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FULL PAPER

Antioxidant and antibacterial activities in the fruit peel, flesh and seed of *Ceri Terengganu* (*Lepisanthes alata* Leenh.)

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Abstract

Malay cherry fruit or locally known as *Ceri Terengganu* (*Lepisanthes alata* Leenh.) is a local tropical exotic fruit and it is native to Malaysia. The *Ceri Terengganu* tree is widely distributed in the east coast of Peninsular Malaysia particularly in Terengganu, Pahang and Johor and commonly cultivated as an ornamental plant in the villages and gardens. A limited number of studies had been done on the proximate analysis and postharvest quality of *Ceri Terengganu* fruit, but the studies on the antioxidant and antibacterial activities of *Ceri Terengganu* fruit extract are still lacking. Hence, this study aimed to determine the antioxidant and antibacterial activity in the peel, flesh and seed extracts of *Ceri Terengganu*. The *Ceri Terengganu* was extracted using 60% ethanol and the total phenolic content (TPC), total flavonoid content (TFC), total monomeric anthocyanins, antioxidant and antibacterial activities were measured using standard methods. The results showed that the seed of *Ceri Terengganu* had the highest amount of TPC, TFC and antioxidant activity, followed by the peel and flesh extracts whilst the peel extract had the highest total monomeric anthocyanins content. Furthermore, all three extracts of *Ceri Terengganu* showed inhibition against selected pathogens tested. In conclusion, the seed of *Ceri Terengganu* possessed the greatest potential to be explored as a source of natural antioxidant and antibacterial agent in the food industry, and thus warrant further investigation.

1. Introduction

Malay cherry fruit or locally known as *Ceri Terengganu* or *Pokok Johor* (*Lepisanthes alata* Leenh.) is a local tropical exotic fruit and is native to Malaysia (Anuar *et al*., 2014; FRIM, 2019). The *Ceri Terengganu* fruit is a globose berry, arranged closely and attractively in big bunches formed by clusters containing about 20 fruits per cluster. Furthermore, the size of each fruit is about 2 cm to 3 cm in diameter and it is deep red with a pointed tip. Each of the fruit contains one to three seeds and its flesh is soft and tastes fairly sweet (FRIM, 2019). In addition, *Ceri Terengganu* is a non-seasonal fruit which can be found throughout the year. Unfortunately, *Ceri Terengganu* is not consumed by the locals but instead planted as an ornamental plant.

Several studies had been done using *Ceri Terengganu* fruit such as on the proximate analysis, total flavonoid content, antioxidants and antibacterial activities. A recent study by Anggraini *et al*. (2019) used different parts of *Ceri Terengganu* plant (whole mature fruit, young leaves and bark) and fruit (rind, flesh and seed) from Indonesia on the total phenolic content, total monomeric anthocyanin and antioxidant activity analysis. Another study by Rahmadi *et al*. (2016) proved that *Ceri Terengganu* fruit peel exerts antimicrobial effect against *Escherichia coli* and *Staphylococcus aureus.* However, none reported on the antibacterial activity of the seed and the methods of analysing the antioxidant activities varied with this study. In addition to that, these previous studies showed that, instead of being an ornamental plant in garden landscapes, the *Ceri Terengganu* fruit can be utilised for its natural antioxidant and antimicrobial activities to be used in the growing food industry. Hence, this study was aimed to determine the chemical properties, antioxidant and antibacterial activities in the peel, flesh and seed extracts of *Ceri Terengganu*.

2. Materials and methods

2.1 Chemicals

The chemicals used for extraction and analysis were

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60% ethanol, 7.5% sodium carbonate, 0.1 M Folin-Ciocalteu reagent, 5% sodium nitrite ($NaNO_2$), 10% aluminium chloride ($AlCl_3$), 1 M sodium hydroxide (NaOH), potassium chloride, sodium acetate, 2, 6-Dichlorophenolindophenol (DCPIP), DPPH and ABTS reagent. The standard used for calibration curve were gallic acid, quercetin and ascorbic acid while butylated hydroxytoluene (BHT), Vitamin C, Vitamin E and Trolox were used as standard antioxidant. All reagents used were of analytical grades.

