrate value was also higher when compared to other *Artocarpus* species, such as breadfruit (58.9%) and Tarap (49.65%) seed flour (Akubor *et al.*, 2000; Masri *et al.*, 2017).

Protein represents 13.67% of the total composition of the jackfruit seed powder in the present study. This value is higher than the protein values observed in the *Artocarpus odoratissimus* (8.78%), *A. altilis* (8.12%) and *A. integer* seed flour (Tukura and Obliva, 2015; Masri *et al.*, 2017). Furthermore, the protein content reported for Mastura jackfruit cultivar seed flour was also higher than the other jackfruit clones such as J29 (7.62%), J31 (8.46%) and J33 (8.24%) seed flour samples (Azeez *et al.*, 2015). Comparable values of 12.25-16.80% and 13.50% protein were also reported by Eke-Ejiofor *et al.* (2014) and Ocloo *et al.* (2010), respectively. Moisture content provides the quantity of water contained in the seed flour as well as its total solid content (Ocloo *et al.*, 2014). In this study, the moisture content of the jackfruit seed powder (Mastura cultivar) was reported as 10.78%, lower than those obtained by Azeez *et al.* (2015) for jackfruit clones of J29 (24.08%), J31 (14.26%) and J33 (14.26). Conversely, the moisture value reported in this study was higher than the amount of 6.09% found by Ocloo *et al.* (2010). Lower moisture content increased the shelf stability and the quality of the flour. The differences in moisture content might have resulted from the different methods of the drying process and its duration employed in the preparation of the seed flour samples.

Ash content is the residual of inorganic materials remaining after the organic matter has been removed away by heating (Ocloo *et al.*, 2010). The reported ash content of the JSP used in this study was 2.41%, in line to the value of 2.70% and 2.45-2.76% as reported by Ocloo *et al.* (2010) and Eke-Ejiofor *et al.* (2014), respectively. The fat content of the JSP was 0.75%, close to the value of 0.78% (Kumar *et al.*, 1988). The result is slightly higher than 0.4% fat reported by Sreeletha *et al.* (2017) relatively low when compared to other *Artocarpus* species seed flour such as, Tarap (15.60%) and breadfruit (9.0%) seed flour (Akubor *et al.*, 2000; Masri *et al.*, 2017), yet still within the range of 0.13%-0.77% as reported by Ejeifor *et al.* (2014). The relatively low-fat value suggest that the flour will be less prone to lipid-related forms of deterioration (Okpala and Gibson-Umeh, 2013). The crude fiber of the JSP was 3.00%, comparable to 3.19% reported by Ocloo *et al.* (2010). The energy value of the JSP was 345 kcal/100g, which is

Table 1. Proximate composition of *A. heterophyllus* seed powder

Composition	Current study	Zuwariah <i>et al.</i> (2018)	Sreeletha <i>et al.</i> (2017)	Sultana <i>et al.</i> (2017)	Azeez <i>et al.</i> (2015)	Eke-Ejiofor <i>et al.</i> (2014)
Ash (g/100 g)	2.41±0.00	2.75±0.04	N.R.	1.30±0.10	3.19±0.00	2.46±0.28
Carbohydrate (g/100 g)	69.39±0.00	83.68±0.13	19.20±0.00	42.49±0.21	66.20±0.00	70.76±7.07
Energy (kcal/100 g)	344.99±0.00	429.50±0.71	206.00±0.00	N.R.	N.R.	N.R.
Fat (g/100 g)	0.75±0.00	0.56±0.01	0.40±0.00	0.98±0.02	1.17±0.17	0.77±0.85
Moisture (g/100 g)	10.78±0.00	3.22±0.16	49.59±0.00	39.22±0.18	14.26±0.08	5.07±0.47
Protein (g/100 g)	13.67±0.00	9.78±0.00	13.60±0.00	16.01±0.11	8.24±0.54	12.45±0.54
Crude Fiber (g/100 g)	3.00±0.00	25.43±0.54	N.R.	3.56±0.14	5.70±0.00	3.53±0.71

