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Enzyme inhibiting, antibacterial and antioxidant activities of peel, pulp and seed extracts from *dau* Ha Chau (*Baccaurea ramiflora* Lour.) fruit of Vietnam

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Abstract

Dau Ha Chau (DHC) was produced by selecting and growing native kinds of *Baccaurea ramiflora* Lour. in Vietnam, where the fruit is utilized for fresh eating not only due to its distinctive sour-sweet flavor, but also because of its vitamins and minerals for the body. With the aim to enhance the value of this fruit, the peel, pulp, and seed extracts were determined antioxidant, antibacterial and antidiabetic activities. The filtrates from ethanol extract of peel and seed powders and fruit juice from squeezing pulp were evaporated at 58°C to create the extracts. The six methods of TAC, DPPH, $ABTS^{\bullet+}$, RP, FRAP, and $NO^{\bullet}$ (antioxidant activities), four bacterial strains and two enzymes (associated with type 2 diabetes mellitus) were evaluated. The results showed that the extract of DHC peel exhibited the highest antioxidant activity (EC_{50} value from the six methods), antibacterial activity (MIC value), enzyme inhibiting activity (IC_{50} value), total phenolics content and total flavonoids content, at 643.81 µg/mL, 787.42 µg/mL, 651.10 µg/mL, 718.22 µg/mL, 270.38 µg/mL, 662.44 µg/mL, 16 < MIC ≤ 32 mg/mL (against *Propionibacterium acnes, Escherichia coli* and *Bacillus cereus*), 32 < MIC ≤ 64 mg/mL (against *Staphylococcus aureus*), 2780.91 µg/mL (against α-amylase), 541.24 µg/mL (against α-glucosidase), 119.43 mg GAE/g and 207.17 mg QE/g, respectively, whereas the opposite was true for extract of DHC seed. DHC pulp extract came second in all analytical methods. Pearson correlation coefficients with r value (0.63-0.99) showed a reasonably strong relationship between total phenolics and flavonoids contents, and antioxidant and enzyme inhibiting activities. This is the first report on the antioxidant, antibacterial and antidiabetic activities of DHC Ha Chau fruit extracts, which could be developed for medicinal, pharmaceutical, food preservative purposes, or industrial foods in the future.

1. Introduction

Dau Ha Chau (DHC) (*Baccaurea ramiflora* Lour.) originates from selecting and propagating the local varieties of *B. ramiflora* in Phong Dien district, Can Tho city, Vietnam. DHC fruits with a pale yellow color possess a sour-sweet taste and have been recognized as a trademark by the Intellectual Property Office of Viet Nam in 2006. According to Goyal *et al*. (2013) and Uddin *et al*. (2018), *B. ramiflora* juice contains a high concentration of phenolic and flavonoid components with possible natural antioxidant activity and is biologically active with adequate antioxidant capacity. Phenolics and flavonoids present in plants are the largest phytochemical molecules with antioxidant properties (Andreu *et al*., 2018).

There were different antioxidant assays for determining the antioxidant activity due to different types of oxidants that represented a certain type of oxidant. The total antioxidant capacity (TAC) assay was based on reducing Mo (VI) to Mo (V) by the compounds from the extracts to the formation of the green phosphate/ Mo (V) complex. The absorbance of the solution was measured at 695 nm (Prieto *et al*., 1999). The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and the 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) assays were used to verify the radical scavenging capacity of the sample with the absorbance taken at 517 nm and 734 nm, respectively. The DPPH free radical-scavenging model is widely used because of the relatively short time required for the analysis and is

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based on the scavenging of DPPH by antioxidants, which was a reduction reaction decolorizing the DPPH solution through the donation of hydrogen to form the stable DPPH–H molecule (Fenglin *et al*., 2004). In the reducing power (RP) capacity assessment, the compounds from the samples donate electrons, causing the conversion of Fe^{3+} to Fe^{2+} in the complex and spectrophotometrically measured at 700 nm (Perl's Prussian blue). The FRAP ferric iron (Fe^{3+}) was reduced to ferrous iron (Fe^{2+}) by reducing the compounds of the different extracts and spectrophotometrically measured at 593 nm (Benzie and Strain, 1996). The nitric oxide ($NO^{\bullet}$) scavenging ability assay was analyzed using the colorimetric Griess reaction method with the absorbance taken at 546 nm (Alisi and Onyeze, 2008).

Researchers have uncovered the potential health advantages of plant polyphenols due to their high antioxidant qualities, which help prevent chronic diseases related to oxidative stress (Dai and Mumper, 2010). Moreover, bioactive substances, including flavonoids and polyphenolic compounds, have demonstrated their antibacterial and antidiabetic activities (Gill and Holley, 2006; Tadera *et al*., 2006; Kwon *et al*., 2008). However, the antibacterial and antidiabetic activities of *B. ramiflora*, especially DHC, have not been studied thoroughly. Bacteria are one of the leading causes of human infections that greatly impact public health. *Propionibacterium acnes*, previously known as *Corynebacterium parvum*, is a Gram-positive human skin commensal, anaerobic growth and causes acne (Kirschbaum and Kligman, 1963). Acne can persist for many years and is involved in severe psychosocial effects such as reduced self-esteem, depression, frustration, and social withdrawal (Bhatia *et al*., 2004). *Bacillus cereus* is a Gram-positive, endospore-forming, motile, aerobic, and anaerobic rod. *Bacillus cereus* causes two types of foodborne disease (a diarrheal syndrome and emetic syndrome) due to the production of distinct toxins. *B. cereus* is becoming one of the most common bacteria of increasing concern in the food industry (Griffiths and Schraft, 2017). According to the WHO priority diseases list for new antibiotic research and development, *Staphylococcus aureus* and *Escherichia coli* are Gram-positive and Gram-negative, respectively, and in the list of antibiotic-resistant bacteria, following in carbapenem-resistant, the 3rd generation cephalosporin-resistant with Enterobacteriaceae including *E. coli* and methicillin-resistant, vancomycin-intermediate and resistant with *S. aureus* (Shrivastava *et al*., 2018). Alpha-amylase and α-glucosidase are the primary enzymes associated with type-2 diabetes mellitus (T2DM). They are a group of enzymes that catalyzes the hydrolysis of starch into sugars. Preventive treatment for T2DM is to reduce carbohydrate digestibility by reducing their activity to manage postprandial hyperglycemia (Kwon *et al*., 2008; Gong *et al*., 2020).