2.2 Plant material and sample preparation

Fresh *Ceri Terengganu* fruits were harvested and collected from State Agriculture Complex's orchard located in Ajil Terengganu. The samples were freshly harvested based on the size uniformity and external colour. The collected *Ceri Terengganu* fruits were washed thoroughly under running tap water and dried with tissues. Each of the fruit parts (peel, flesh and seed) were then separated manually. The peel obtained was cut into small pieces while the flesh and seed obtained were homogenised separately in a waring commercial blender. Weighed portions of the peel, flesh and seed of the fruit samples were dried in the drying cabinet with a temperature of 60°C for 2 days until the moisture content reached approximately 10%. Then, the samples were grounded into a fine powder by using a commercial blender and was sieved through 250 µm laboratory test siever. The powder obtained was stored in airtight and amber containers at room temperature (25°C) until further analysis.

2.3 Solvent extraction

The extraction of the samples was done according to Anzian *et al*. (2017) and Ghasemzadeh *et al*. (2015). Briefly, 10 g samples powder of each part (seeds, flesh and peel) from *Ceri Terengganu* fruit was mixed with 100 mL of 60% ethanol aqueous solution for 2 hrs at 65° C using water bath shaker to maintain the temperature. Each sample was then centrifuged at 4000 x *g* for 10 mins (Liang *et al*., 2012). The suspension was filtered through Whatman No. 1 filter paper in a filter funnel. The filtrate was pooled and the solvent was evaporated and concentrated using rotary vacuum evaporator at 40° C to obtain a final volume of concentrate crude extract (Anzian *et al*., 2017). The filtrate was then stored at -20° C.

2.5 Chemical analysis

2.5.1 Total phenolic content (TPC)

The total phenolic contents of different parts of *Ceri Terengganu* fruit extract was measured using the Folin-Ciocalteu method (Rakitzis, 1975) with some modifications. Briefly, an aliquot of 1 mL sample solution was mixed with 1.5 mL of 7.5% sodium carbonate and 1 mL of 0.1M Folin-Ciocalteu reagent. After incubation at room temperature for 30 mins in the dark, the absorbance of the reaction mixture was measured at 765nm against reagent blank. Gallic acid was used to obtain a standard curve. The results were presented in mg gallic acid equivalent (GAE)/100 g of sample on a dry weight basis (DW).

2.5.2 Total flavonoid content (TFC)

The total flavonoids content of different parts of *Ceri Terengganu* fruit extract was determined by the aluminium chloride colourimetric method described by Makris *et al*. (2007) with slight modification. An aliquot of 1 mL sample solution was mixed with 4 mL of distilled water in a tube. 0.3 mL of 5% sodium nitrite ($NaNO_2$) was then added and allowed to react for 5 mins. Next, 0.3 mL of10 % aluminium chloride ($AlCl_3$) was added and the mixture was allowed to stand for further 5 mins before adding 2 mL of 1 M sodium hydroxide (NaOH) and 2.4 mL of distilled water to the reaction mixture. The absorbance was measured at 510 nm against a blank of distilled water. Quercetin was used as a standard compound for the quantification of total flavonoids. All values were expressed as mg of quercetin equivalent (QE)/100 g of sample on a dry weight basis (DW).

2.5.3 Total anthocyanin content (TAC)

Total anthocyanin content of different parts of *Ceri Terengganu* fruit extract was measured according to the pH differential method by using two buffer systems. The potassium chloride buffer solution (0.025M, pH= 1) and sodium acetate trihydrate (0.4 M, pH = 4.5) was prepared. Briefly, 1mL test sample extracts were diluted with 3mL of the corresponding buffer for 15 mins. The absorbance of each solution was measured at 512nm and 700nm (for haze correction) against a blank cell filled with distilled water (Moldovan *et al*., 2016). The concentration (mg/L) of total monomeric anthocyanin for each extract was calculated according to the following formula and expressed as Cy-3-glc equivalents:

$$TAC\ (mg/L) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where MW is the molecular weight (g/mol) = 449.2 g/mol for Cy-3-glc, DF is the dilution factor (1 mL sample is diluted to 4 mL, DF= 4); and is the extinction coefficient (L cm^{-1} mol^{-1}) = 26,900 for Cy-3-glc, where L (pathlength in cm) = 1 while 1000 = conversion factor from gram to milligram and A was the nett absorbance.

2.5.4 Total ascorbic acid content (TAA)

The ascorbic acid concentration was measured based

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on the reduction of the dye 2,6-dichlorophenolindophenol (DCPIP) by ascorbic acid described by Fattahi *et al*. (2011) with slight modification. Briefly, 0.5 g of samples were mixed in 3 mL metaphosphoric acid (1%). Then, 0.5 mL of DCPIP was added to the supernatant and measured at 520 nm spectrophotometrically. Ascorbic acid standard curve was prepared. All values were expressed as mg of ascorbic acid equivalent (AA)/100 g of sample on a dry weight basis (DW).

