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Comparative nutritional and toxicity analyses of beverages from date seed and barley powders as caffeine-free coffee alternatives

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Abstract

Coffee is one of the most preferred beverages due to its flavour and aroma, which is partially contributed by the presence of caffeine. However, there are many negative effects of caffeine on human health. Alternative products like date seed and barley beverage have become an interest to switch from caffeine to caffeine-free beverages. The present work thus aimed to evaluate the nutritional properties and toxicity of date seed and barley powders as compared to Arabica coffee powder. Samples were analysed for its caffeine content, antioxidant activity, and toxicity activity from boiled water extract, whereas the nutrition compositions and heavy metal contents were analysed based on respective extraction method performed. A mass spectral peak of caffeine was detected in the Arabica coffee but not in the date seed and barley powders. All three samples were shown to possess antioxidant activities with Arabica coffee yielding the highest. Arabica coffee, however, exhibited a moderate level of toxicity to human lung fibroblast (MRC-5) cell line with IC_{50} of 230 ± 40 µg/mL at the extract concentration. There was no inhibition on 50% MRC-5 cell viability showed by the date seed and barley powders up to 10 mg/mL extract concentration. The abundance of heavy metals detected in all samples was lower than the regulatory limits. Our findings therefore further supported the advantages of date seed and barley powders as alternatives to coffee beverage as both contained undetected amount of caffeine, low fat and high carbohydrate contents, and possessed good antioxidant activity with low potential health risks.

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Introduction

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid that occurs naturally in coffee beans, tea leaves, and in more than 60 other plant species. Coffee has been found to contain 31 to 124 mg of caffeine for each 100 g of coffee (Higdon and Frei, 2006). Caffeine has been mostly used as dietary ingredient and psychostimulant by about 80% of the world population (Willson, 2018). Caffeine has been associated with some beneficial effects in adults; however, regular consumption higher than the recommended intake may cause undesirable effects such as tension and anxiety (Jin *et al.*, 2016), headache (Espinosa Jovel and Sobrino Mejía, 2017), insomnia (Jin *et al.*, 2016), infertility (Lyngsø *et al.*, 2017), inhibition of collagen biosynthesis, as well as generating toxicity effect. Caffeine also acts as an antagonist to adenosine receptor that causes stimulatory effects of most biological functions including stimulation of central nervous system, acute elevation of blood pressure, and increment in metabolic rate (Higdon and Frei, 2006; Willson, 2018). Caffeine intakes higher than 300 mg a day also lead to spontaneous abortion in pregnant women, and affect foetal growth and lactating conditions (Higdon

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and Frei, 2006; Lyngsø *et al.*, 2017). Negative effects of caffeine are more obvious in older adults under medication as a high concentration of caffeine in plasma could increase the risk of interaction with the drugs. Some medications such as cimetidine, disulfiram, oestrogens, and quinolone class antibiotics have the potential to increase caffeine-related side effects (Higdon and Frei, 2006).

Date seed, a wasted by-product of date (*Phoenix dactylifera* L.) fruit industry, has been widely utilised in food products such as in the production of cereal snacks and bakery products (Najjar *et al.*, 2020). Date seed powder has been reported to contain high dietary fibres and phenolic compounds. The higher content of dietary fibres has provided various therapeutic functions against diabetes, obesity, hypertension, and coronary heart diseases (Fikry *et al.*, 2019), while the phenolic compounds play important roles in protecting against free radicals (Rahmani *et al.*, 2014), hypercholesterolemia (Takaeidi *et al.*, 2014), cancers, asthma, and coughing (Fikry *et al.*, 2019). Date seed powder has also been used in non-caffeinated drinks (Venkatachalam and Sengottian, 2016). In Arab countries, roasted date seed powder is consumed as a coffee-like drink to avoid bad impacts of high caffeine intake from coffee (Fikry *et al.*, 2019). The date seed is processed into powder for a coffee substitute, and being commercialised by the Arab people as a plain product, and also as a mixture with coffee.