Nowadays, healthy food is a primary concern for many people. Many fruits have been studied for their effects on human health. Yet up to now, there have been no studies regarding the antioxidant, antibacterial and enzyme-inhibitory capacities of DHC despite it being a typical fruit of the region. Therefore, the study was conducted to provide scientific data to evaluate the potential of this fruit as a natural source of antioxidants, antibacterial agents, and enzyme inhibitors for humans. In this study, the antioxidant, antibacterial and enzyme-inhibiting activities of different DHC fruit parts (peel, pulp and seed) were determined, compared and analyzed the correlation coefficient among them for the value of the fruit and for the purpose of identifying the fraction with high these activities. This would have further studies focusing on the development of natural antioxidants, antibacterial agents or antidiabetic for food and beverage processing or therapeutic and pharmaceutical preparations.

2. Materials and methods

2.1 Plant collection

The ripe fresh DHC fruits were harvested with the bunches in lunar July from the local garden in Phong Dien District, Can Tho City. The bunches of fruits were packed in plastic bags covered with some DHC leaves inside and transported quickly to the laboratory of Can Tho University, Vietnam. Before rinsing in tap water, the diseased, decayed, defective fruits were removed to eliminate foreign matter and dust. After that, ripe DHC fruits were separated into parts including peel, juice, and seed.

2.2 Preparation of the extract

DHC peel samples were sliced into small pieces using a stainless-steel knife and dried in a hot-air oven. (Memmert KS19, Germany) at 50±2°C for 8 hrs. DHC seed samples (500 g) were also dried in a hot-air oven at 50±2°C for 12 hrs. After being dried to an 8-10% moisture content, the two samples were ground into raw powders. Each type of powder was weighed 200 g, soaked in 4 L of 96% ethanol for 24 hrs at room temperature, and then filtered through Whatman No. 1 paper. The extraction was repeated three times. The filtrate of two extracts of each type was collected and evaporated by a rotary evaporator (Ika, model RV10 digital V, Germany) at 58±2°C (Ministry of Health Vietnam, 2017; Bahrin *et al*., 2018) to dryness to obtain 33.21 g peel extract and 20.91 g seed extract. Fruit juice from squeezing DHC pulp was also filtered through

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Whatman No. 1 paper and then evaporated at 58±2°C under reduced pressure to obtain the juice extract below approximately 10% moisture content. The extracts were used to determine chemical composition, antioxidant, antibacterial and antidiabetic activities.

2.3 Chemical composition

2.3.1 Total phenolics content

The total phenolics content (TPC) was determined by the Folin-Ciocalteu method (Singleton *et al*., 1999) and slight modifications with gallic acid (GAE) as a standard. The absorbance was 765 nm using UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Different concentrations from the standard stock solution of gallic acid (1 mg/mL) were prepared in 1, 2, 4, 6, 8, and 10 µg/mL. The TPC in the sample was calculated as gallic acid equivalent (mg GAE/mL)

2.3.2 Total flavonoid content

The total flavonoid content was determined by the $AlCl_3$ complexation method of Bag *et al*. (2015) and slight modifications using quercetin (QE) as a standard. The absorbance was 510 nm using UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Different concentrations from the standard stock solution of quercetin (1 mg/mL) were prepared in 10, 20, 40, 60, 80, and 100 µg/mL. The TFC in the sample was expressed as quercetin equivalent (mg QE/mL).

2.4 Determination of antioxidant activity

2.4.1 Total antioxidant capacity assay

The TAC was tested according to the method of Prieto *et al*. (1999). The extract with 300 µL was added to 900 µL of reagent solution containing 0.6 M acid sulfuric, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After incubated at 95°C for 90 mins in the dark, the solution was spectrophotometrically recorded at 695 nm by the UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). The TAC was calculated by the Trolox standard curve and expressed in µg/mL Trolox. The EC_{50} value was the concentration of the extract that had an absorbance of 0.50 (Piaru *et al*., 2012).

2.4.2 1,1-Diphenyl-2-picrylhydrazyl assay

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay was determined by the method of Sharma and Bhat (2009) with suitable modifications using Trolox as a standard. A 40 µL DPPH (1,000 µg/mL) reagent solution was added to 960 µL of the extract. A blank control was 960 µL of methanol and 40 µL DPPH solution. After leaving in the dark for 30 mins, the mixture was spectrophotometrically measured at 517 nm by UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). DPPH scavenging activity was calculated as in the equation:

$$\%AA = [(Ao - As)/Ao] \times 100\%$$

where As is the absorbance of the sample, Ao is the absorbance of the blank sample, and %AA is the percentage of inhibition of DPPH. The EC_{50} value was defined as the minimal extract concentration required to absorb 50% of the DPPH in the test solution.