Table 2. Comparison of mineral contents in jackfruit seed powder

Elements	Value (mg/100 g)			
	Current study	Sreeletha et al. (2017)	Baliga et al. (2011)	Ocloo et al. (2010)
Phosphorus	128.83±0.00	177.00±0.00	Not reported	Not reported
Potassium	769.32±0.00	972.00±0.00	246.00±0.00	1478.10±25.60
Aluminum	1.67±0.00	Not reported	Not reported	Not reported
Calcium	40.86±0.00	182.00±0.00	50.00±0.00	308.70±16.60
Magnesium	102.94±0.00	130.00±0.00	54.00±0.00	338.00±38.80
Manganese	0.58±0.00	Not reported	Not reported	0.11±0.11
Iron	1.00±0.00	Not reported	1.50±0.00	13.07±12.37
Copper	0.88±0.00	1.93±0.00	Not reported	1.05±0.89
Zinc	1.36±0.00	5.17±0.00	Not reported	<0.001
Boron	0.92±0.00	Not reported	Not reported	Not reported
Sodium	5.39±0.00	Not reported	63.20±0.00	6.07±2.01

higher than the range of 292 – 313 kcal/100g reported by Akinmutimi (2006) but lower than the value 383 kcal/100 g reported by Ocloo et al. (2010).

Table 2 shows the mineral contents of the *A. heterophyllus* (Mastura) seed powder. Potassium (7.69 mg/g) represents the major elements reported in the flour, followed by phosphorus (1.29 mg/g), magnesium (1.03 mg/g), calcium (0.41 mg/g) and sodium (0.05 mg/g). The results obtained which reported potassium as the most abundant mineral present in the seed powder are in line with previous quantification reports of jackfruit seeds flour by Sreeletha et al. (2017) and Ocloo et al. (2010). However, it is worth noting that various amounts of proximate and mineral contents analysis of jackfruit seed powder were available in the literature. The variations in proximate compositions were also observed in the seed flour from other fruits of *Artocarpus* species. All these differences might be due to the effect of different cultivars of jackfruit, different maturity stages and other factors influencing the growing environment of jackfruit in the plantation area such as the soil, climate and agriculture practice (Madrigal-Aldana et al., 2011).

### 3.2 Functional properties of jackfruit seed powder

Table 3 demonstrates the functional properties of Mastura jackfruit seed powder used in this study. The seed flour exhibited a low value of bulk density with only 0.67 g/cm<sup>3</sup>. The bulk density results from this study were comparable to other reported values. Bulk density is a measure of the heaviness of a flour sample and is dependent on the particle size of the sample (Ocloo et al., 2014). The relatively low value of bulk density reported suggesting that the potential to utilize the jackfruit seed flour in the formulation and development of complementary foods where high nutrient to low bulk density is needed (Mepba et al., 2007). Besides that, bulk density also influences the type of packaging materials used for the flour. Low bulk density needs less dense packaging, which would be cost-effective for the packaging cost (Masri et al., 2017).

Water absorption capability (WAC) indicates the ability of flour to associate with water under a limited supply of water. The WAC recorded for the jackfruit seed powder used in this study was 2.35 g/g. The value obtained is relatively high than amounts reported for wheat flour (0.75 g/g) (Akubor et al., 2014) and breadfruit seed flour (1.55 g/g) (Adepeju et al., 2011). However, the observed WAC value is lower than reported by Eijofor et al. (2014) but close to 2.61 g/g reported for Tarap seed flour (Masri et al., 2017), 2.30 g/g and 2.05 g/g of the seed flour from other jackfruit varieties (Tulyathan et al., 2002; Odoemelam, 2005). The WAC is mostly attributed to the high content of hydrophilic components in the seed flour such as, carbohydrates and proteins, which have a high affinity for water molecules (Roy Chowdhury et al., 2012). Flours with high WAC is useful in the applications as functional ingredients in bakery product as this could prevent staling by reducing moisture loss (Obatolu et al., 2007), hence maintaining the soft texture of the resulting food product (Al-Farga et al., 2016).

Table 3. Functional properties *A. heterophyllus* Mastura cultivar seed powder

Functional parameters	Value (dry weight basis)
Bulk density (g/mL)	0.67±0.00
Water absorption capacity (g/g)	2.35±0.17
Oil absorption capacity (g/g)	1.14±0.07
Swelling power (%)	4.12±0.07
Solubility (%)	9.98±0.80

