



## **Inhibitory Potency of Indonesian Tamarillo (*Solanum betaceum* Cav) Crude Extract Against $\alpha$ -Glucosidase Enzyme Activity**

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### **Abstract**

The aims of the research were to determine the inhibitory potency of the Indonesian Tamarillo crude extracts against the  $\alpha$ -glucosidase activity and identify their major anthocyanin and carotenoid content using LC-MS. In vitro assay was used to treat Tamarillo crude extracts which consisted of four levels: (1) Acarbose, positive control; (2) Tamarillo anthocyanin crude extract; (3) Tamarillo carotenoid crude extract; and (4) combination of Tamarillo anthocyanin crude extract and Tamarillo carotenoid crude extract. The results showed that the three crude extracts: the Tamarillo anthocyanin crude extract, the Tamarillo carotenoid crude extract and combination of Tamarillo anthocyanin and carotenoid crude extract could inhibit  $\alpha$ -glucosidase activity in 30.59%, 42.14% and 48.08% respectively. All of the Tamarillo crude extract inhibited mixed inhibition (noncompetitive and competitive inhibitor). Identification of the Tamarillo anthocyanin crude extracts showed six major compounds of anthocyanin type and four major compounds of carotenoid type. Three major anthocyanins type (pelargonidin-3-rutinoside, cyanidin-3-rutinoside and delphinidin-3-rutinoside) and three major carotenoids type ( $\beta$ -carotene,  $\beta$ -cryptoxanthin, zeaxanthin) were a tentative component of Tamarillo which is thought to play a role in inhibiting  $\alpha$ -glucosidase enzyme activity. Tamarillo extract can be alternative to prevent the development of postprandial hyperglycemic in type 2 diabetes.



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## Introduction

Diabetes is one of the noncommunicable diseases with prevalence and death risk continues to increase every year. One of diabetes types that prevalence reached 90% is of type 2 diabetes<sup>1</sup>. The development of type 2 diabetes which to be diabetic complications can be inhibited with dietary therapy using bioactive compounds.

According to<sup>2</sup> adherence to diet therapy will be inversely related to the level of blood sugar which means more obedient to run a diet therapy then blood sugar levels can be suppressed in type 2 diabetes.

Anthocyanin and carotenoid compounds belong to bioactive compound that potentially work as anti-diabetes especially in type 2<sup>3,4</sup>. One of the type 2 anti-diabetes potencies of anthocyanin or carotenoid is the inhibitory potency against  $\alpha$ -glucosidase<sup>3</sup>.

Inhibition of  $\alpha$ -glucosidase activity is one of the mechanisms in decreasing the blood sugar in diabetes. It causes the inhibition of carbohydrate hydrolysis to glucose in the brush border of the intestine<sup>5</sup>. The carbohydrates (oligosaccharides, disaccharides) cannot be hydrolyzed into glucose, therefore, no sugar transferred into the bloodstream system, affecting to the reduction of supply glucose in the blood, so the postprandial hyperglycemic on diabetes will decrease. Inhibition ability against  $\alpha$ -glucosidase enzyme activity from the material used as oral anti-diabetes drugs for treating type 2 diabetes that major potential as prevention and treatment of diabetes especially on postprandial hyperglycemic condition<sup>5,6,7</sup>. Although, the inhibition of  $\alpha$ -glucosidase enzymes is not the only digestive enzyme of carbohydrates, but this enzyme is an enzyme plays a direct important role in the digestion of carbohydrates into only glucose in intestine<sup>8</sup>, compared to salivary and pancreatic  $\alpha$ -amylase that can digest carbohydrates into dextrin, oligosaccharides, maltose and glucose<sup>9</sup>. In addition, the ability of phenolic compounds such as anthocyanin in inhibiting  $\alpha$ -glucosidase is higher than inhibiting the  $\alpha$ -amylase<sup>10</sup>.

The role of anti-diabetes especially as an inhibitor of  $\alpha$ -glucosidase enzyme activity from anthocyanin

and carotenoids in material is determined by the anthocyanin or carotenoid type. Anthocyanin and carotenoid of natural material consist of several types, but not all anthocyanin or carotenoid types are known to have potential in inhibiting  $\alpha$ -glucosidase enzyme activity. Anthocyanin types that have inhibitory potency against  $\alpha$ -glucosidase enzyme activity were cyanidin-3-rutinoside, delphinidin-3-rutinoside and anthocyanidin i.e., cyanidin, delphinidin and pelargonidin<sup>11,12</sup>, but for the carotenoid type, there has been no found yet about inhibitory potency against  $\alpha$ -glucosidase enzyme activity, but there was only reported as anti-diabetes on type 2 diabetes from  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein and zeaxanthin<sup>13,4,14</sup>. Carotenoids can bind to proteins<sup>15</sup>. The ability of compounds can bind to proteins, causing them can bind to enzymes such as  $\alpha$ -glucosidase enzyme<sup>16</sup>, therefore carotenoids probably capable of inhibiting  $\alpha$ -glucosidase enzyme activity.

One source of food that has more than one bioactive compound i.e., anthocyanin and carotenoid is Tamarillo<sup>17</sup>. Tamarillo (*Solanum betaceum* Cav.) is a fruit that is oval-shaped and sweet acidic taste which is originated from the Andes Mountains of Peru, which then spread to Brazil, Ecuador, Colombia New Zealand<sup>18</sup> and in Indonesia. Tamarillo is a unique fruit because it has a source of anthocyanin and carotenoids together in the fruit that can be consumed<sup>19</sup>, generally sources of anthocyanins and carotenoids come from one type of fruit or vegetable such as blackberries, strawberries and blackcurrant were anthocyanin sources; while tomatoes, carrots, and mango were carotenoid sources.