2.4.3 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid assay

The 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) assay was implemented using the method of Nenadis *et al*. (2004) with some modifications using Trolox as a standard. First, a solution containing 7 mM ABTS and 2.45 mM Potassium persulfate was prepared and stored in the dark at room temperature for 12-16 hrs. This solution was then diluted with ethanol to obtain an absorbance at 734 nm using UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). A reagent solution of 990 µL of the diluted $ABTS^{\bullet+}$ and 10 µL of the extract was left for 6 min at room temperature and then spectrophotometrically measured at 734 nm. $ABTS^{\bullet+}$ scavenging activity was calculated in the same way as DPPH scavenging activity. The EC_{50} value was the minimum extract concentration required to scavenge 50% of the $ABTS^{\bullet+}$ present in the test solution.

2.4.4 Reducing power capacity assessment

The reducing power (RP) capacity was evaluated according to the modified method of Oyaizu (1986). The extract solution (500 µL), potassium buffer (0.2 M, pH 6.6) (500 µL) and potassium ferricyanide 1% (500 µL) were incubated at 50°C for 20 mins. Secondly, trichloroacetic acid 10% (500 µL) was added before the mixture was centrifuged for 10 mins at 3,000×*g*. The supernatant (500 µL) was mixed with 100 µL $FeCl_3$ 0.1% and 500 µL distilled water and spectrophotometrically measured at 700 nm by UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Finally, antioxidant activity was calculated using the Trolox standard curve and expressed in g/mL. The EC_{50} value was the concentration of the extract that had an absorbance of 0.50 (Piaru *et al*., 2012).

2.4.5 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) was determined by the method of Benzie and Strain (1996) with modifications. In the first step, $FeCl_3$ 20 mM (0.25 mL), TPTZ solution 10 mM in HCl 40 mM (2.5 mL) and acetate buffer 0.2 M, pH = 3.6 (2.5 mL) were mixed to prepare the FRAP reagent solution and incubated at

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37°C. In the following step, the mixture of 10 µL extract solution with different concentrations and 990 µL FRAP reagent solution was left reacted at room temperature for half an hour and then spectrophotometrically measured at 593 nm UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Finally, the antioxidant activity was calculated by the Trolox standard curve and expressed in µg/mL of Trolox. The EC_{50} value was the concentration of the extract that had an absorbance of 0.50 (Piaru *et al*., 2012).

2.4.6 Nitric oxide scavenging ability assay

The Nitric oxide (NO•) scavenging ability was analyzed using the colorimetric Griess reaction method (Alisi and Onyeze, 2008) with modifications using Trolox. The reaction mixture of 200 µL extract solution and 400 µL sodium nitroprusside (5 mM) was incubated at 25°C for 60 mins before being centrifuged for 15 mins at 11000×*g*. The supernatant was added to 600 µL of Griess reagent (1% sulphanilamide/0.1% N-(1-naphthyl) ethylenediamine and 2.5% H_3PO_4) in a 96-well plate for 5 mins at room temperature. The absorbance was taken at 546 nm using a UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). The $NO^{\bullet}$ scavenging ability was calculated by the Trolox standard curve and expressed in µg/mL Trolox. The EC_{50} value was the concentration of the extract with an absorbance value of 0.50 (Piaru *et al*., 2012).

2.5 Determination of antibacterial activity

Antibacterial activity was evaluated against four bacterial strains (*Staphylococcus aureus* ATCC® 6538, *Bacillus cereus* ATCC® 10876TM, *Escherichia coli* ATCC® 25922TM, and *Propionibacterium acnes* (isolated from patients with acne in Can Tho University of Medicine and Pharmacy) by determining the zone of inhibition using the agar well diffusion method described by Sambrook and Russell (2006) and Srinivasan *et al*. (2001) with modifications. The bacterial culture was diluted to 10^6 bacteria/mL, and 100 µL was spread on the Luria-Bertani (LB) agar plate surface. After the LB agar plate dried, holes were drilled on the surface with a diameter of 7 mm. The extract samples were diluted in 10% dimethyl sulfoxide (DMSO) to create the extract solutions at concentrations of 160; 320; 640; 1,280 and 2,560 µg/mL. Each extract solution at each concentration was pipetted into agar wells. After 24 hrs incubation at 30°C, the diameter of the incubation zones was measured in mm.

The bacterial cell concentrations were measured using a combination of two methods as plate counting cell numbers and optical density (OD) measurement (Mangoni *et al*., 2004; Mohammed Sadiq *et al*., 2010; Pan *et al*., 2014). First, each bacterial species in Luria-Bertani broth was serially diluted from 0 to -10 fold in saline solution in triplicate. Aliquots of 10 ml diluted sample were spread on LB plates and then incubated overnight at 37°C. The colonies on the plates were counted to evaluate the number of colony-forming units (CFU). Next, aliquots of 200 µL diluted sample were added to a 96-well plate and immediately assayed by measuring OD using a UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland) at 600 nm. Sterile saline solution was used as blanks. Finally, the standard curve of OD versus bacterial cell number (CFU) was used to determine the cell of bacteria.