The oil absorption capacity (OAC) of Mastura jackfruit seed powder was recorded as 1.15 g/g, slightly higher than the wheat flour (0.91 g/g) (Akubor et al., 2014). This value is close to the amounts reported for Tarap seed flour (1.69 g/g) (Masri et al., 2017) and Tigernut seed flour (1.07-1.13 g/g) (Oladele and Aina, 2007). OAC is one of the desired properties in food formulations because of its potential use in retaining the flavor of food products, useful in improving palatability and increasing the shelf life of bakery and meat products (Aremu and Akintayo, 2007). In this study, since the flour content a negligible amount of fat, OAC is

dependent on the existence of lipid-binding surface of hydrophobic protein subunits in the jackfruit seed flour (Roy Chowdhury *et al.*, 2012). This result suggests the potential use of jackfruit seed flour in food formulations particularly in retaining the flavor of food products, improving palatability and increasing the shelf life of bakery and meat products (Aremu and Akintayo, 2007).

The jackfruit seed powder employed in this study has a swelling power of 4.12 g/g, which is close to the value of 4.77 g/g as reported by Ocloo *et al.* (2010) for another cultivar of jackfruit seed flour. However, this result is lower than those reported swelling power values of 2.47 and 2.10 for yellow and black tiger nut flours (Oladele and Aina, 2007). Swelling power is a measure of hydration capacity because the determination is a weight measure of swollen starch granules and their occluded water (Ocloo *et al.*, 2010). Meanwhile, the solubility value reported in this study was 9.98%. The result was within the range of 8.24-14.48% reported by Eke-Ejiofor *et al.* (2014) for samples of jackfruit seed flour. Solubility reflects the extent of intermolecular cross-bonding with the granule (Hari *et al.*, 1989).

### 3.3 Lethal effects of jackfruit seeds crude extract on embryonic development of zebrafish

The lethal effects observed in the control and exposed zebrafish embryos in response to exposure of various concentrations of crude extract for 96 hours are shown in Figure 1. Embryos of the control group in embryo medium showed normal embryonic development (Nagel, 2002) with zero embryo mortality through the 96 hours exposure period, thereby satisfying the validation criteria of the OECD 236 test guideline (OECD, 2013). Meanwhile, exposure to the highest level of jackfruit seed crude extract tested in this study (125 µg/ml) resulted in 100% mortality on the zebrafish embryos within 24 hours of exposure (Figure 1a). In contrast, the lower concentrations ( $\leq 31.25$  µg/ml) did not induce any significant lethal effects even after exposure for 48 h (Figure 1b). However, after exposure for 72 h until 96 h, the lower concentrations (15.625 and 31.25 µg/ml) resulted in significant mortality on the zebrafish embryos. Hence, the lowest tested concentration (7.8125 µg/ml), did not significantly affect the survival of zebrafish embryos until the end of the 96 hours exposure.