2.6 Antioxidant activity analysis

2.6.1 DPPH free radical scavenging capacity

The DPPH scavenging activity of different parts of *Ceri Terengganu* fruit extract was determined as described by Liang *et al*. (2012) with some modification. Briefly, 2 mL of sample solution was added to 2 mL of the DPPH solution (0.1 mM). The mixture was shaken and incubated for 30 mins at room temperature in the dark. The absorbance of the resulting solution was measured at 517 nm against a blank. Butylated hydroxytoluene (BHT), Vitamin C and Vitamin E were used as standard antioxidant. The radical-scavenging activity was calculated as a percentage of inhibition using the equation below:

$$Inhibition\ (\%) = \frac{A_0 - As}{A_0} \times 100$$

Where A_0 is the absorbance of the blank sample and As is the absorbance in the presence of the different test samples.

2.6.2 ABTS radical scavenging assay

ABTS assay of different parts of *Ceri Terengganu* fruit extract was determined according to the method of Fattahi *et al*. (2011) and Rajurkar and Hande (2011) with some modifications. ABTS radical cation ($ABTS^{\bullet+}$) was prepared by mixing 100mL of potassium persulfate solution (2.45 mM) and 100 mL of $ABTS^{+}$ solution (7 mM) in dark for 24 hrs. The ABTS solution was diluted with 95% ethanol to an absorption value of 0.70±0.02 at 734nm. Briefly, 0.5 mL of sample extract was added to 4 mL $ABTS^{\bullet+}$ radical cation solution for 5 mins before the absorbance being measured at 734 nm. Trolox was used as standard antioxidant. The ABTS scavenging effect was calculated as a percentage of $ABTS^{\bullet+}$ discoloration using the equation below:

$$Inhibition\ (\%) = \frac{A_c - As}{A_c} \times 100$$

Where A_c is the absorbance of the control and As is the absorbance of the sample plus ABTS radical after 5 mins incubation.

2.7 Antibacterial activity analysis

2.7.1 Test microorganisms

Gram-positive bacteria were *Bacillus subtilis* (ATCC 6051), *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 33862) and *Staphylococcus aureus* (ATCC 33862) while Gram-negative bacteria were *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145).

2.7.2 Preparation of inoculum and inoculation of microorganisms on MHA agar

Preparation of the inoculum was prepared using the method suggested by Gebreyohannes *et al*. (2013). Firstly, the inoculum was standardized by matching the turbidity with a 0.5 McFarland standard (No. 1) in Mueller-Hinton Broth (MHB). The MHA agar was then labelled and divided into five zones (peel, flesh, seed, positive control and negative control). The MHA agar plate surface was then inoculated by spreading 100 µL of the microbial inoculum over the entire agar surface using cotton swab was dried at room temperature for about 3 to 5 mins.

2.7.3 Preparation of wells and incubation of MHA agar plates

Preparation of wells followed the method used in the previous study by Balouiri *et al*. (2016). Firstly, 4 wells were punched aseptically with a sterile tip (8 mm) on each zone of MHA agar. 50 l of each sample extract was added into the wells at its respective zone (peel, flesh and seed). Ampicillin, oxytetracycline and chloramphenicol antibiotic disc was served as a positive control and were placed in the respective zone. Sterile distilled water served as negative control. The MHA agar plates were incubated at 37°C for 18 to 24 hrs. The diameter of the resulting zones of inhibition was measured.

2.8 Statistical analysis

All treatments and analysis were carried out in triplicates. All results were reported as mean standard deviation. One-way ANOVA was carried out to determine the significant difference. The differences between means were determined using Fisher's least significant difference (LSD) test with the degree of significant ($p < 0.05$).