2.6 Determination of antidiabetic activity

2.6.1 Alpha-amylase inhibitory assay

The assay was performed using the method by Rana *et al*. (2019) with modifications using acarbose as a standard. Initially, the reaction mixture of 50 µL phosphate buffer 0.2 M (pH = 7.0), 50 µL of the extract, and 50 µL and 50 µL enzyme α-amylase solution (3 U/mL) were mixed and left for 5 mins at 37°C. After that, 50 µL starch solution (2 mg/mL) was added to the mixture and incubated for 15 mins at 37°C. The reaction was terminated by adding 200 L of concentrated HCl. Finally, the 300 µL of 1% iodine solution was added to the mixture. The absorbance was taken at 660 nm using a UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Alpha-amylase inhibitory activity was determined by the formula:

$$(\%)\ Inhibition = \left[1 - \left(Abs_{sample}/Abs_{control}\right)\right] \times 100$$

where $Abs_{control}$ and Abs_{sample} were the absorbances of the control (without the extracts or acarbose) and the plant extract or acarbose, respectively. The IC_{50} value was the sample concentration providing 50% inhibition.

2.6.2 Alpha-glucosidase inhibitory assay

The assay was performed by the method of Shai *et al*. (2011) with modifications using acarbose as a standard. Initially, the reaction mixture of 100 µL phosphate buffer 100 mM (pH = 6.8), 40 µL of the extract, and 20 µL enzyme α-glucosidase solution (1 U/mL) were mixed and left for 15 mins at 37°C. After that, 40 µL p-nitrophenyl-α-D-glucopyranoside (5 mM) was added to the mixture and incubated for 20 mins at 37°C. The reaction was stopped with 100 µL Na_2CO_3 (0,1 M). The absorbance was taken at 405 nm using a UV-VIS Thermo Scientific (Miltiskan GO/Cat No: 51119200, Finland). Alpha-glucosidase inhibitory activity was calculated by the formula:

$$(\%)\ Inhibition = \left[1 - \left(Abs_{sample}/Abs_{control}\right)\right] \times 100$$

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where $Abs_{control}$ and Abs_{sample} represented the absorbances of the control (without plant extract or acarbose) and the plant extract or acarbose, respectively. The IC_{50} value was the sample concentration at which 50% inhibition occurred.

2.7 Statistical analysis

The analysis methods and Pearson correlation coefficients in this study were results from the Minitab 16 software. The assay was performed three times for each extract concentration. One-way analysis of variance and Tukey's tests were used to determine significant group differences. The results of all analyses were expressed as mean ± standard deviation (SD). *P* values less than 0.05 were deemed statistically significant.

3. Results and discussion

3.1 Evolution of total phenolics and flavonoids contents

The large groups of compounds commonly found in plants are phenolics and flavonoids. Regarding Figure 1, the content of the two categories under the survey showed a sharp climb from the fresh fruit to the extracts of DHC. While the concentrations of fresh peel, pulp, and seed were only 99.02×10^{-2}, $81.62\times10^{-2,}$ and 52.52×10^{-2} mg GAE/g of total phenolics content, respectively, they increased by over 120 times (peel), 84 times (pulp) and 113 times (seed) in the fruit extracts. The same pattern was observed in the flavonoid content, characterized by an increase of 162 times in the figure for peel extract to 207.17 mg QE/g, a rise of 102 times in that of pulp extract to 116.69 mg QE/g, and a growth of 120 times in the figure for seed extract to 74.24 mg QE/g. The peel contained the most total phenolics and flavonoid concentrations among the three main portions of the DHC fruit. The pulp came second, and the seed had the lowest level. The results of this investigation were consistent with previous studies regarding the antioxidant capability of peel, pulp, and seed fractions of *Canarium odontophyllum* Miq. fruit (Prasad *et al*., 2010). The content of phenolic compounds (phenolic acids and flavonoids) changed due to different varieties of fruit species or growing conditions (Dixon and Paiva, 1995) or fruit growth stages (Amiot *et al*., 1995).

3.2 Antioxidant activity

As regards the evaluation of antioxidant activity in food and biological systems, many methods have been developed for different reactions. This facilitates interpreting the results of the antioxidant potential of a sample. The antioxidant activity of DHC fruit was assessed by six different methods (TAC, DPPH, $ABTS^{\bullet+}$, RP, FRAP, and $NO^{\bullet}$). In Figure 2, the measured antioxidant activity is proportional to the extract concentration.

In Figure 2A, the TAC of peel and pulp extracts was higher than that of seed extract at the concentrations tested. While the EC_{50} value of Trolox was 53.46 µg/mL, there was no statistical difference between the EC_{50} value (about 658.61 µg/mL) of peel and pulp extracts (Table 1). The DPPH and $ABTS^{\bullet+}$ assays in Figure 2B and 2C proved the radical scavenging capacity of the extracts. The peel extract ranked first in DPPH radical-scavenging and $ABTS^{\bullet+}$ scavenging ability with 787.42 and 651.10 µg/mL on average EC_{50} values in Table 1. The opposite was true for seed extract with 1058.96 and 930.19 µg/mL of EC_{50} values. However, Trolox made up the highest EC_{50} value in this method. The data reported