Barley (*Hordeum vulgare* L.) also has been used as a coffee-like beverage. In Ethiopia, barley is roasted and served as a coffee-like beverage. Barley is a type of cereals that has high dietary fibre content, and often used in Chinese traditional medicine (Minaiyan *et al.*, 2014; Zeng *et al.*, 2020). The presence of β-glucan in barley also makes it medicinally useful as this bioactive compound can reduce the risk of coronary heart diseases (Minaiyan *et al.*, 2014). Barley β-glucan (1/3, 1/4-β-D-glucan) could decrease the cholesterol level in hypercholesterolaemic hamster (Tong *et al.*, 2015). Barley has also been reported to be a good source of magnesium which plays important roles in glucose metabolism and the secretion of insulin (Minaiyan *et al.*, 2014). Some studies showed the effectiveness of barley to control blood glucose level (Minaiyan *et al.*, 2014), atherosclerotic cardiovascular disease (Gangopadhyay *et al.*, 2015), and cancers such as colon, lung (Czerwonka *et al.*, 2017), breast, and prostate (Woo *et al.*, 2017) cancers.

To evaluate the date seed and barley powders potential as caffeine-free drinks as less harmful alternative drinks for coffee consumers, the present work compared the nutritional properties and safety of both date seed and barley powders with the commercially available Arabica coffee in terms of their caffeine contents, antioxidant activities, proximate compositions, heavy metals, as well as toxicity levels.

Materials and methods

Sample collection

Pure roasted date seed and barley powder with no additives added were provided by a factory in Jordan. Ground pure Arabica coffee powder was purchased from a local market in Kuala Lumpur, Malaysia, as a control.

Human embryonal lung fibroblast (MRC-5) preparation

Human embryonal lung fibroblast (MRC-5) cell line was maintained in RPMI 1640 complete culture media enriched with 10% foetal bovine serum (FBS), 1% antibacterial (penicillin-streptomycin), and 0.1% antifungal (amphotericin-B).

Caffeine detection using LCMS-QToF

Samples were extracted in the ultrapure water at boiling temperature for 30 min, twice, according to Nyoro *et al.* (2018) with slight modifications. The supernatant was collected by centrifugation at 4,000 rpm for 5 min, followed by freeze-drying to obtain constant dry weight. The extract was re-dissolved in ultrapure water at 1 mg/mL, and directly filtered into 2 mL vial using a 0.2-µm syringe filter. The sample was injected into an Agilent 1290 Rapid Resolution Liquid Chromatography equipped with 6550 iFunnel Mass Spectrometry - Quadrupole Time of Flight (LCMS-QToF) System (Agilent Technologies Inc., USA). The injection volume of 3 µL was run through 1.8- µm × 2.1 mm × 150 mm Agilent Zorbax Rapid Resolution High Definition Eclipse Plus C_{18} column at a flow rate of 0.3 mL/min. The running parameter was set following Jamil *et al.* (2018) with some in-house parameter modification. The mobile phases were (A) ultrapure water and (B) 0.1% formic acid in acetonitrile at reverse-phase gradient of 5% B (0.5

min), 5 - 30% B (4.5 min); 30 - 60% B (4 min), 60 - 95% B (5 min), 95 - 5% B (1 min), and 5% B for calibration (5 min). The thermostat column temperature was fixed at 30°C. Samples were run in positive ionisation mode with the following MS parameters: gas temperature, 290°C; gas flow, 11 L/min; nebuliser pressure (N2), 35 psi; sheath gas, 11 L/min at 350°C; capillary voltage, 3,500 V; nozzle voltage, 1,000 V; and fragmentor voltage, 175 V. Ultrapure water was used as blank. Four technical replicates were used for each sample.