3. Results and discussion

3.1 Chemical analysis

3.1.1 Total phenolic content (TPC)

The results obtained in Figure 1(A) showed that

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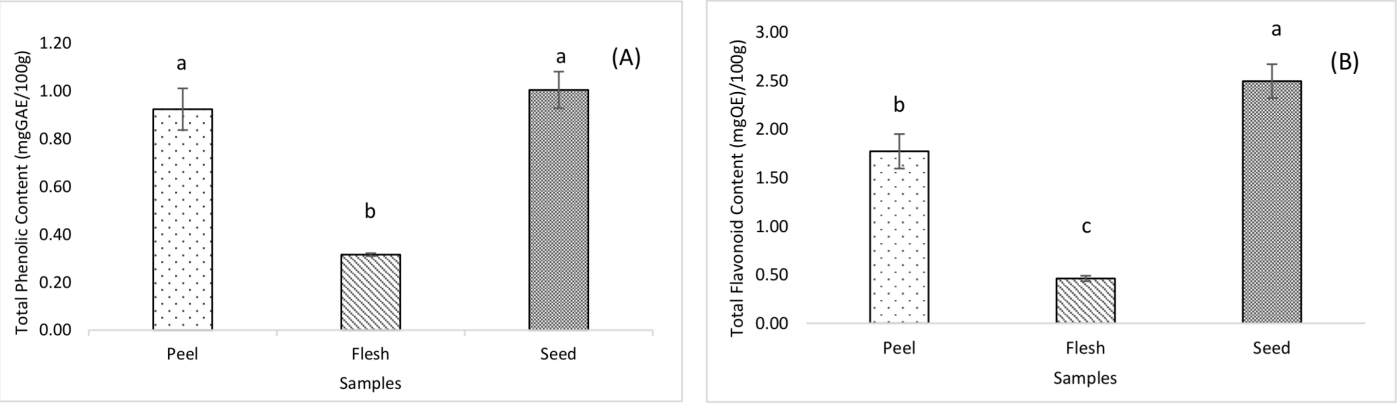

Figure 1. (A) Total phenolic content and (B) total flavonoid content of different parts of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extract. Error bars represent standard deviation values. Different letters indicate significant differences between products at $p<0.05$.

there was no significant difference in the total phenolic content between peel and seed of *Ceri Terengganu* ($p<0.05$) in which the seed (1.00 mg GAE/100 g) extract exhibited the highest total phenolic content followed by the peel (0.92 mg GAE/100 g) and the flesh (0.32 mg GAE/100 g). The findings of this study were in agreement with the previous study by Anggraini *et al*. (2019). The high total phenolic content obtained by Anggraini *et al*. (2019) was primarily due to the different solvent used. The use of 100% methanol in Anggraini *et al*. (2019) study could enable the extraction of low molecular weight phenolic compound due to the high polarity index of methanol compared to ethanol. Additionally, methanol was effective at extracting compounds with higher antioxidant potential (Abarca-Vargas *et al*., 2016). However, in this study, aqueous ethanol was used to extract these compounds primarily due to the ethanol is safer and cheaper as compared to methanol if this study is going to be scaled up.

In other comparisons, the TPC obtained in all three extracts were low as compared to those obtained from grape pomace ethanolic extract (MohdMaidin *et al*., 2018) which showed 2100mg GAE/100 g of TPC values. The reason for this was possibly due to the grape pomace is actually consist of the whole fruit including its seed, peel, flesh and stalk at one extract. Whereas in this study, the TPC values of the different parts were determined, respectively.

3.1.2 Total flavonoid content (TFC)

From Figure 1(B), there was a significant difference in the total flavonoid content of different parts of *Ceri Terengganu* ($p<0.05$) in which the seed exhibited the highest (2.5 mg QE/100 g) total flavonoid content followed by the peel (1.77 mg QE/100 g) and flesh (0.46 mg QE/100 g). Similar findings have been reported by Gokgoz and Pekgoz (2017) using black grapes (*Vitis vinifera* L.) where the seed had higher total flavonoid content than the flesh. The differences between the different parts could possibly contribute by the different composition of the seed of *Ceri Terengganu*. Petkova and Antova (2015) stated that the main composition of melon seed included sterols, tocopherols and also fatty acids. Although the composition of the *Ceri Terengganu* seed was not determined in this study, the differences in TFC values could be explained by these lipids.

Moreover, the seed which is the most important part of the fruit contains the most amount of nutrients which are needed for the germination process. The seed coat/ hull composed of epidermis, hypodermis, chlorenchyma, palisade, parenchyma and endothelial cells, all of which contain most organelles such as vacuoles and cell walls, thus containing high amounts of phenolics in both the soluble and insoluble-bound forms (Shahidi and Yeo, 2016). This might have explained the highest total phenolic acid content and total flavonoid content presented in the seed of the *Ceri Terengganu* fruit.

3.1.3 Total anthocyanin content (TAC)

From Figure 2(A), the total anthocyanin content of the peel exhibited significantly higher total anthocyanin content compared to seed and flesh. The finding of this analysis was in agreement with the previous study by Anggraini *et al*. (2019). These results could be clearly observed in the colour of its peel where it exerted the brightest red colour, followed by the seed and finally flesh which had a soft red colour. Besides that, according to Gao and Mazza (1995), dark-coloured cherries have a total anthocyanin content ranging from 82 to 297 mg/100 g whereas light-coloured cherries have a total anthocyanin content ranging from 2 to 41 mg/100 g which accounts for the major phenolic content in cherries.