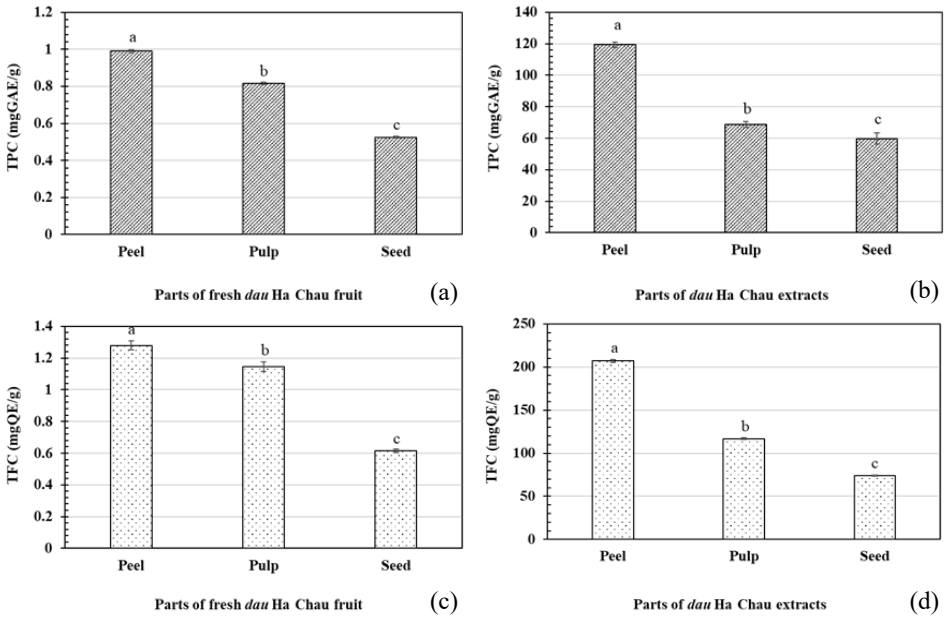

Figure 1. The total polyphenols content in the parts of fresh DHC fruit (A) and extracted forms (B) and the total flavonoids content in the parts of fresh DHC fruit (C) and extracted forms (D). Bars with different notations are statistically significantly different ($p < 0.05$) between groups.

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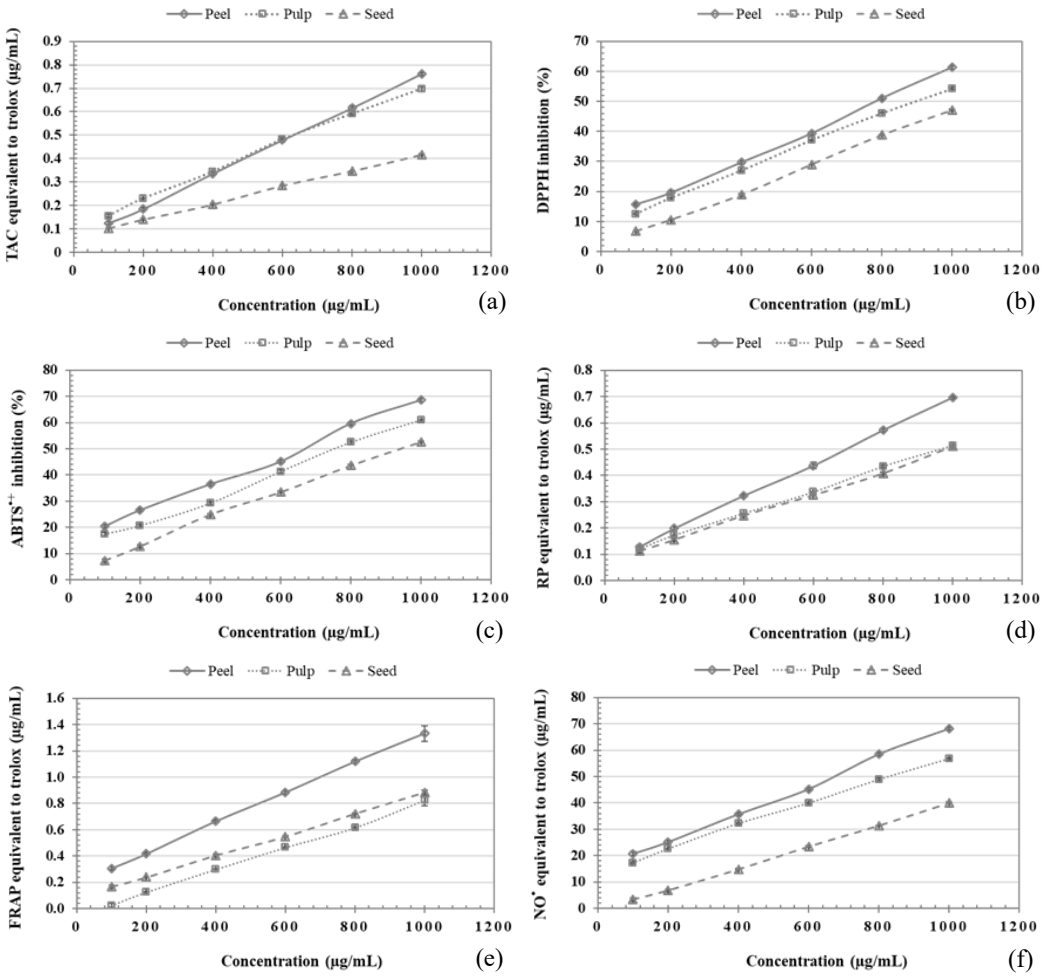