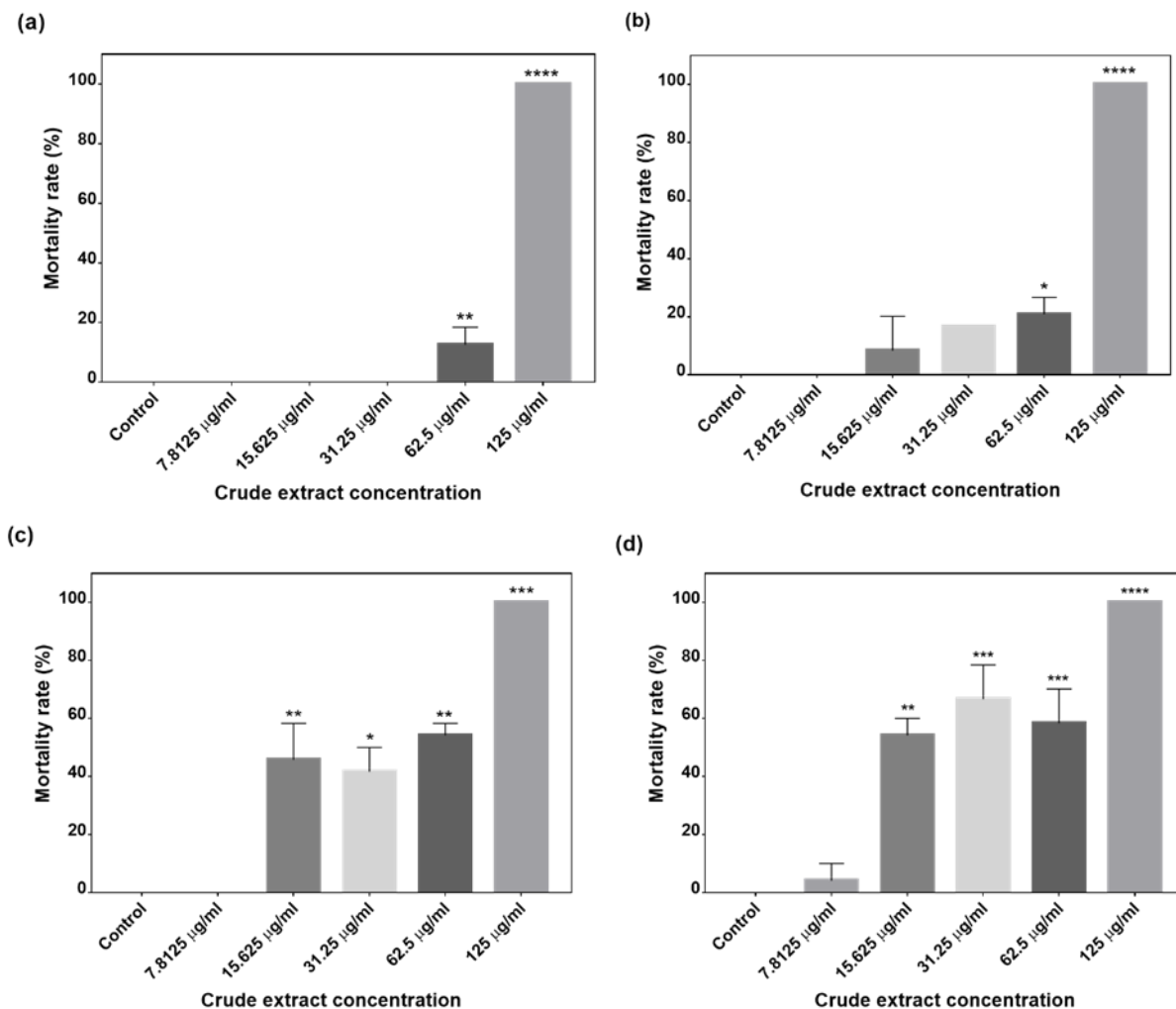


Figure 1. Cumulative mortality rate of zebrafish embryos after exposure to (a) 24 hrs, (b) 48 hrs, (c) 72 hrs and (d) 96 hrs to the indicated concentration of jackfruit seed crude extract. The data are presented as the mean  $\pm$  SEM of duplicate experiments. Asterisks represent significant differences between control and experimental groups within each time. The asterisks are expressed as: \* $P < 0.0332$ , \*\* $P < 0.0021$ , \*\*\* $P < 0.0002$ , \*\*\*\* $P < 0.001$  (one-way ANOVA, followed by Dunnett's post-tests)