Furthermore, the colour of the cherry fruit is determined by the concentration and distribution of different anthocyanins in the skin (Esti *et al*., 2002; Gonçalves *et al*., 2007). The few major anthocyanins

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found in cherries were 3-rutinoside and 3-glucoside of cyanidin whereas minor anthocyanins include 3-rutinoside and 3-glucoside of peonidin as well as pelargonidin 3-rutinoside (Robards *et al*., 1999). In addition, the anthocyanin content may vary between fruits even if they were of the same type. This could be due to the external and internal factors such as genetic and agronomic factors, intensity and type of light, temperature, processing, handling and storage (Kayesh *et al*., 2013).

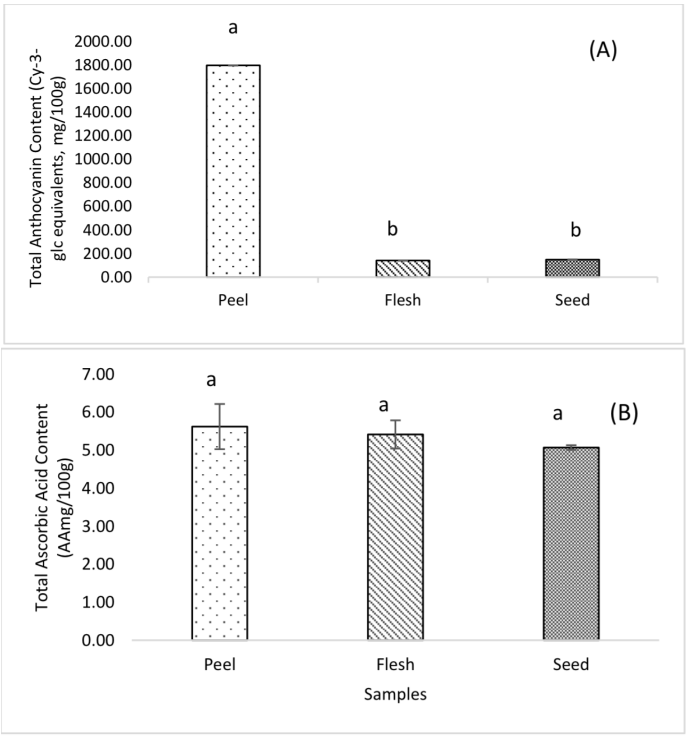

Figure 2. (A) Total anthocyanin content and (B) total ascorbic acid content of different parts of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extract. Error bars represent standard deviation values. Different letters indicate significant differences between products at $p<0.05$.

3.1.4 Total ascorbic acid content (TAA)

From Figure 2(B), the peel showed the highest total ascorbic acid content followed by the flesh and seed. There was no significant difference between total ascorbic acid in the peel, flesh and seed. The ascorbic acid content of *Ceri Terengganu* fruit was previously studied by Anuar *et al*. (2014) and the finding was the ascorbic acid content of *Ceri Terengganu* fruit decreased significantly from green to red stage. The study also showed that green *Ceri Terengganu* fruit contained the highest ascorbic acid content of 6.33 mg/100 g while the ascorbic acid content decreased to 5.36 mg/100 g as it matures towards the red stage.

In addition, the major organic acid present in *Ceri Terengganu* may not be ascorbic acid but instead malic acid, which was not measured in this study (Esti *et al*., 2002; Bernalte *et al*., 2003). Supporting that, Gundogdu and Bilge (2012) also found that the content of malic acid in cherries was identified to be higher in content compared to other organic acids. These organic acids might cause an effect on the antioxidant and antimicrobial activities tested further.

3.2 Antioxidant activity analysis

3.2.1 DPPH free radical scavenging capacity

Figure 3(A) showed the radical scavenging activity of different extracts of *Ceri Terengganu*. The results showed that the seed extract exhibited the highest (83.9%) antioxidant capacity as compared to that of the peel (83.2%) and flesh (52.4%). There was no significant difference between the antioxidant capacity of the peel and seed. The finding of this analysis was in agreement with the previous study by Anggraini *et al*. (2019). In their study, the sample extracted using ethanol showed that the result of the seed of the *Ceri Terengganu* fruit had the highest DPPH radical scavenging activity, followed by the peel and the flesh.