Identification of anthocyanins or carotenoids from Tamarillo is an attempt to determine the type of anthocyanin or its constituent carotenoids, so it can be used as a reference to determine the ability as anti-diabetes especially in inhibiting  $\alpha$ -glucosidase enzyme activity. Tamarillo anthocyanin types have been identified, but the number and type of anthocyanin found variety, due to differences in habitat origin and methods. However, the most of the major anthocyanin types are found cyanidin-3-rutinoside, delphinidin-3-rutinoside, pelargonidin-3-rutinoside and three anthocyanidin types (cyanidin, delphinidin and pelargonidin)<sup>20,21,22,23</sup>. The carotenoids

type of Tamarillo is also different, but still the same on major carotenoids types ( $\beta$ -cryptoxanthin,  $\beta$ -carotene, zeaxanthin and lutein)<sup>19,24</sup>.

Despite the Tamarillo has health benefits e.i., prebiotic, anti-cholesterol and improve SOD activity<sup>25,26</sup>, but research at anti-diabetes function still limited, especially on  $\alpha$ -glucosidase enzyme inhibitory has not been done. The Indonesia Tamarillo has been developed, but still underutilized for food compared to strawberry, dragon fruit and grape. Therefore, the aim of this research were to determine the inhibitory potency of Indonesian Tamarillo crude extracts against  $\alpha$ -glucosidase enzyme and to identify of anthocyanin and carotenoid type.

### Material and Methods

The red variety of Tamarillo fruit was collected from Dieng Hill, Wonosobo, Central Java, Indonesia that harvested in 6 months after anthesis. Acetonitrile, methanol, formic acid for HPLC, *p*-Nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) and  $\alpha$ -glucosidase enzyme were purchased from Sigma Aldrich (St. Louis, MO, USA).

### Preparation of Tamarillo Anthocyanin Crude Extract (TACE)<sup>27</sup>

From about 300 g Tamarillo fruit, the skin of the fruit was removed, then blended for 2 minutes without additional water, then 200 g of fruit pulp was mixed with 70% ethanol containing 3% citric acid (ratio 1: 4). After that, it was extracted by sonicator (Elma D 78224; T760DH; 40 kHz; Germany) at amplitude power of 100% and a temperature of  $27 \pm 2^\circ\text{C}$  (controlled by ice) for 20 minutes, then filtered by a vacuum filter. The filtrate concentrated by the rotatory evaporator was at  $40^\circ\text{C}$ .

### Preparation of Tamarillo Arotenoid Crude Extract (TCCE)<sup>s</sup>

The 200 g of fruit pulp from flesh tamarillo was mixed 80% acetone solvent with ratio of 1: 4, then extracted by sonicator (Elma D 78224; T760DH; 40 kHz, Germany) with amplitude power of 100% and at a temperature of  $27 \pm 2^\circ\text{C}$  (controlled by ice), for 30 minutes, then filtered by a vacuum filter. The filtration was transferred to the separating funnel containing petroleum ether. The organic solvent collected and concentrated by the rotatory evaporator at  $30^\circ\text{C}$ .

### Sample Solution Preparation for $\alpha$ -Glucosidase inhibitory assay

For Tamarillo anthocyanin crude extract (TACE) solution; 100 mg was TACE dissolved by 1 ml DMSO and ethanol solvent to 5 ml and mixed. For Tamarillo carotenoid crude extract (TCCE) solution 100 mg TCCE dissolved by 1 ml DMSO and acetone solvent to 5 ml and mixed. Every sample solutions were diluted to a concentration of 100  $\mu\text{g}/\text{mL}$ . Preparation of combination of Tamarillo anthocyanin crude extract and Tamarillo carotenoid crude extract (TCACEC) solutions: 2.5 ml of TACE solution added with 2.5 ml of TCCE solution.

### $\alpha$ -Glucosidase inhibitory assay<sup>29</sup>

The reaction mixture contained 490  $\mu\text{l}$  of 0.1 M phosphate buffer (pH 6,8), 250  $\mu\text{l}$  of 0,625 mM *p*-nitro phenyl  $\alpha$ -D-glucopyranoside (PNPG), and 10  $\mu\text{l}$  of test samples (TACE or TCCE or CTACCE) solution was incubated at  $37^\circ\text{C}$  for 5 minutes, then 250  $\mu\text{L}$  of  $\alpha$ -glucosidase solution (0.25 Unit/ml) was added, after that, incubated at  $37^\circ\text{C}$  for 15 minutes. Termination of reaction was done by adding 1000  $\mu\text{l}$  of 0.2 M sodium carbonate ( $\text{NaCO}_3$ ) solution. The amount of *p*-nitro phenol released in the reaction mixture, red at 400 nm using spectrophotometer UV Vis. Blanco was prepared for correcting the background absorbance, where the enzymes were replaced with the buffer. Controls were conducted to replace the samples with aquabidestilata steril (500 ml aqua pro injection). Acarbose was used as a positive control. All experiments were carried out in triplicates. The inhibition percentage of  $\alpha$ -glucosidase activity was assessed by the following formula:

$$\text{Inhibitory (\%)} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100$$

$\text{IC}_{50}$  of each extract was determined by making the linear regression equation ( $y = ax + b$ ) of 5 different concentrations of samples. The x-axis was the sample concentration, and the y-axis was the % inhibitory of the  $\alpha$ -glucosidase activity. Inhibitory concentration ( $\text{IC}_{50}$ ) was formulated as follows:

$$\text{IC}_{50} = (50-b)/a$$

### Kinetics of Inhibition on the $\alpha$ -glucosidase activity<sup>7,29</sup>

The inhibition mode of the extracts against  $\alpha$ -glucosidase was evaluated with the 6 different concentrations of *p*-nitrophenyl  $\alpha$ -D-glucopyranoside (PNPG) (50-100  $\mu$ g/mL) as substrate. The enzyme reaction was assayed with the method similar to  $\alpha$ -Glucosidase inhibitory assay<sup>29</sup>. The inhibition type of the data was determined by Lineweaver-Burk plot analysis. The obtained regression equation was used to calculate the Michaelis Menten constant ( $K_m$ ) and maximum enzyme reaction rate ( $V_{max}$ ).