Figure 2. The antioxidant activities of the different parts extracts of DHC fruit: (A) TAC, (B) DPPH, (C) $ABTS^{\bullet+}$, (D) RP, (E) FRAP and (F) $NO^{\bullet}$.

on the RP assay in Figure 2D showed an increase, reducing the extracts' power with the rise of extract concentrations. The pulp and seed extract experienced lower reducing power (1049.92 – 1079.08 µg/mL) than the peel extract (718.22 µg/mL) (Table 1). According to the FRAP assay, raising the concentration of the extracts increased the reduction of Fe^{3+} (Figure 2E). In this method, the most decrease in Fe^{3+} (the least EC_{50} value) was observed in Trolox, at 3.17 µg/mL. The peel extract registered twice the reduction of Fe^{3+} compared to the seed extract. The pulp extract showed the weakest antioxidant activities equivalent to the highest EC_{50} value (Table 1). It was also revealed that fruit peel and seed fractions, including date, guava, kiwifruit, purple mulberry, strawberry, and white pomegranate, were stronger than the pulp fraction based on their FRAP values (Guo *et al*., 2003). In Figure 2F and Table 1, the $NO^{\bullet}$ scavenging ability of peel extract ranked first in terms of the EC_{50} value among three different sections, at 662.44 µg/mL, whereas the opposite was true for seed extract, at 1253.38 µg/mL. The pulp extract came second, at 831.76 µg/mL.

Significant variances exist between the antioxidant activity of the various DHC fruit extracts. The greatest number of antioxidant activities went to the peel extract, demonstrating the activities in all six methods (TAC,

Table 1. Antioxidant activity (EC_{50} values) of the different parts extracts of DHC fruit.

Parameters	EC_{50} (µg/mL)			
	Peel extract	Pulp extract	Seed extract	Trolox
TAC	643.81±6.14^{b}	658.61±8.11^{b}	1436.56±8.70^{a}	53.46±0.20^{c}
DPPH	787.42±8.65^{c}	893.95±2.42^{b}	1058.96±3.21^{a}	0.69±0.01^{d}
$ABTS^{\bullet+}$	651.10±2.66^{c}	774.03±2.83^{b}	930.19±3.27^{a}	2.74±0.02^{d}
RP	718.22±13.00^{b}	1049.92±12.76^{a}	1079.08 ±27.55^{a}	5.08±0.02^{c}
FRAP	270.38±10.10^{c}	642.42±33.46^{a}	522.92±7.71^{b}	3.17±0.02^{d}
$NO^{\bullet}$	662.44±5.67^{c}	831.76±5.93^{b}	1253.38±1.4^{a}	72.86±1.12^{d}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$) between groups.

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DPPH, ABTS$^{\bullet+}$, RP, FRAP, and NO$^{\bullet}$). The pulp extract came second in these activities in DPPH, ABTS$^{\bullet+}$ and NO$^{\bullet}$ methods, followed by the seed extract in TAC, DPPH, ABTS$^{\bullet+}$, RP, and NO$^{\bullet}$. This was the same in total antioxidant capacity and reducing power assay with a higher potential antioxidant capacity in *B. ramiflora* peel than in seed. The Trolox experienced strong standard antioxidants to compare the potential of test compounds in the six antioxidant assays. It is well-documented that the antioxidant activities of DHC fruit extracts were closely correlated with the content of polyphenols and flavonoids (two important antioxidant components) (Table 4). This reduced effect of free radicals is based on their ability to transfer hydrogen atoms to free radicals (Aryal *et al*., 2019). In addition, antioxidant compounds acting as reducing agents gave hydrogen atoms to the ferric complex to halt the radical chain reaction (Singh and Rajini, 2004).

3.3 Antibacterial activity

As shown in Figure 3, the inhibition zone diameter fluctuated from 8.47 to 26.01 mm. While DHC peel extract showed the strongest activity against the bacteria *S. aureus* with an inhibition zone diameter of 26.01 mm at the highest concentration (256 mg/mL) surveyed, seed extract experienced no zone of inhibition at this concentration. Moreover, in Table 2, the minimum inhibitory concentration (MIC) value of DHC peel extract was 16 < MIC ≤ 32 mg/mL for *P. acnes*, *E. coli* and *B. cereus* and 32 < MIC ≤ 64 mg/mL for *S. aureus*. DHC pulp extract was effective with MIC value at 32< MIC ≤ 64 mg/mL against *P. acnes*. The MIC of DHC pulp extract against bacterial strains (*S. aureus*, *E. coli,* and *B. cereus*) and seed extract against all bacterial strains surveyed were higher than 256 mg/mL. Recently, Jusuf *et al*. (2020) demonstrated the antibacterial efficacy of plant extracts against *P. acnes* and food poisoning pathogens (*S. aureus*, *E. coli*, and *B. cereus*).

Plant extract antibacterial components such as terpenoid, alkaloid, and phenolic chemicals have been demonstrated to interact with enzymes and proteins of the microbial cell membrane, allowing for the breaking and scattering of large amounts of protons towards the cell exterior, causing cell death or inhibiting enzymes required for amino acid biosynthesis (Gill and Holley, 2006); extracts of eight Finnish berries suppressed the growth of specific Gram-negative bacteria (Puupponen-Pimiä *et al*., 2001). According to the findings of Puupponen-Pimiä *et al*. (2001), different bacterial species have varying sensitivity to phenolics and flavonoids, and berry extracts inhibited Gram-negative but not Gram-positive bacteria. In contrast, DHC extracts inhibited both three Gram-positive and one Gram-negative pathogen tested as the data reported in Figure 3 and Table 2.