Moreover, the results obtained in this study showed that the antioxidant capacity of peel and seed was significantly higher than that of BHT (58.2%) and vitamin E (42.7%) but was similar to that of vitamin C (88.2%). This showed that the peel and seed had a comparable scavenging property as compared to synthetic antioxidants. Interestingly, from Figure 2(B), there was no significant difference between total ascorbic acid in the peel, flesh and seed but the total antioxidant content was significantly higher in the peel and seed. Therefore, it can be said that the high antioxidant capacity of peel and seed of *Ceri Terengganu* was not contributed by vitamin C (ascorbic acid) but could possibly be due to the actions of other antioxidants such as phenolic and flavonoid as depicted in the total phenolic and flavonoid content shown in Figure 1(A) and 1(B). In addition, Deighton *et al*. (2000) have also stated that ascorbic acid only made a minor contribution to the total antioxidant capacity.

3.2.2 ABTS radical scavenging assay

Figure 3(B) showed the ABTS radical scavenging activity of different parts of *Ceri Terengganu*. The results showed that the seed extract (48.2%) showed the highest antioxidant activity followed closely by peel (45.1%) and flesh (33.9%) extracts. Both the peel and the seed extracts achieved significantly higher antioxidant activity as compared to the flesh extract, indicating the antioxidant content was higher in the peel and seed compared to the flesh. All parts of the *Ceri Terengganu* have significantly lower antioxidant activity compared to Trolox (64.7%), a water-soluble analogue of vitamin E.

Both DPPH and ABTS are similar tests for the

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antioxidant activity where both tests use strongly coloured stable radical compounds (Holtz, 2009). Owing to the same principles, similar trends of antioxidant activity results were obtained in this study hence, further strengthened the results obtained by both tests. Therefore, a Pearson correlation test was done to understand the correlation between each test and the results was tabulated in Table 1.

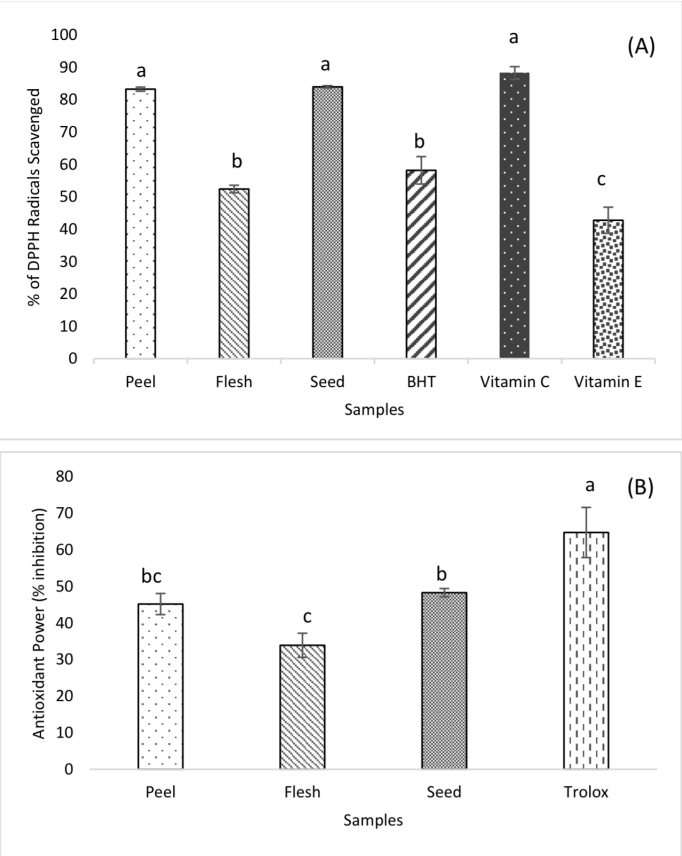

Figure 3. (A) Antioxidant activity in scavenging DPPH free radical from different parts of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extracts, BHT, Vitamin C and Vitamin E and (B) antioxidant power in ABTS Radical Scavenging Assay from different parts of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extracts and trolox. Error bars represent standard deviation values. Different letters indicate significant differences between products at $p<0.05$.

From Table 1, DPPH and ABTS were highly correlated with each other with a correlation coefficient of 0.933. Another point to note was that both DPPH and ABTS was highly correlated with TPC and TFC (both with more than 0.9). By referring to the results obtained for TPC and TFC in Figure 1(A) and 1(B), both tests showed that the peel and seed of *Ceri Terengganu* contained high total phenolic content and total flavonoid content. These results might possibly explain the high antioxidant activity of the peel and seed observed in ABTS and DPPH. This correlation between antioxidant capacity and phenolics was also reported in previous studies. González-Gómez *et al*. (2010) pointed out that the content of flavonoids was correlated with the antioxidant capacity in the sweet cherry extract. Similarly, Vangdal and Slimestad (2006) also found a correlation between the antioxidant capacity of sweet cherry fruits and the content of phenolic compounds and anthocyanin present in these fruits (Lima *et al*., 2002).