### Anthocyanin Identification<sup>30</sup>

Amount 0,1 mg anthocyanin crude extracts were diluted in 10 ml acetonitrile (MeCN contain 0.1 % formic acid) and 5  $\mu$ L of the solution was injected to UPLC- QToF-MS System (Waters). The UPLC was equipped with BEH C18 column (1.7x2.1x50  $\mu$ m) and temperature column was 40°C, flow rate 0.3 mL/min. The eluents were: A: H<sub>2</sub>O contain 0.1% formic acid and B: Acetonitrile containing 0.1% formic acids. The program was set as the gradient methods were at 0-1 min 95% solvent A: 5% solvent B; at 6-7 min 0% solvent A: 100% solvent B; at 7.5-9 min 95% solvent A: 5% solvent B. Identification of anthocyanin compounds was carried out using MassLynk software version 4.1.

### Carotenoid Identification<sup>31,32</sup>

Amount 20  $\mu$ L crude carotenoid extract was diluted in 2 mL methanol, then 5  $\mu$ L of solution was injected

in LC-MS (TSQ Quantum – APCI), flow rate 300  $\mu$ L/s. The LC-MS was equipped with Hypersil C18 column (1.9 x 2.1x 50  $\mu$ m) and pump (Accela 1250 Pump). The tray temperature control was 10oC and with pump at low pressure 700 PSI. The eluent solvent: A (0.1% FA in water), solvent B (0.1% FA in acetonitrile), solvent C (20 mMol; 50% IPA: 50% methanol) and solvent D (0.1% in methanol). The gradient was at 0-0,6 min, solvent A : 50%, solvent B: 0%, solvent C:50%, solvent D: 0%; at 2,5-3,0 min solvent A: 0%, solvent B: 0%, solvent C: 100%, solvent D: 0%; at 3,5-5,0 min solvent A: 50%, solvent B: 0%, solvent C: 50%, solvent D: 0%;. Identification of carotenoid compounds was carried out by using MassLynk software version 4.1.

### Data analysis

The data were analyzed by using analysis of variance (ANOVA) and continued by Duncan's Multiple Range Test (DMRT) at 5% significance level according to IBM SPSS Statistic 20.

### Results

#### Inhibitory of $\alpha$ -glucosidase

The result of inhibitory  $\alpha$ -glucosidase of all treatments showed in the Table 1. The result  $IC_{50}$  of all treatments showed at Figure 1

**Table 1: Percentage inhibitory  $\alpha$ -glucosidase activity on all treatments**

Treatments	Average value of % inhibitory activity (%)
C0 (acarbose drug)	73.763 $\pm$ 4.041 <sup>a</sup>
TACE (Tamarillo anthocyanin crude extract)	48.079 $\pm$ 1.869 <sup>b</sup>
TCCE (Tamarillo carotenoid crude extract)	30.593 $\pm$ 1.719 <sup>d</sup>
TCACEC (Combination of Tamarillo anthocyanin crude extract and tamarillo carotenoid crude extract)	42.144 $\pm$ 1.472 <sup>c</sup>

Values with different letters within similar column are significantly different at  $p < 0.05$

### The mode of inhibition

The mode or mechanism of inhibition of  $\alpha$ -glucosidase from sampels is used Lineweaver Burk plots (1/[S] versus 1/v), where [S] is substrate concentration as

x axis and v is reaction velocity as y axis on non inhibitor and all samples inhibitor demonstated in Figure 2.