Proximate composition

Proximate analysis was conducted according to Association of Official Analytical Chemists methods for moisture, crude fat, crude protein, crude fibre, ash, and carbohydrate contents (Jauhari *et al.*, 2013). Briefly, the moisture content was determined by oven-drying 5 g of samples at 105°C for 18 h, and the dry weight differences were measured. The crude fat was extracted from the samples in petroleum ether at 160°C for 3 h, oven-dried, and the percentages of crude fat were determined by calculating the percentage differences in dry weight. The crude protein content (% N in protein) was analysed using the Kjeldahl method, where samples (0.5 g) were added with 7 g of potassium sulphate and 0.8 g of copper sulphate, followed by 12 mL of concentrated sulphuric acid. Digestion process was performed until the blue-green clear solution appeared. The solution was allowed to cool before distilling with 40% sodium hydroxide for 5 h, followed by titration with 0.1 N hydrochloric acid for the production of pink colour solution. Crude protein content was calculated using Eq. 1:

$$Crude\ protein(\%) = \frac{(Titrant_{sample} - Titrant_{blank}) \times 0.1\ N \times 6.25 \times 14.007}{Sample\ Weight_{mg}} \times 100 \quad \text{(Eq. 1)}$$

The crude fibre content was determined where the residues obtained from the extraction of 5 g of sample with 200 mL of petroleum ether were digested with 200 mL of 0.255 N sulphuric acid by boiling for 1 h. The residue was collected and further digested with 200 mL of 0.3 N sodium hydroxide by boiling for another hour. The residue was collected on an ashless filter paper, dried overnight, and followed by ashing for 18 h at 550°C. The weights of fibre after drying and ashing was collected. The crude fibre content was calculated using Eq. 2:

$$Crude\ fibre\ (\%) = \frac{Total\ weight\ after\ drying - Total\ weight\ after\ ashing}{Sample\ Weight_g} \times 100 \quad \text{(Eq. 2)}$$

The ash content of the samples was measured where a sample (5 g) was dried in a furnace at 550°C for 18 h to form white or nearly white ash. The sample was then left to cool for 1 h. The ash content of the sample was calculated using Eq. 3:

$$Ash\ yield\ (\%) = \frac{Weight\ remaining\ ash_g}{Weight\ of\ pre-combustion\ sample_g} \times \frac{100}{100 - Moisture\ content} \times 100 \quad \text{(Eq. 3)}$$

The total carbohydrate content of the samples was determined by weight differences.

Antioxidant activity

The antioxidant activity of each sample was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to procedures described by Sahu *et al.* (2013) with slight modifications. Briefly, the dried extracts of barley and date seed powders were prepared at 1,000 µg/mL by dissolving both powders, respectively, in ultrapure water and followed by two-time dilution to final concentrations of 1,000, 500, 250, 125, 62.5, 31.25, and 15.63 µg/mL; while the dried extract of Arabica coffee was dissolved in ultrapure water at 100 µg/mL followed by two-time dilution to final concentrations of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µg/mL. The sample concentrations, each at 1.5 mL volume, were mixed with 0.1 mM DPPH reagent at 1:1 ratio, and incubated at room temperature in a dark condition for 30 min. The amount of DPPH radicals that have been reduced in the presence of antioxidant molecules in the samples were measured using UV-visible spectrophotometer at 517 nm against water as blank. Negative control was the DPPH solution without any samples. DPPH radical scavenging (%) was calculated using Eq. 4:

$$Inhibition\ in\ DPPH\ (\%) = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100 \quad \text{(Eq. 4)}$$

Toxicity

The extracted samples were re-dissolved in complete culture media (under an aseptic condition at 10 mg/mL, and sterile filtered using a 0.2-µm syringe filter). Toxicity activity was determined through the 3,(4,5-dimethylthiazol-2-yl)-2,3-diphenyltetrazolium bromide (MTT) assay using human embryonal lung fibroblast (MRC-5) cell line, as described by previous studies (Banimustafa *et al.*, 2013) with slight modification. Each well of 96 well plates was loaded with 100 µL of MRC-5 monolayer cell at 1×10^4 cells/mL, and incubated for 24 h at 37°C in the presence of 5% CO_2. The media in the three wells of the first row was removed, and re-filled with 100 µL of the 10,000 µg/mL extract. Two-time serial dilution was applied directly on the wells with the highest extract concentration was at the top rows of the plate with the final concentrations of 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5 µg/mL. The plate was incubated for 72 h at 37°C with 5% CO_2. The media in each well was removed, and 20 µL of 5 mg/mL MTT solution was added, followed by incubation for 4 h under the same incubation conditions. Next, 100 µL of DMSO was then added into each well to dissolve any purple formazan crystal produced. The absorbance of the solution was measured at 590 nm using a microplate reader. The cell viability (%) was determined using Eq. 5:

$$Cell\ viability\ (\%) = \frac{Absorbance_{sample} - Absorbance_{blank}}{Absorbance_{control} - Absorbance_{blank}} \times 100 \qquad \text{(Eq. 5)}$$

Results

Determination of caffeine content in the boiled extracts of date seed, barley, and Arabica coffee powders

Analysis of caffeine content on the boiled water extract of each powder was performed using Agilent MassHunter Qualitative Analysis software. The identification of peak of caffeine was determined through database search (DB search) using Agilent Personal Compound Database and Library Manager Software (PCDL Manager) that provides complete information on the caffeine molecular formula ($C_8H_{10}N_4O_2$), average mass (194.0804 Dalton), and molecular structure which are linked to CAS reference number of 58-08-2, and ChemSpider ID of 2424. From the mass chromatogram in Figure 1 (Ai), areas of 1.05 × 107 and 7.68 × 107 were detected at minute 4.598 ± 0.006 in the boiled water extract of Arabica coffee. The peak matched (93.09%) with caffeine standard from the database based on the molecular weight of 194.0804 Dalton, MS spectrum of 195.0882 m/z [M+H]+, and the molecular structure (Figure 1A). Interestingly, there was no peak of caffeine detected in the boiled water extract of date seed (Figure 1B) and barley (Figure 1C) at the same retention time. The same result was also obtained from single molecular searching on all samples.

Proximate compositions of date seed, barley, and Arabica coffee powders

The proximate compositions of date seed, barley, and Arabica coffee powders were determined in percentage (%). As shown in Table 1, date seed and barley powder contained lower fat and higher carbohydrate as compared to Arabica coffee powder. Against Arabica coffee, barley powder showed higher fibre content, and date seed powder showed higher total protein content. Water extracts of date seed, barley, and Arabica coffee powders obtained through the boiling process were used in the determination of antioxidant activity and toxicity activity.

Antioxidant activity and toxicity of date seed, barley, and Arabica coffee powders

Antioxidant activity was represented by the concentration of extract that inhibited 50% (IC_{50}) and 25% (IC_{25}) of DPPH radicals obtained from the graph of DPPH antioxidant radical scavenging activity versus sample concentration. Antioxidant activity of Arabica coffee powder at 25 and 50% DPPH radicals (Figures 2B and 2D) was greater than date seed and barley powders (Figures 2A and 2D). There was no DPPH radical scavenging activity by barley powder detected at 50% DPPH radicals up to 1,000 µg/mL extract concentration, while exhibiting almost 1,000 times lower antioxidant activity at 25% DPPH radicals (980.0 ± 28.3 µg/mL) as compared to date seed powder. Lethal effect on 50% cell viability of those powders was determined on human normal lung cell line (MRC-5) using MTT Assay from the graph of cell viability (%) versus sample concentration. In contrast to the boiled water extracts of date seed and barley powders which did not show any inhibition at 50% cell viability up to 10,000 µg/mL extract concentration, the boiled water extract of Arabica coffee powder inhibited 50% of the cell viability at the extract concentration as low as 230 ± 40 µg/mL, as shown in Figures 2C and 2D.