3.3 Antimicrobial activity analysis

Table 2 shows the antibacterial activity of different parts of *Ceri Terengganu* against selected foodborne pathogens. The results obtained showed inhibition of all peel, flesh and seed extracts against *S. aureus*, *B. cereus* and *B. subtilis*. However, no inhibition was observed against *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *S. enterica* ser. Typhimurium. Additionally, the seed extract showed the largest zone of inhibition against *S. aureus*, *B. cereus* and *B. subtilis* followed by the peel and the flesh extracts. This indicated that the seed of *Ceri Terengganu* had the highest antimicrobial activity among all parts.

The difference in the inhibitory effect of different parts of *Ceri Terengganu* against different bacteria can be explained by the structural differences in the bacteria. From the results obtained, *Ceri Terengganu* was effective against most gram-positive bacteria but not against *L. monocytogenes*. In addition, all the three extracts of *Ceri Terengganu* had no inhibitory effect against all gram-negative bacteria tested in this study. These differences in sensitivity between gram-positive and gram-negative bacteria to *Ceri Terengganu* extract was probably being attributed to the structural and compositional differences in membranes between the two groups (Lambert *et al.,* 2001). Gram-negative bacteria have an outer membrane that served as an impermeable barrier for many small molecules while gram-positive bacteria were generally lacked outer membrane making gram-positive bacteria more prone to antimicrobial agents (Wendakoon *et al*., 2012). The barrier formed surrounding gram negative bacteria contains lipopolysaccharides which protects the bacteria membrane from antimicrobial agents (Cui *et al*., 2012; Nohynek *et al*., 2006).

Although *L. monocytogenes* is a gram positive bacteria, its resistance towards *Ceri Terengganu* extract can be explained by the formation of biofilm making the bacteria become less susceptible to antimicrobial agents (Olaimat *et al*., 2018). The biofilm promotes the formation of persister cells with improved efflux pump activity which are responsible for removing harmful materials from bacteria such as antimicrobial agents. Besides that, the biofilm may also provide an environment for the transfer of resistant genes across bacteria (Walsh *et al*., 2001; Wilson *et al*., 2018). This clearly explained the antimicrobial test results obtained using *Ceri Terengganu*.

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Table 1. Pearson Correlation between bioactive compound and antioxidant analysis

		Correlation					
		TPC	TFC	TAC	TAA	DPPH	ABTS
TPC	Correlation Coefficient (r)	1	0.968*	0.406	-0.073	0.990*	0.912*
	p- value	-	0.002	0.424	0.891	0	0.011
TFC	Correlation Coefficient (r)	0.968*	1	0.169	-0.228	0.945*	0.921*
	p- value	0.002	-	0.749	0.664	0.005	0.009
TAC	Correlation Coefficient (r)	0.406	0.169	1	0.506	0.468	0.3
	p- value	0.424	0.749	-	0.306	0.35	0.563
TAA	Correlation Coefficient (r)	-0.073	-0.228	0.506	1	-0.114	-0.244
	p- value	0.891	0.664	0.306	-	0.83	0.641
DPPH	Correlation Coefficient (r)	0.990*	0.945*	0.468	-0.114	1	0.933*
	p- value	0	0.005	0.35	0.83	-	0.007
ABTS	Correlation Coefficient (r)	0.912*	0.921*	0.3	-0.244	0.933*	1
	p- value	0.011	0.009	0.563	0.641	0.007	-

*Correlation is significant at the 0.05 level (2-tailed)

Table 2. Antimicrobial activities of different parts (peel, flesh and seed) of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extract and controls (n=3) against different microbes

Types of Microbes	Zone of Inhibition (mm)						
	Samples			Negative Control	Positive Control		
	Peel	Flesh	Seed	Sterile Distilled Water	Penicillin (10 µg)	Oxytetracycline (30 µg)	Chloramphenicol (50 µg)
S. aureus	12.17±0.94	11.50±0.24	14.58±0.12	NI	16.67±1.53	12.25±0.35	30.25±0.35
L. monocytogenes	NI	NI	NI	NI	15.50±0.50	26.75±1.06	26.25±1.06
B. cereus	11.75±1.06	11.75±0.59	15.00±0.70	NI	10.00±0.00	13.75±0.35	29.00±1.41
B. subtilis	11.92±0.59	10.58±0.35	13.33±0.71	NI	24.00±0.00	24.50±0.71	25.50±0.71
E. coli	NI	NI	NI	NI	12.00±0.50	22.00±1.41	30.50±0.71
P. aeruginosa	NI	NI	NI	NI	NI	11.50±0.71	14.00±1.41
S. enterica ser. Typhimurium	NI	NI	NI	NI	16.17±1.61	19.50±0.71	30.50±0.71