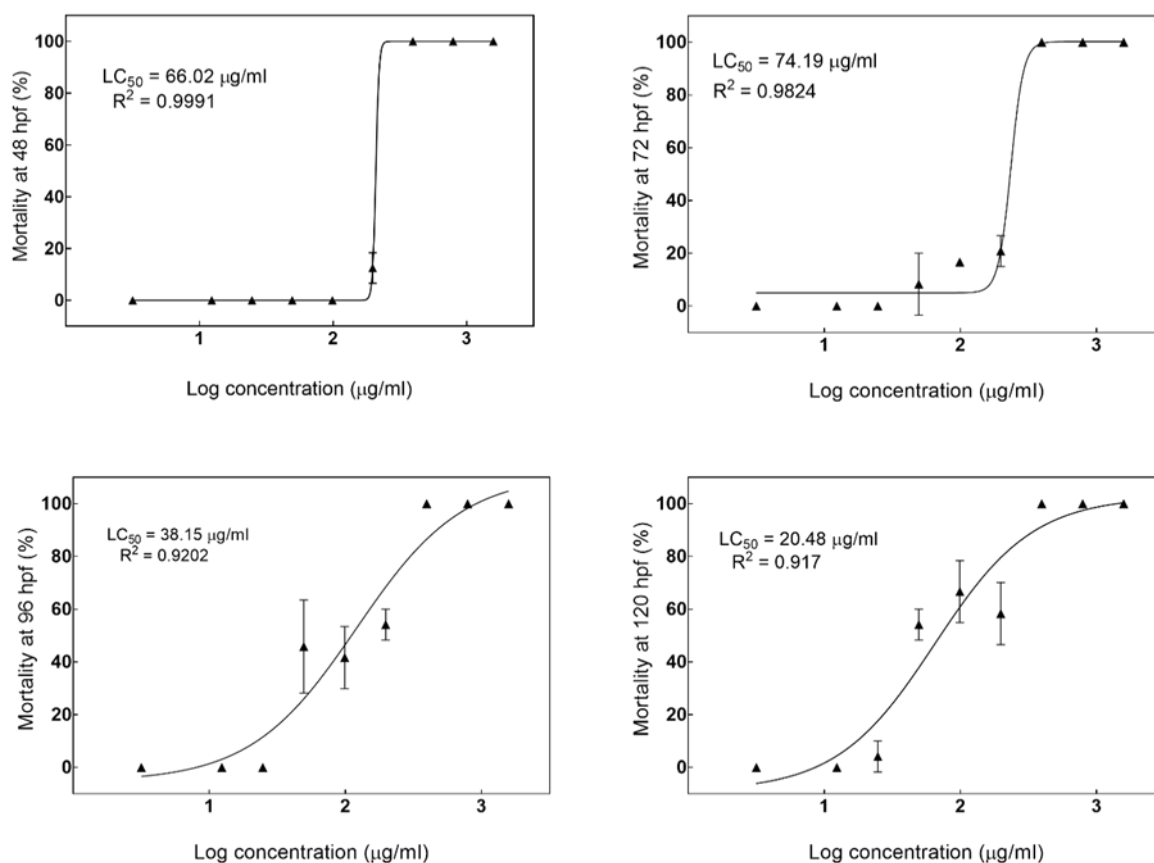


Figure 2.  $LC_{50}$  values for jackfruit seed crude extract against developing zebrafish embryos calculated at different hours of exposure

The median lethal concentration ( $LC_{50}$ ) values of jackfruit seed crude extract against zebrafish embryos at 48 hpf, 72 hpf, 96 hpf and 120 hpf were also calculated and presented in Figure 2. Another study on *in vivo* toxicity and teratogenic effects of *Thuja orientalis* L. extract on zebrafish embryos revealed 0.7029 mg/mL as  $LC_{50}$  (Breeta *et al.*, 2018).

The present study is the first report on the impact of jackfruit seed crude extract on the early life stages of the zebrafish. Overall, we found that the jackfruit seed crude extract showed toxic effects on the embryonic development of zebrafish in a concentration and time-dependent manner. Similar results were also demonstrated by Ismail *et al.* (2017) who evaluated the safety and toxicity of *Andrographis paniculata*, *Cinnamomum zeylanicum*, *Curcuma xanthorrhiza*, *Eugenia polyantha* and *Orthosiphon stamineus* water extracts using the zebrafish embryo model. The increased embryos mortality observed at the highest concentration indicates that the early development of zebrafish embryos was sensitive towards the higher concentration of jackfruit seed crude extract tested in this study. According to Lam *et al.* (2005), the significant toxic effects exhibited by several bioactive compounds, chemicals or drugs derived from plant sources against zebrafish embryos might be due to the presence of toxic compounds in the extracts. Previously, the *A.*

*heterophyllum* phosphate buffer saline (PBS) extract has been reported to induce antiproliferation effects against human breast cancer (MCF7) and non-small lung carcinoma (H1299) cells (Mohd Ali, 2014). The cytotoxic activity may be due to the presence of jacalin, a galactose-binding lectin in the crude extract of jackfruit seed (Kabir, 1994). Besides that, this could also mean that the surrounding chorion failed to protect the exposed embryos against the harsh environment. Therefore, toxic compounds contained in the jackfruit seed crude extract might easily diffuse to enter the cellular compartments in the zebrafish embryos (Majewski *et al.*, 2017).

#### 4. Conclusion

This study revealed that the jackfruit seed powder from the Malaysian cultivar of *A. heterophyllum* (Mastura) was rich in carbohydrates, proteins and minerals elements. Hence, the seed powder has a lot of potentials to be manipulated into various applications in the food and pharmaceutical industry. We also demonstrated that high concentrations of jackfruit seed crude extract were toxic to the development of zebrafish embryos in a concentration and time-dependent manner. In the future, investigation on the identification of the compound responsible for the toxic effects observed in the exposed zebrafish embryos as well as the underlying toxic mechanism should be carried out.

## Conflict of Interest

The authors declare no conflict of interest.

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