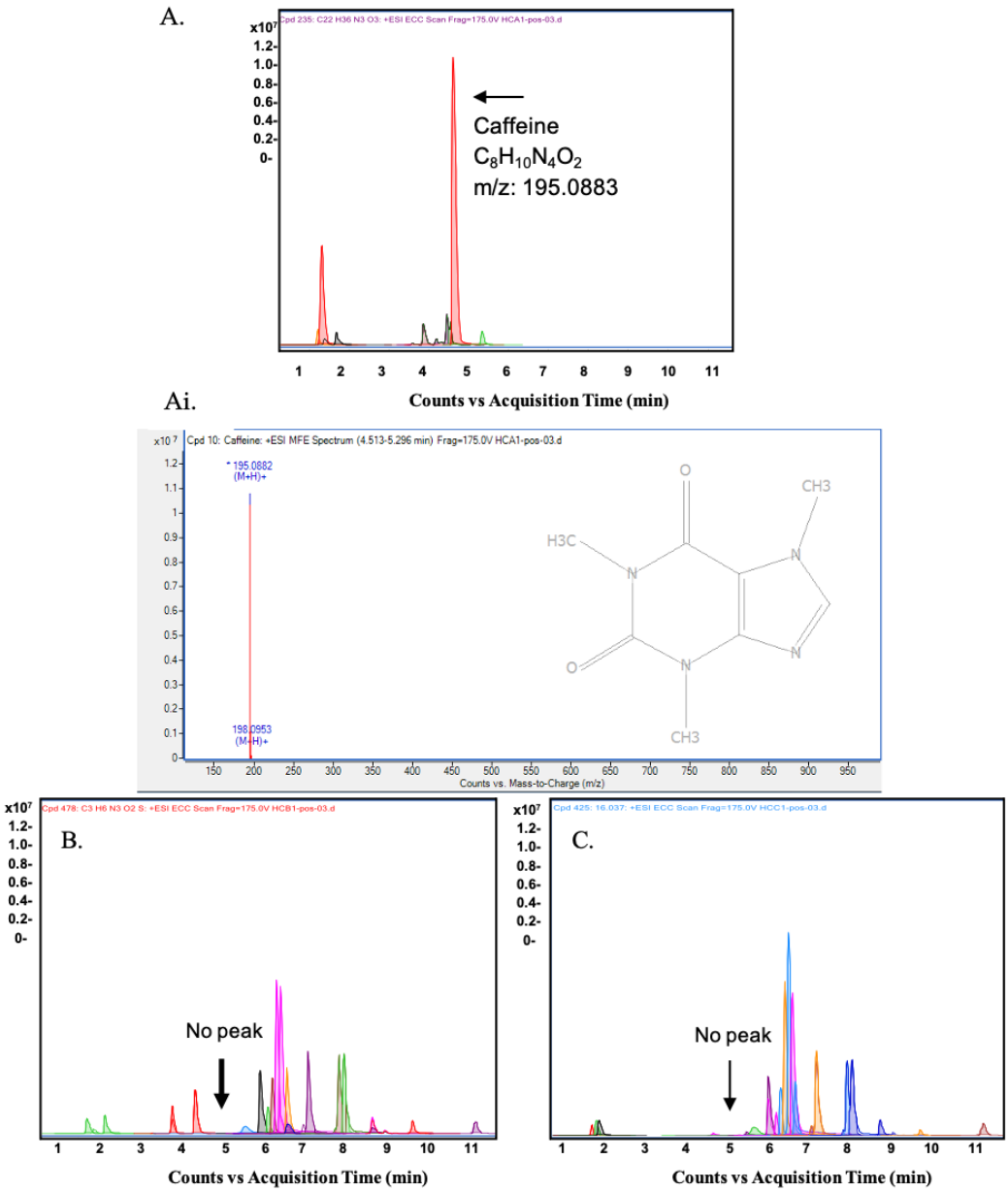
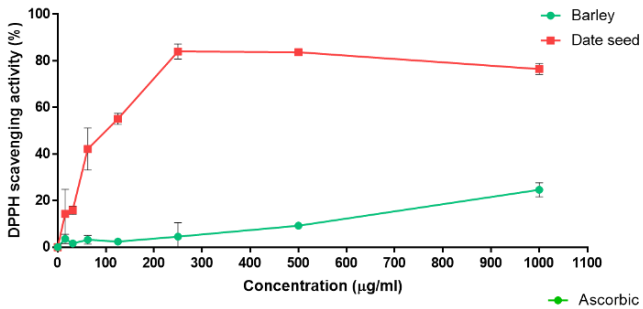


Figure 1. Full mass spectrometry compound chromatogram of the boiled water extract of Arabica coffee powder **(A)**, date seed powder **(B)**, and barley powder **(C)** generated by LC/MS-QToF system.