*NI= No Inhibition. Value are expressed as mean±SD

Furthermore, the difference in antimicrobial effect portrayed by each part of *Ceri Terengganu* fruit was due to the phytochemical content present in the fruit. Phytochemicals are bioactive non-nutrient plant compounds that occur naturally in plants (Diep *et al*., 2014; Huang *et al*., 2016). Phytochemicals present in *Ceri Terengganu* might possibly possess hydrophobic characteristics which enable them to embed into lipid components of bacterial cell membrane and mitochondria, thus resulting in the leakage of intracellular material (Carson *et al*., 2002). Moreover, the phytochemicals can also affect the enzymatic action in the bacteria thus blocking their virulence (Ankri and Mirelman, 1999). This can be done by affecting the structure like flagella that can inhibit bacterial adhesion (Burt *et al*., 2007). In addition, polyphenols which are a class of phytochemical was found to demonstrate the antimicrobial activity by interacting with the bacteria cell membrane. Inouye *et al*. (2001) stated that the polyphenols were more effective against gram positive bacteria compared to gram negative bacteria mainly due to the difference in composition in the cell membrane of both bacteria type. Besides that, the presence of an organic acid such as malic acid that presented in the *Ceri Terengganu* fruit also acted as an antimicrobial barrier against the microbes. This acid was often used in the food industry as preservative agents, attributing their antimicrobial efficacy to the pH changes of the treated media (Joshi *et al*., 2012) and might help in the antimicrobial efficiency by attacking the cell walls, cell membranes, metabolic enzymes, protein synthesis systems and the genetic material of microorganisms and thus preventing them to grow (Tripathi and Dubey, 2004).

On the other hand, the seed extract of *Ceri Terengganu* fruit showed the highest antimicrobial activity compared to the flesh and the peel. This was possibly due to the seed possessed the highest amount of phytochemical compounds as compared to other parts of the fruit. Joshi *et al*. (2012) had stated that phenolic compounds such as phenolic acids and flavonoids protect

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the fruit against pathogenic agents by penetrating the cell membrane of microorganisms, causing cell lysis. Moreover, flavones, flavonoids and flavonols were also some of the effective antimicrobial substances against a wide range of microorganisms due to their ability to complex microbial cell walls (Takahashi *et al*., 1995; Cowan, 1999; Zhao *et al*., 2001). Furthermore, Lambert *et al*. (2001) have stated that the higher the phenolic content, the higher the antimicrobial effect which could possibly explain the high antimicrobial effect of the *Ceri Terengganu* seed against the microbes.

4. Conclusion

In conclusion, the bioactive compound of different parts of *Ceri Terengganu* (*Lepisanthes alata* Leenh.) fruit extract has been determined. The *Ceri Terengganu* seed exhibited the highest total phenolic content and total flavonoid content among all parts of *Ceri Terengganu* fruit followed by the peel and the flesh. In addition, the seed of *Ceri Terengganu* expressed the highest antioxidant capacity in DPPH free radical scavenging capacity test and ABTS assay followed by the peel and the flesh. The antioxidant activity of the *Ceri Terengganu* seed was found to be higher than synthetic antioxidant (BHT). Hence, *Ceri Terengganu* seed would be a promising source of natural antioxidant replacing the synthetic antioxidants in the food industry. Furthermore, the peel, seed and flesh of *Ceri Terengganu* fruit showed inhibition against most gram positive bacteria such as *S. aureus*, *B. cereus* and *B. subtilis* with the seed showing the highest antimicrobial activity followed by the peel and flesh but there was no inhibition for *L. monocytogenes*, *E. coli, P. aeruginosa* and *S. enterica* ser. Typhimurium. Therefore, the *Ceri Terengganu* fruit extract could be a choice of natural antimicrobial agent that can be used in the food industry. The seed particularly should be studied in detail for its composition, including its lipid content, that might have contributed to its excellent bioactivities showed in this study. Finally, the seed of *Ceri Terengganu* possessed a great potential to be explored as a source of natural antioxidant and antimicrobial agent in the food industry, and thus warrant further investigation.

Conflict of interest

The authors declare no conflict of interest.

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