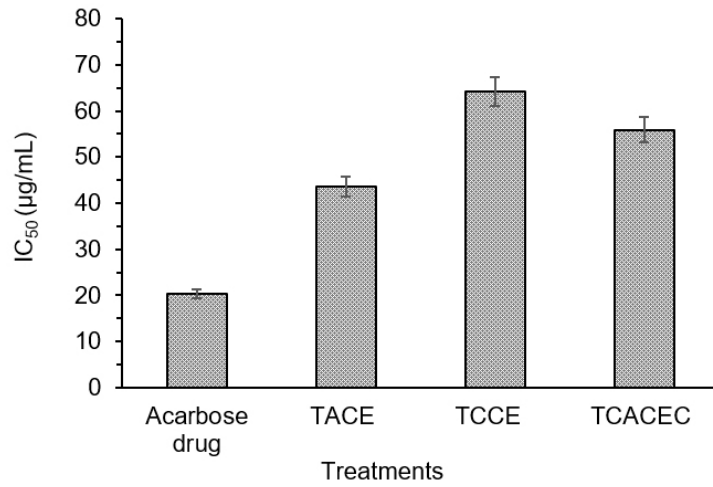


Fig.1: IC<sub>50</sub> value of all treatments

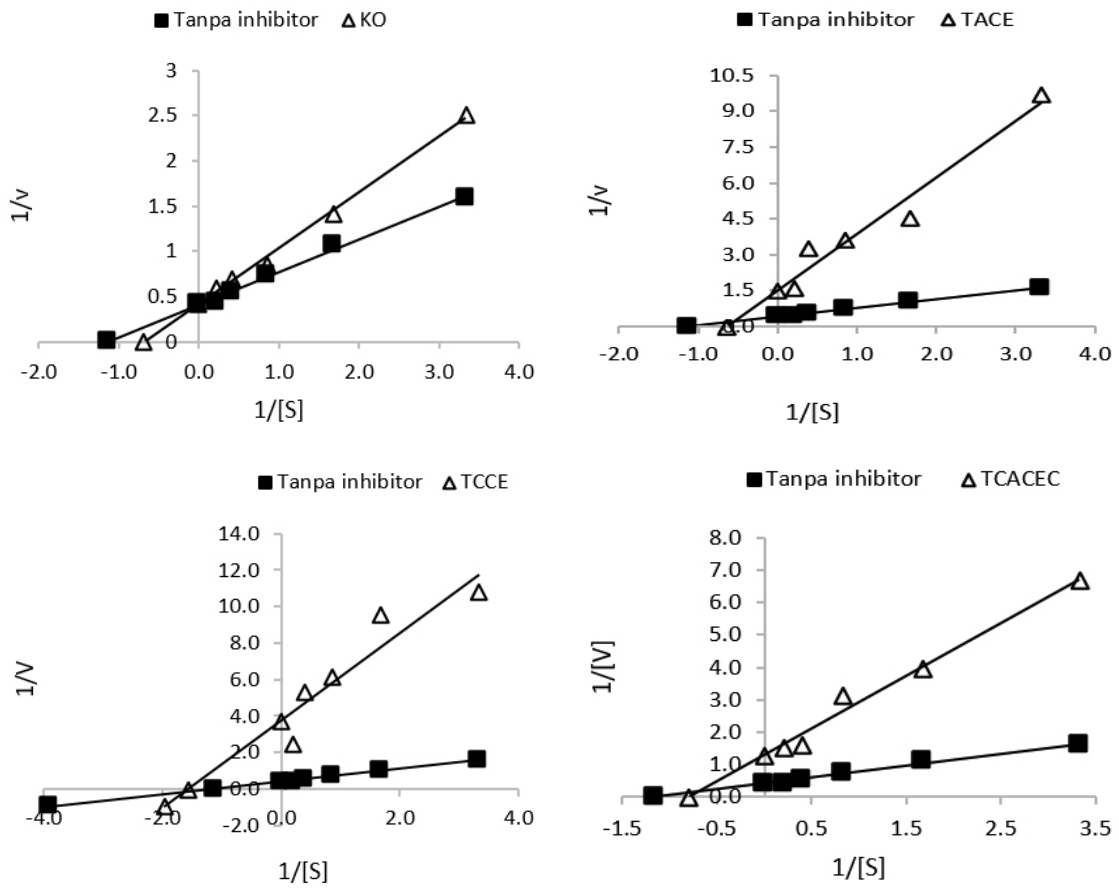
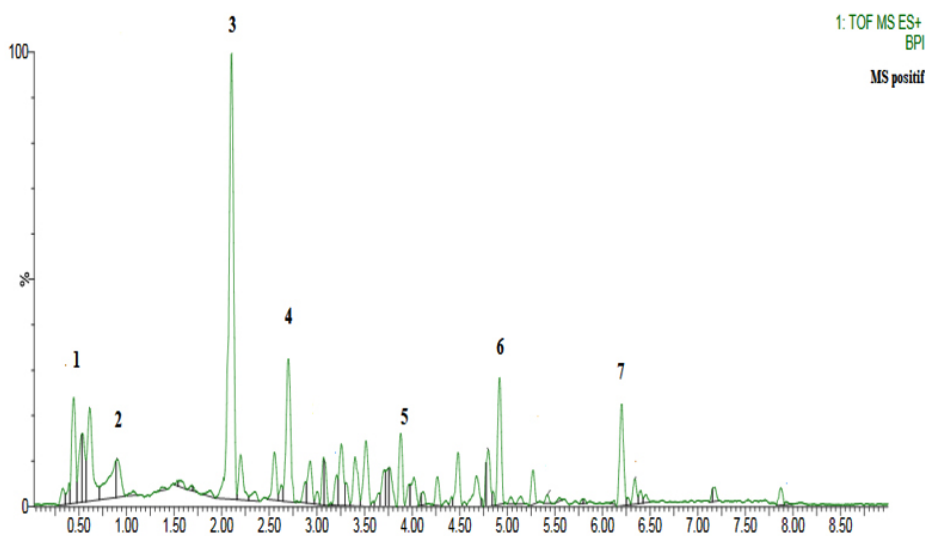


Fig.2: IC<sub>50</sub> Lineweaver-Burk plot of  $\alpha$ -glucosidase inhibition by (A) acarbose drug; (B) TACE; (C) TCCE; and (D) TCACEC

**Major Anthocyanin types of Indonesian Tamarillo**

The chromatogram of major anthocyanin types In Indonesian Tamarillo using UPLC-MS demonstrated

in Figure 3 and the characteristic of it showed in Table 2.



**Fig.3: IC<sub>50</sub> Chromatograms of major anthocyanin types in Indonesian Tamarillo**

**Table 2: Characteristic of chromatogram result of tentative anthocyanin types in Indonesian Tamarillo**

Peak Num.	t <sub>R</sub> (min)	PeakArea (A)	[M+H] <sup>+</sup>	MS1MS2	Tentative Anthocyanins Type	Ref.
1	0,45	154	611	303	Dp-3-rhamnosylglucoside	33
2	0,90	66	611	303	Dp-3-rutinoside	22 <sup>19</sup>
3	2,1	828	579	433/271	Pg-3-rutinoside	22
4	2,7	245	595	449/287	Cy-3-rutinoside	22 <sup>19</sup>
5	3,88	98	403	342/239	Ni	-
6	4,92	171	757	611/287	Cyanidin 3-O-glucosyl-rutinoside)	21
7	6,20	154	859	611/303	Dp-3-(6-z-p-coumaroylglucoside)-5-(6-malonylglucoside)	33

Ni: not identified

**Major carotenoid types of Indonesian Tamarillo**

The chromatogram of major carotenoid types In Indonesian Tamarillo using LC-MS demonstrated in

Figure 4 and thier chromatogram characteristic result of it demonstrated in Table 3.