3.4 Antidiabetic activity

The antidiabetic activity of DHC fruit was evaluated on the inhibitory effect against two types of carbohydrate hydrolyzing enzymes (α-amylase and α -glucosidase) associated with type-2 diabetes mellitus. As shown in Figure 4, the more concentrated the extracts under study were, the greater their inhibitory action against α-amylase and α -glucosidase. Over the three extracts, DHC peel showed the strongest capacity for generating these enzyme inhibitors with IC_{50} values at 2,780.91 and 541.24 µg/mL, respectively (Table 3). However, The

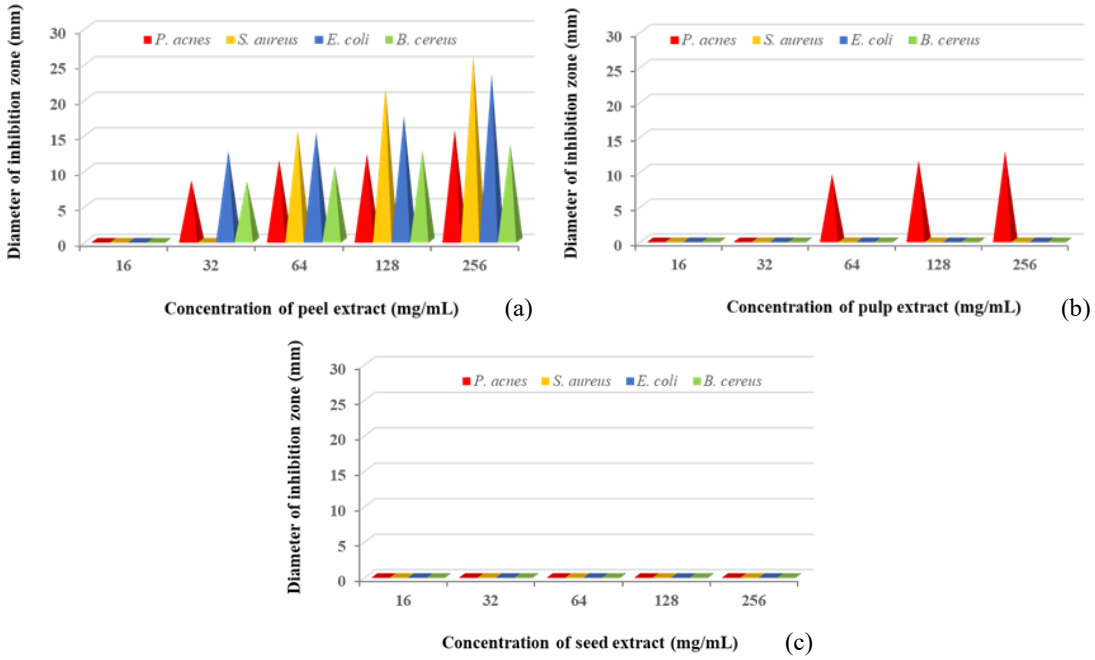

Figure 3. The antibacterial effect of the different parts of extracts (A) peel, (B) pulp and (C) seed) of DHC fruit on *P. acnes*, *S. aureus*, *E. coli* and *B. cereus*.

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Table 2. Minimum inhibitory concentration (MIC) of the different parts of extracts of DHC fruit.

Organism	MIC value of the extracts		
	Peel	Pulp	Seed
P. acnes	16 < MIC ≤ 3 mg/mL	32 < MIC ≤ 6 mg/mL	MIC > 25 mg/mL
S. aureus	32 < MIC ≤ 64 mg/mL	MIC > 256 mg/mL	MIC > 256 mg/mL
E. coli	16 < MIC ≤ 32mg/mL	MIC > 256 mg/mL	MIC > 256 mg/mL
B. cereus	16 < MIC ≤ 32 mg/mL	MIC > 256 mg/mL	MIC > 256 mg/mL

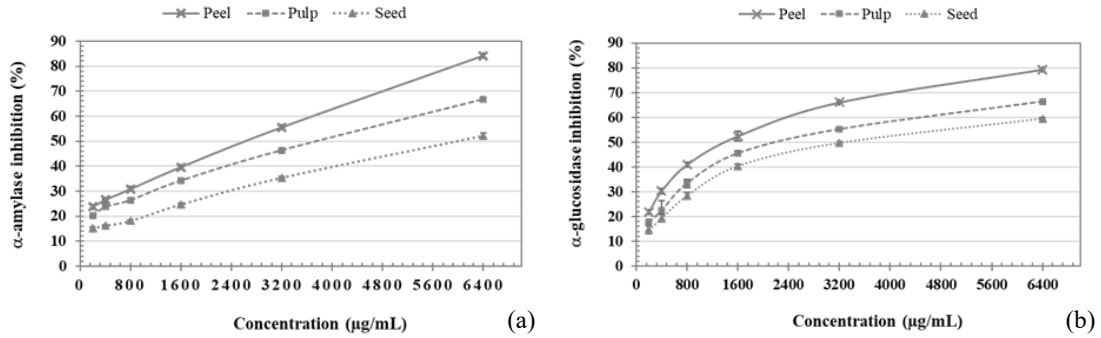

Figure 4. Inhibition (%) of α-amylase (A) and α-glucosidase (B) activities by the parts of DHC fruit extracts at different concentrations.