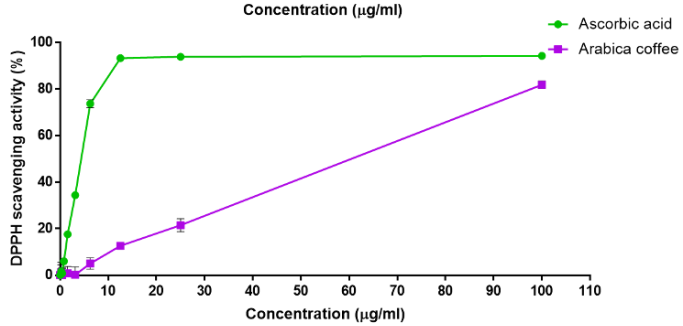
Table 1. Proximate compositions (%) of Arabica coffee, date seed, and barley powders.

	Proximate composition (%)		
	Arabica coffee	**Date seed**	**Barley**
Moisture	3.3	2.9	1.9
Crude fat	4.4	0.8	1.9
Crude protein	15.7	11.7	2.4
Crude fibre	16.0	6.7	10.1
Ash	18.7	17.2	5.5
Total carbohydrate	41.8	60.8	78.2

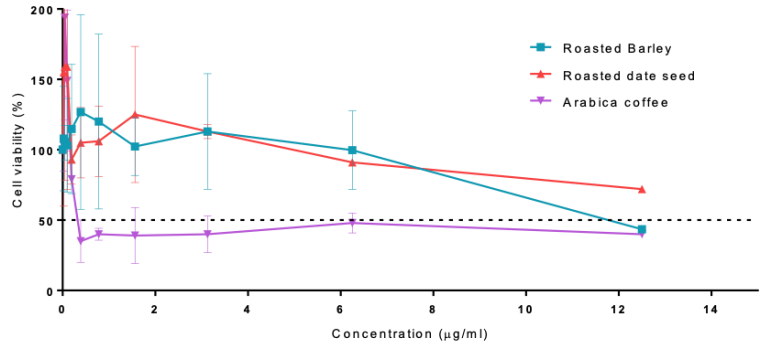
A.



B.



C.



D.

	Inhibition Concentration, µg/mL		
	Arabica coffee	**Date seed**	**Barley**
Antioxidant, (IC_{50})	39.0±4.2	85.0±21.2	ND*
(IC_{25})	25.3±4.6	37.5±9.6	980.0±28.3
Toxicity, (IC_{50})	230±40	ND**	ND**

ND*: Not detected up to 1,000 µg/mL extract concentration

ND**: Not detected up to 10 mg/mL extract concentration

Figure 2. DPPH antioxidant radical scavenging and toxicity activities of boiled water extract of barley, date seed, and Arabica coffee powders. DPPH antioxidant radical scavenging activity of barley and date seed powders **(A)** and Arabica coffee powder **(B)**; toxicity activity of barley, date seed, and Arabica coffee powders on MRC-5 cell line **(C)**; and summarised data on antioxidant and toxicity activities of barley, date seed, and Arabica coffee powders **(D)**.