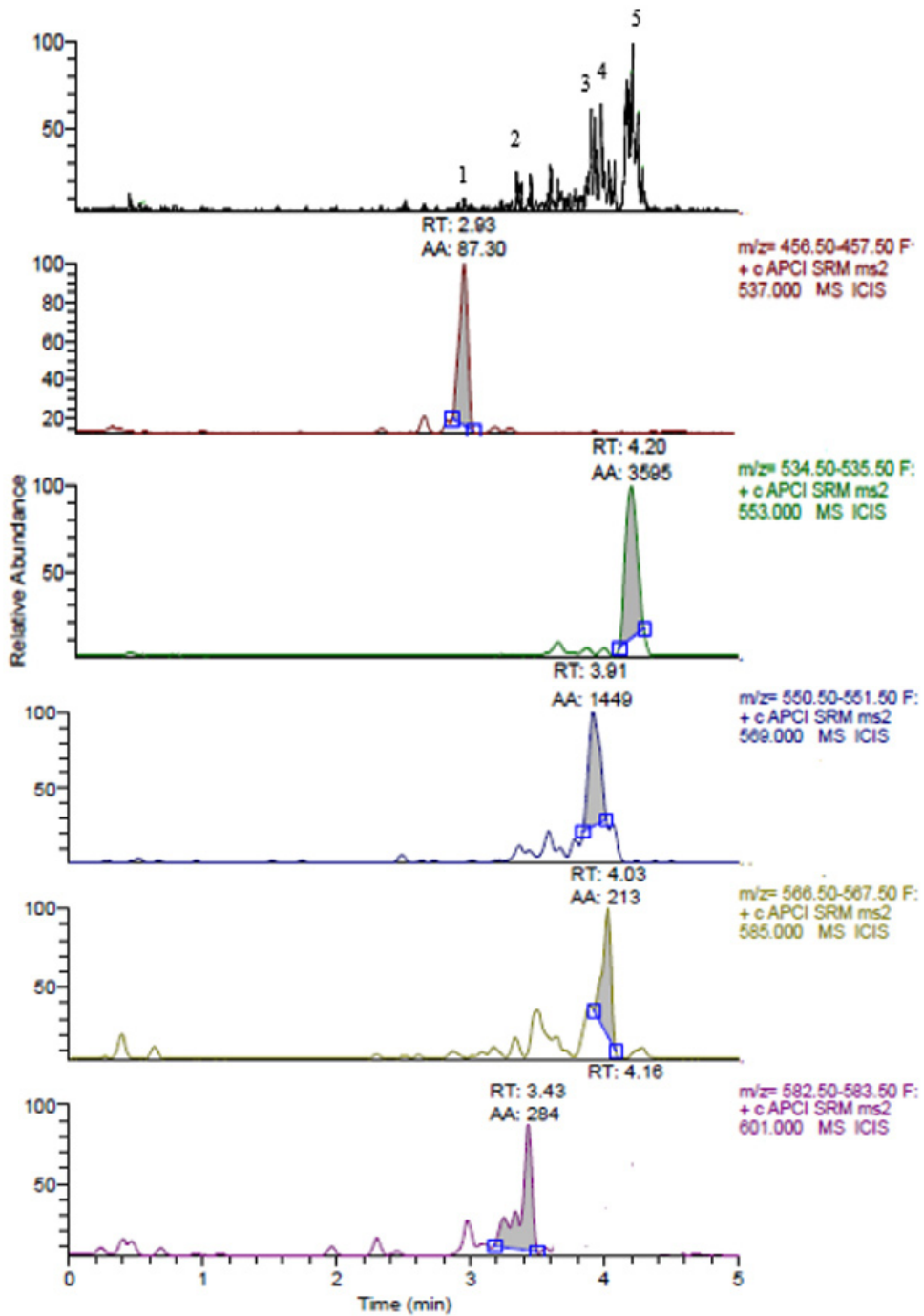


Fig.4: Chromatograms of major carotenoid types in Indonesian Tamarillo

**Table 3: Chromatogram characteristic of tentative anthocyanin types in Indonesian Tamarillo**

Peak no.	Rt.	Peak area	[M+ H] <sup>+</sup>	MS1/MS2	Tentative Carotenoids Compounds	Ref.
1	2,93	1,55	537	456,50-457,50	β-carotene	34
2	3,34	3,69	601	582,50-583,50	Neoxanthin	35
3	3,91	26,11	569	550,50-551,50	Zeaxanthin	34
4	4,03	3,84	585	566,50-567,50	Lutein	35
5	4,20	64,78	553	534,50-535,50	β-cryptoxanthin	34

## Discussion

### α-glucosidase inhibitory activity

The results showed that Tamarillo anthocyanin crude extract (TACE), Tamarillo carotenoid crude extract (TCCE) and their combination (TCACEC) could inhibit α-glucosidase activity, but still lower than acarbose (positive control) (Table 1). The result of percentage inhibition on α-glucosidase activity by TACE, TCCE and TCACEC were 48.08%; 30.59% and 42.14%, respectively. These results were correlated with the IC<sub>50</sub> value. The IC<sub>50</sub> of the TACE, TCCE and TCACEC were: 43.52 µg/mL; 64.20 µg/mL; and 55.88 µg/mL, respectively (Figure 1). The TACE was the most α-glucosidase inhibitor than other extracts. IC<sub>50</sub> value of all Tamarillo extract types were higher than IC<sub>50</sub> value of acarbose (20,40 µg/mL). This means that the ability to inhibit α-glucosidase from all Tamarillo extracts is still lower than acarbose, but acarbose's ability is still lower than quercetin<sup>36</sup>. This result is supported by<sup>37</sup>, state that quercetin which is as positive control on α-glucosidase inhibition of several tropical and subtropical plants has IC<sub>50</sub> value of 4.2 µg/mL. All Tamarillo extracts are still classified as strong glucosidase inhibitors because IC<sub>50</sub> values are still below 100 µg/mL<sup>38</sup>.

Inhibitor potency of all Tamarillo extracts against α-glucosidase is lower than acarbose which is similar to research of<sup>39</sup>. They stated that purple sweet potato extract at concentration of 100 µg/mL could inhibit α-glucosidase by 56.37% while acarbose is 62.82%.

The potential of TACE inhibition against α-glucosidase, probably due to the presence of cyanidin 3-rutinoside, delphinidin 3-rutinoside and anthocyanidins (pelargonidin, delphinidin and cyanidin). Their ability had been proved in inhibiting α-glucosidase activity<sup>40,12</sup>. Pelargonidin 3-rutinoside as the most major anthocyanin in this study, may play a role in inhibiting α-glucosidase activity because if it is hydrolyzed it becomes pelargonidine<sup>41,42</sup>. This requires further study to isolate the pelargonidine 3-rutinoside from Tamarillo and test its ability as α-glucosidase inhibitor. TCCE may inhibit recommended α-glucosidase activity due to the presence of β-cryptoxanthin, β-carotene, zeaxanthin and lutein components. These components have been shown to have the potential as anti-diabetes type 2<sup>43,44,45</sup>, one possibility through the mechanism of α-glucosidase activity inhibitory. TCACEC was also able to inhibit α-glucosidase activity and was significantly higher (P<0.05) than TCCE. It was suspected that there was a synergistic inhibition among anthocyanin and carotenoid compounds in inhibiting α-glucosidase activity.