IC_{50} value of acarbose (the positive control) for α-amylase and α-glucosidase was deficient at 18.71 and 7.67 µg/mL, respectively. This demonstrated that acarbose is capable of blocking these enzymes. Results of a recent survey conducted by a group of researchers reveal that the antioxidant ability of the extracts encouraged the inhibition of α-amylase and α-glucosidase activities (Thilagam *et al*., 2013). Besides, polyphenols and flavonoids have α-amylase and α-glucosidase inhibitory activity (Tadera *et al*., 2006; Kwon *et al*., 2008). Flavonoids have an inhibitory effect because the hydroxyl groups of flavonoids form hydrogen bonds with the active side chains of the enzyme, and the AC ring system of flavonoids forms a conjugated π system with the indole Trp59 in the enzyme. This renders the reaction between α-amylase and starch for starch digestion impossible (Gu *et al*., 2015).

Furthermore, Mbhele *et al*. (2015) demonstrated that a phytochemical has mild to severe inhibitory effects on α -amylase, and α -glucosidase, making it a promising antidiabetic agent. The α-glucosidase enzyme promotes carbohydrate digestion and the release of glucose. Because of the suppression of this enzyme, glucose uptake was postponed. This led to slowing down the blood glucose level after a meal and retard the progression of diabetes. According to Levetan (2007), the effective administration of diabetes can be achieved by combining a light inhibition of α-amylase and a dramatic inhibition of α-glucosidase. The consequences of Table 3 were compatible with these findings because the IC_{50} (µg/mL) against α-amylase was about 5.14; 5.7, and 7.31 times as much as α-glucosidase in DHC peel, pulp, and seed, respectively.

3.5 Correlation coefficients of total phenolics content, total flavonoids content, antioxidant and antidiabetic activities

As reported in Table 4, the r value from 0.63 to 0.99 with $p < 0.05$ showed that the total phenolics and flavonoids contents correlated highly with TAC, DPPH, $ABTS^{\bullet+}$, RP, FRAP, $NO^{\bullet}$, α-amylase, and α-glucosidase. This indicated that the total phenolics and flavonoid contents of DHC fruit contributed to the antioxidant and antidiabetic activities. The minus (-) sign in front of the correlation value means that two variables negatively correlate. The higher the concentration of total phenolics and flavonoids, the lower the EC_{50} and IC_{50} value gain. Polyphenol and flavonoid compounds contain antioxidant properties that can neutralise free radicals, preventing them from surrendering their hydrogen atoms and electrons (Aryal *et al*., 2019). Kwon *et al*. (2008) reported that polyphenols inhibited α-amylase and α-glucosidase based on their chemical profiles. Tadera *et*

Table 3. Enzyme inhibiting activity (IC_{50}) of the different parts of extracts of DHC fruit.

Enzymes	IC_{50} (µg/mL)			
	Peel extract	Pulp extract	Seed extract	Acarbose
α-amylase	2,780.91±17.19^{c}	3,976.07±53.44^{b}	5,904.95±143.86^{a}	18.71±0.06^{d}
α-glucosidase	541.24±10.10^{c}	697.33±14.00^{b}	807.65±13.32^{a}	7.67±0.10^{d}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p < 0.05$) between groups.

https://doi.org/10.26656/fr.2017.8(4).395

al. (2006) demonstrated that flavonoids have α-amylase and α-glucosidase inhibitory activity. The results of this study implied that DHC fruit (especially the pulp) might be a potential resource for developing antioxidant and antidiabetic function drinks.

In terms of chemical composition, DHC peel contained the highest concentration of total phenolics and flavonoids (Figure 1) while DHC pulp ranked second, and DHC seed had the lowest of these two ingredients. Besides, the result from Table 4 indicated the height correlation between total phenolics content, total flavonoids content, antioxidant and antidiabetic activities. Because DHC peel contains the highest concentration of total phenolics and flavonoids, the enzyme inhibiting, antibacterial and antioxidant activities of the peel are the highest when compare with the pulp and seed.

Table 4. Correlation coefficients of the contents of total phenolics, total flavonoids to activities of antioxidant and enzyme inhibiting.

Parameters	Correlation value*	
	Total phenolics content	Total flavonoids content
Antioxidant activities (EC_{50})		
TAC	-0.63	-0.76
DPPH	-0.87	-0.95
$ABTS^{\bullet+}$	-0.89	-0.96
RP	-0.99	-0.97
FRAP	-0.89	-0.8
$NO^{\bullet}$	-0.81	-0.9
Enzyme inhibiting activities (IC_{50})		
α-amylase	-0.87	-0.94
α-glucosidase	-0.96	-0.99

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

4. Conclusion

This study revealed that the peel extract of DHC fruit had a higher content of phenolics and flavonoids and stronger antioxidant, antibacterial and enzyme-inhibiting activities than pulp and seed extracts. The pulp extract came second in these contents and activities. In addition, correlation analysis indicated that total phenolics and flavonoids contents in DHC had a reasonably strong relationship for TAC, DPPH, $ABTS^{\bullet+}$, RP, FRAP, $NO^{\bullet}$, α-amylase, and α-glucosidase. This shows that the peel and pulp extract of DHC could be used as a source of natural antioxidants, antibacterials and enzyme inhibitors.

Conflict of interest

The authors declare no conflict of interest.

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