Heavy metals in date seed, barley, and Arabica coffee powders

The analysis of heavy metal contents in the powders of date seed, barley, and Arabica coffee was focused on six heavy metals found in food and beverages products, as stated in the Food Act 1983 and Food Regulations 1985 namely mercury, arsenic, lead, stanum, cadmium, and antimony at maximum levels of 1, 1, 2, 40, 1, and 1 mg/kg, respectively (Alina *et al.*, 2012). Each powder contained all the six heavy metals, but were below the maximum permissible limits (Table 2). Mercury and antimony contents in the date seed and barley powders were not significantly different from each other, but were higher in Arabica coffee powder. Cadmium and stanum contents were comparable between date seed and Arabica coffee powder, with barley powder yielding the lowest value for cadmium, and the highest for stanum. The lead content was not significantly different in all the three samples, whereas the presence of arsenic was the highest in barley powder, followed by date seed and Arabica coffee powder.

Table 2. Heavy metals contents (mg/kg) of Arabica coffee, date seed, and barley powders.

	Heavy metal content (mg/kg)		
	Arabica coffee	**Date seed**	**Barley**
Mercury, Hg	0.044 ± 0.011	0.026 ± 0.004	0.023 ± 0.002
Arsenic, Ar	0.010 ± 0.002	0.017 ± 0.002	0.026 ± 0.005
Lead, Pb	0.156 ± 0.003	0.196 ± 0.006	0.161 ± 0.015
Tin, Sn	5.980 ± 0.841*	6.427 ± 0.811*	11.600 ± 1.145*
Cadmium, Cd	0.017 ± 0.003	0.016 ± 0.001	0.009 ± 0.002
Antimony, Sb	0.073 ± 0.008	0.059 ± 0.005	0.054 ± 0.004

* p value < 0.001.

Discussion

Many ethnic groups worldwide consider coffee as a drink of choice during formal and non-formal occasions. The benefits of drinking coffee, however, have been criticised for some negative biological effects to human health especially due to the high content of caffeine. This issue has encouraged researchers and healthcare professionals to recommend caffeine-free beverages. Discontinuation of caffeine intake has also been prescribed to patients with specific health conditions. Due to this, many people have decided to change their lifestyle from caffeinated coffee to decaffeinated types without realising that decaffeinated coffee is not completely free of caffeine. About 0.6 to 3.09% of caffeine has been detected in commercial decaffeinated coffee powder (Nyoro *et al.,* 2018). The caffeine content in coffee after decaffeination is reduced only at 0.02 to 2% (Farah, 2009). Superko *et al.* (1991) reported an increment in plasma low-density lipoprotein (LDL), a type of bad cholesterol, by consuming decaffeinated coffee.

Barley and date seed have gained interest as an alternative coffee beverage that was claimed free from caffeine (Zeng *et al.,* 2020). It has been proven in the present work that no caffeine was detected in the boiled water extract of barley and date seed powders by the highly sensitive instrument capable of detecting any metabolites at femtogram level. The results of zero (0%) caffeine content were also found previously in date seed powder (Venkatachalam and Sengottian, 2016) and barley powder (USDA, 2018). As expected, the level of caffeine content in the Arabica coffee powder based on the area of the peak was very high as it reached up to tens of millions for 3 µg sample, which was equivalent to 3 µL volume injection of 1 mg/mL sample. This result was also supported by a study of Nyoro *et al.* (2018). Low content of fat and high content of carbohydrate in barley and date seed powders based on the nutrient data can make them more preferable as compared to the Arabica coffee, as both could be healthy to the heart muscle (Hamdy *et al.,* 2018). The consumption of coffee has been reported to cause an increment in serum lipid (including cholesterol and triglycerides) and low-density lipoprotein (bad cholesterol) (Buscemi *et al.,* 2016).

High coffee intake also has been observed to cause an increment in the risk of getting coronary heart diseases (Higdon and Frei, 2006). In contrast, the consumption of date seed powder through a study on hyperlipidemic rabbits has reduced the serum lipid (cholesterol and triglycerides) and low-density lipoprotein (LDL), while increasing the serum level of high-density lipoprotein (HDL), also known as good cholesterol (Mushtaq *et al.,* 2017). The decrease in serum lipid (cholesterol and triglycerides) and LDL, and increase in serum HDL were also observed with the consumption of barley by hypercholesterolaemic men (Behall *et al.,* 2004; Wang *et al*., 2017). DPPH radical scavenging activity of barley powder in the present work was comparable with a previous study by Djordjevic *et al.* (2011), where no inhibition at 50% DPPH radicals by barley extract was detected, as well as by wheat (cereal) and soybean (legume). The present work also showed that antioxidant activity of barley was considered promising at 25% DPPH radicals, two-time higher than wheat, and almost the same as compared to soybean.