Acarbose as an anti-diabetic agent (positive control) had potential as an inhibitor of α-glucosidase activity because it has a similar structure with the substrate, resulting in competition with the substrate to bind to the active side on the enzyme. The acarbose bonding effects with the enzyme cause the disruption of the enzyme activity, resulting in hydrolysis of carbohydrates into glucose in the wall of the intestine



delayed. In fact, the effect on the supply of glucose into blood is reduced and postprandial blood sugar in type 2 diabetes will decrease<sup>46</sup>.

### Mode of Inhibition Against $\alpha$ -glucosidase Activity

The mode of inhibition of all Tamarillo crude extracts (TACE, TCEC, TCACEC) against  $\alpha$ -glucosidase activity which determined with Lineweaver-Burk plot analysis data, which calculated from the results according to Michaelis-Menten Kinetics showed difference with the acarbose (Figure 2). The acarbose was a competitive inhibitor. The TACE, TCEC and TCACEC were mixed inhibitor. They were seen from the intersection of the line of the non-inhibitor and the inhibitor. The line intersection of acarbose exactly on the y-axis (Figure 2A), whereas the TACE (Figure 2B), TCCE (Fig. 2D) and TCACEC (Fig. 2C) were located between x and y axis that more near to x axis.

The competitive inhibitor was shown as acarbose indicated that the presence of inhibitor had no effect on  $V_{max}$  but increased Km value or  $K_{app} > K$  (data not shown) The competitive inhibitor mechanism, the present of inhibitor could bind to the active site of the enzyme and compete with the substrate for binding to the active site of the enzyme. Competitive inhibitor property often resembles the substrate in the case of chemical structure, shape and polarity pattern. The competitive inhibitor mode of acarbose had similar result of the research of<sup>12</sup>.

The mixed inhibitor of TACE, TCCE and TCACEC had effect on  $V_{max}$  and Km.  $V_{max}$  of TACE and TCACEC decreased and Km increased ( $V_{app} < V$ , and  $K_{app} > K$ ) means they can bind to free enzyme and enzyme-substrate complex, but  $V_{max}$  and Km TCCE decreased ( $V_{app} < V$ , and  $K_{app} < K$ ) means TCCE with mixed inhibitor not affected by  $V_{max}$  and Km. This may be due to the extract containing more than one glucosidase inhibitor. The mixed inhibitor of all Tamarillo extract similar to quercetin that has mixed inhibitor mode<sup>37</sup>.

### Major Tentative Anthocyanin Type in Indonesian Tamarillo

Figure 3 illustrated major chromatogram peaks of anthocyanin. There were 7 peaks and the identified 6 tentative anthocyanin types that were shown in Table

1. The peaks identified as tentative anthocyanin types: (1) peak 1 (rt:0,45), m/z 611 of  $[M+H]^+$ , m/z 303 of MS1, identified as delphinidin 3-rhamnoylglucoside (Ivanova *et al.*, 2011), (2) peak 2, 0,9 of rt, m/z 611 of  $[M+H]^+$ , m/z 3023 of MS2 611, identified as delphinidin-3-glucoside-5-rhamnose), (3) peak 3, 2,1 of rt, m/z 579 of  $[M-H]^+$ , m/z 433 of MS1, m/z 271 of MS2, identified as pelargonidin-3-glucoside-5-rhamnose), (4) peak 4, 2,7 of rt, m/z 595 of  $[M+H]^+$ , m/z 499 of MS1 and m/z 287 of MS2, identified as cyanidin-3-rutinoside); (5) peak 5, 3,88 of rt, m/z 403 of  $[M+H]^+$  and m/z 146 of MS1, no-identified; (6). Peak 6, 4,92 of rt, m/z 757 of  $[M+H]^+$ , m/z 611 of MS1 and m/z 287 of MS2, identified as cyanidin 3-O-glucosyl-rutinoside; and 7). peak 7, 6,2 of rt, m/z 859 of  $[M+H]^+$ , m/z 611 of MS1 and m/z 303 of MS2, identified as Dp-3-(6-z-p-coumaroylglucoside)-5-(6-malonylglucoside).

The three types of anthocyanins in Indonesian Tamarillo (delphinidin-3-rutinoside, pelargonidin-3-rutinoside cyanidin-3-rutinoside) similar to anthocyanin present in New Zealand<sup>20</sup>. Ecuador and Colombia<sup>21,22,23</sup>. The observed three carotenoids compounds differed with Brazilian Tamarillo. Brazilian Tamarillo just had only delphinidin-3-rutinoside and cyanidin-3-rutinoside<sup>17</sup>, while the major anthocyanin (pelargonidin-3-rutinoside) was similar within Ecuador Tamarillo, but differed with Brazil and New Zealand Tamarillo (delphinidin-3-rutinoside)<sup>20,19,21</sup>. The difference of the amount and major of anthocyanin may be due to different the place of growing habitats that could affect different compositions and different analysis methods<sup>17</sup>.

The major anthocyanidin (pelargonidin) compound in Indonesian Tamarillo was also observed as the major compounds in the strawberry<sup>47</sup>. The other compounds i.e. delphinidin-3-rutinoside was similar with the major anthocyanin in Blackcurrant extract<sup>12</sup> and cyanidin-3-rutinoside was similar with Black rice<sup>48</sup>.

### Major Tentative Carotenoid Type in Indonesian Tamarillo

The major tentative carotenoids in Indonesian Tamarillo were 5 carotenoid types: (1) peak 1 was m/z 537 with fragment identified as  $\beta$ -carotene; (2) peak 2 was m/z 569, identified as zeaxanthin; (3) peak 3 was m/z 569, identified as lutein; (4) peak

4 was m/z 601 identified as neoxanthin and (5) peak 5 was m/z 553, identified as  $\beta$ -cryptoxanthin. The major carotenoid in Indonesian Tamarillo was  $\beta$ -cryptoxanthin. The amount reached 64.79% of the total.

The major carotenoid in Tamarillo ( $\beta$ -cryptoxanthin) and main carotenoids were  $\beta$ -cryptoxanthin,  $\beta$ -carotene, zeaxanthin, neoxanthin and lutein were similar within Brazilia Tamarillo, whereas it was different at the amount of carotenoids. In Brazil, Tamarillo was identified to have 17 carotenoids<sup>17</sup>. This difference is affected by the different environment and habitats, so they can affect their composition and different analysis method<sup>19,23</sup>.