Lung cancer is considered the highest type of cancer affecting men in Malaysia; in the present work, the inhibition of 50% viability on lung cancer cell line MRC-5 cell was observed following the treatment by Arabica coffee extract; but not the date

seed and barley extracts. This could be due to the presence of caffeine in the Arabica coffee extract. In a study that used mammalian cell line (CHO), caffeine has been found to exhibit acute cellular toxicity by inhibiting the growth and causing death to the cells (Kuwayama, 2012). *In vivo* study on the acute toxicity of caffeine was reported in animals such as fish (*Leuciscus idus*) with LC_{50} of 87 µg/mL after 96 h exposure, rats with LD_{50} of 200 - 400 mg/kg, and mice with LD_{50} of 185 mg/kg (CCOHS, 2012). The report suggested that the oral intake of caffeine caused moderate toxicity to mammals. In another study, two-week oral consumption of an aqueous extract of Robusta coffee beans has been reported to cause low acute toxicity on Wistar rats with LD_{50} of 2,000 mg/kg for roasted beans, and LD_{50} of 5,000 mg/kg for ground beans. The acute toxicity of Arabica coffee was contrary to the antioxidant activity at almost 1,000 times higher than that of the barley powder extract. There was a claim that the consumption of caffeine at certain concentration is very crucial since caffeine is very beneficial at low concentration as it can act as antioxidant; however, apoptosis may occur at high dosage (Liu *et al.*, 2017).

The higher antioxidant activity of Arabica coffee powder observed in the present work was probably due to its intense dark colour. DPPH absorption is proportionally interrupted by the colour intensity, as the darker the colour, the lower the absorbance value (Liang and Kitts, 2014). As a result, the darker sample could possess a greater value of DPPH radical scavenging activity. This was observed in an earlier study (Yashin *et al.*, 2013) in which the antioxidant activity of Arabica coffee increased as the colour of coffee extract increased.

The lower concentration than the regulatory limits of six heavy metals in the date seed and barley has fulfilled the requirement of Food Act 1983 and Food Regulations 1985 (Alina *et al.*, 2012). This has made both the caffeine-free powders suitable for daily consumption as suggested in a study that found the safe level of heavy metals in some commercially roasted ground coffee. However, other studies have detected the presence of heavy metals in certain samples of date seeds (Aldjain *et al.*, 2011), barley (Stanisˇić Stojić *et al.*, 2016), and coffee beans (da Silva *et al.*, 2017). This could be due to the varieties and origins of the food/plant samples as stated previously (da Silva *et al.*, 2017), or human activities such as waste mining and application of pesticides (Aldjain *et al.*, 2011; da Silva *et al.*, 2017).

Conclusion

Coffee is one of the products that contain a high amount of caffeine. Caffeine has been reported to contribute a lot of beneficial effects of coffee including energy and alertness booster. However, the intake of caffeine, especially at high dosage, may contribute to negative impacts on human health. Therefore, the search for alternative sources to serve as caffeine-free coffee is vital for better health. Date seed and barley have been suggested to provide great coffee-like taste, and increasingly used in few communities. The present work has demonstrated that date seed and barley powders have promising medicinal properties in comparison to Arabica coffee, with lower fat and higher carbohydrate, no toxic heavy metals, promising antioxidant activity, and harmless to human normal cell line. Future studies on the metabolites from both date seed and barley powders will provide complementary information and deeper characterisation of their key metabolites. Further research should be performed on exploring the potential antioxidant activity on a range of different cancer cell lines.

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