### Conclusion

Indonesian Tamarillo extract that unique contains two components (anthocyanin and carotenoid) has potency as inhibitory on  $\alpha$ -glucosidase activity. Percentage of inhibition against  $\alpha$ -glucosidase activity from the three crude extracts: combination of the anthocyanin and carotenoid crude extract, the anthocyanin crude extract, the carotenoid

crude extract were 42,14%, 48,08% and 30,59% respectively with the mode inhibition modes were mixed inhibitor, 1 type on Tamarillo anthocyanin crude extract and thier combination and mixed inhibitor 2 type on Tamarillo carotenoid crude extract. The major tentative anthocyanin an carotenoid in Tamarillo Indonesian were in 6 types and 4 types, respectively. The two major anthocyanin types (cyanidin-3-rutinoside and delphinidin-3-rutinoside) and three carotenoid types ( $\beta$ -carotene,  $\beta$ -cryptoxanthin and zeaxanthin) were components that act as inhibitors of glucosidase enzyme activity. Pelargonidin 3-rutinoside is the most major anthocyanin type that found in Tamarillo Indonesia and tentative potential to act as an inhibitor of  $\alpha$ -glucosidase enzyme activity.

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### References

1. IDF. Eighth edition 2017. (2017).
2. Kumari, G., Singh, V. & Jhingan, A. K. Effectiveness of Lifestyle Modification Counseling on Glycemic Control in Type 2 Diabetes Mellitus Patients. *Curr. Res. Nutr. Food Sci.* 2018; **06**:70–82.
3. Sancho, R. A. S. & Pastore, G. M. Evaluation of the effects of anthocyanins in type 2 diabetes. *FRIN*. 2012; **46**:378–386
4. Mohammed, H. A., Al-Shamma, G. A. & Hashim, H. M. Beta-Carotene, Glycemic Control And Dyslipidemia In Type 2 Diabetes Mellitus Summary : *J Fac Med Baghdad*. 2006; **48**:435–441.
5. Nair, S. S., Kavrekar, V. & Mishra, A. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *Eur. J. Exp. Biol.* 2013; **3**: 128–132.
6. Park, H. *et al.*, Discovery and biological evaluation of novel  $\alpha$ -glucosidase inhibitors with in vivo antidiabetic effect. *Bioorg. Med. Chem. Lett.* 2008; **18**:3711–3715.
7. Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, W. Y. & Rhee, H. I. Inhibitory effect of pine extract on  $\alpha$ -glucosidase activity and postprandial hyperglycemia. *Nutrition*. 2005; **21**:756–761.
8. Rouzbehan, S., Moein, S., Homaei, A. & Moein, M. R. Kinetics of  $\alpha$ -glucosidase inhibition by different fractions of three species of Labiatae extracts: A new diabetes treatment model. *Pharm. Biol.* 2017; **55**: 1483–1488.
9. Kolusheva, T. & Marinova, A. A Study of the Optimal Conditions for Starch Hydrolysis Through Thermostable  $\alpha$  - Amylase. *J. Univ. Chem. Technol. Metall.* 2007; **42**:93–96.
10. McDougall, G. J. *et al.*, Different polyphenolic components of soft fruits inhibit  $\alpha$ -amylase and  $\alpha$ -glycosidase. *J. Agric. Food Chem.* 2005; **53**:2760–2766.
11. Adisakwattana, S., Anun, S. Y., Charoenlertkul,

- P. & Wongsasiripat, N. Cyanidin alleviates postprandial hyperglycemia and its synergism with acarbose by inhibition of intestinal  $\alpha$ -glucosidase. *J. Clin. Biochem. Nutr.*2011;**49**, 36–41.
12. Boath, A. S., Stewart, D. & McDougall, G. J. Berry components inhibit  $\alpha$ -glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chem.*2012;**135**:929–936.
  13. Montenen, J. *et al.*, Dietary Antioxidant Intake and Risk of Type 2 Diabetes. *Diabetes Care.*2004;**27**:362–366.
  14. Wang, L. *et al.*, Plasma Lycopene , Other Carotenoids, and the Risk of Type 2 Diabetes in Women. *Am. J. Epidemiology.*2006;**164**: 576–585.
  15. Reszczynska, E., Welc, R., Grudzinski, W., Trebacz, K. & Gruszecki, W. I. Carotenoid binding to proteins: Modeling pigment transport to lipid membranes. *Arch. Biochem. Biophys.*2015;**584**:125–133.
  16. Griffiths, D. W. & Moseley, G. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *J. Sci. Food Agric.*1980;**31**:255–259.
  17. De Rosso, V.V. & Mercadante, A. Z. Identification and Quantification of Carotenoids , By HPLC-PDA-MS/MS, from Amazonian Fruits. *J. Agric. Food Chem.*2007;**55**:5062–5072.
  18. Correia, S. I. & Canhoto, J. M. Biotechnology of tamarillo (*Cyphomandra betacea*): From in vitro cloning to genetic transformation. *Sci. Hortic. (Amsterdam)*.2012;**148**:161–168.
  19. De Rosso, V. V. & Mercadante, A. Z. HPLC – PDA – MS / MS of Anthocyanins and Carotenoids from Dovyalis and Tamarillo Fruits AND. *J. Agric. Food Chem.*2007;**55**, 9135–9141.
  20. Wrolstad, R. E. & Heatherbell, D. A. Identification of anthocyanins and distribution of flavonoids in tamarillo fruit (*Cyphomandra betaceae* (Cav.) Sendt.). *J. Sci. Food Agric.*1974;**25**, 1221–1228.
  21. Mertz, C. *et al.*, Phenolic compounds , carotenoids and antioxidant activity of three tropical fruits. *J. Food Compos. Anal.*2009;**22**:381–387.
  22. Osorio, C. *et al.*, Chemical characterisation of anthocyanins in tamarillo (*Solanum betaceum* Cav.) and Andes berry (*Rubus glaucus* Benth.) fruits. *Food Chem.*2012;**132**, 1915–1921.
  23. Espin, S. *et al.* Phenolic composition and antioxidant capacity of yellow and purple-red Ecuadorian cultivars of tree tomato (*Solanum betaceum* Cav.). *Food Chem.*2016;**194**:1073–1080.
  24. Mertz, C., Brat, P., Caris-veyrat, C. & Gunata, Z. Characterization and thermal lability of carotenoids and vitamin C of tamarillo fruit (*Solanum betaceum* Cav. ). *Food Chem.*2010;**119**:653–659.
  25. Kou, M. *et al.*, *Cyphomandra betacea* Sendt. phenolics protect LDL from oxidation and PC12 cells from oxidative stress. *LWT - Food Sci. Technol.*2009;**42**:458–463.
  26. Gannasin, S. P., Adzahan, N. M., Mustafa, S. & Muhammad, K. Techno-functional properties and in vitro bile acid-binding capacities of tamarillo (*Solanum betaceum* Cav.) hydrocolloids. *Food Chem.*2016;**196**, 903–909.
  27. Chen, F. *et al.*, Optimization of ultrasound-assisted extraction of anthocyanins in red raspberries and identification of anthocyanins in extract using high-performance liquid chromatography – mass spectrometry. *Ultrason. Sonochemistry.*2007;**14**:767–778.
  28. Santos, L. E. O., Zarate, L. X. P. & Salcedo, L. O. G. Optimization of ultrasonic-assisted extraction of total carotenoids from peach palm fruit (*Bactris gasipaes*) by-products with sunflower oil using response surface methodology. *Ultrason. Sonochem.* 1–8 (2015). doi:10.1016/j.ultsonch.2015.04.010
  29. Mayur, B., Sandesh, S., Shruti, S. & Sung-yum, S. Antioxidant and -glucosidase inhibitory properties of *Carpesium abrotanoides* L. *J. Med. Plants.*2010;**4**:1547–1553.
  30. Liao, Z.-X., Zhang, B.-B., Ding, L.-S. & Zhou, Y. Development of an UPLC-QTOF-MS method for qualitative and quantitative analysis of *Saussurea eopygmaea*. *Acta Chromatogr.*2013;**25**:171–180.
  31. Breemen, R. B. Van, Huang, C., Tan, Y., Sander, L. C. & Alexander, B. Liquid Chromatography / Mass Spectrometry of Carotenoids Using Atmospheric Pressure Chemical Ionization.

- J. Mass Spectrom.*1996; **31**:975–981.
32. Breemen, R. B. Van. Liquid chromatography / mass spectrometry of carotenoids.1997;**69**, 2061–2066.
  33. Schütz, K., Persike, M., Carle, R. & Schieber, A. Characterization and quantification of anthocyanins in selected artichoke (*Cynara scolymus* L.) cultivars by HPLC-DAD-ESI-MSn. *Anal. Bioanal. Chem.*2006;**384**, 1511–1517.
  34. Breemen, R. B. Van, Dong, L. & Pajkovic, N. D. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids Lycopene. *Int. J. Mass Spectrom.*2012;**312**, 163–172.
  35. Rivera, S. M., Christou, P., Canela-garayoa, R. & Introduction, I. Identification of Carotenoid Using *Mass Spectrometry*.2013;1–20. doi:10.1002/mas
  36. Li, Y. Q., Zhou, F. C., Gao, F., Bian, J. S. & Shan, F. Comparative Evaluation of Quercetin, Isoquercetin and Rutin as Inhibitors of  $\alpha$ -Glucosidase. *J. Agric. Food Chem.*2009;**57**, 11463–11468.
  37. Indrianingsih, A. W., Tachibana, S. & Itoh, K. In Vitro Evaluation of Antioxidant and  $\alpha$ -Glucosidase Inhibitory Assay of Several Tropical and Subtropical Plants. *Procedia Environ. Sci.*2015;**28**:639–648.
  38. Molyneux, P. The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity. *J. Sci. Technol.*2004;**26**: 212–219.
  39. Elmaniar, R. & Muhtadi. Aktivitas Penghambatan Enzim  $\alpha$ - Glukosidase oleh Ekstrak Etanol Ubi Jalar Ungu (*Ipomoea batatas* L.). in The 5<sup>th</sup> *Urecol Proceeding*.2017; 825–831.
  40. Adisakwattana Sirichai, Sirintorn Yibchock Anun, Piyawan Charoenlertkul, N. W. Cyanidin-3-rutinoside alleviates postprandial hyperglycemia and its synergism with acarbose by inhibition of intestinal glucosidase. *J. Clin. Biochem. Nutr.*2011;**48**:107–111.
  41. Fang, J. Bioavailability of anthocyanins. *Inf. Heal. Drug Metab Rev.*2014;**46**:508–520.
  42. Fernandes, I., Faria, A., Calhau, C., Freitas, V. de. & Mateus, N. Bioavailability of anthocyanins and derivatives. *J. Fuctional Foods.*2014;**7**: 54–66.
  43. Yaqub, S. *et al.*, Chemistry and Functionality of Bioactive Compounds Present in Persimmon. *J. Chem.*2016;1–14. doi:10.1155/2016/3424025
  44. Ernawita *et al.*, Carotenoids of indigenous citrus species from Aceh and its in vitro antioxidant, antidiabetic and antibacterial activities. *Eur. Food Res. Technol.*2016;**242**: 1869–1881.
  45. Roohbakhsh, A., Karimi, G. & Iranshahi, M. Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. *Biomed. Pharmacother.*2017;**91**: 31–42.
  46. Bischoff, B. Pharmacology of  $\alpha$ -glucosidase inhibition. *Eur. J. Clin. Invest.*1994;**24**:3–10.
  47. Cerezo, A. B., Cuevas, E., Winterhalter, P., Garcia-Parilla, M. C. & Troncoso, A. M. Isolation, identification, and antioxidant activity of anthocyanin compounds in Camarosa strawberry. *Food Chem.*2010;**123**:574–582.
  48. Hou, Z., Qin, P., Zhang, Y., Cui, S. & Ren, G. Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. *Food Res. Int.*2013;**50**: 691–697.