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RESEARCH ARTICLE

Antioxidant and α-glucosidase Inhibitory Activities of Four Types of Chrysophyllum cainito L. Fruit

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Antioxidant and α-glucosidase Inhibitory Activities of Four Types of Chrysophyllum cainito L. Fruit

SUMMARY

Chrysophyllum cainito L., locally grown in East Java, Indonesia, was used traditionally in diabetes treatment. The study investigated antioxidant and antidiabetic activity of four morphologically-classified C. cainito fruit. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging and inhibition of α-glucosidase assay were applied to determine antioxidant and antidiabetic activities. 70% ethanolic extract showed higher radical scavenging activity than another extracts. Type 1 exhibited the strongest antioxidant capacity with an IC50 of 24.469 ± 0.065 µg/mL. Nevertheless, type 3 showed the most potent activity in α-glucosidase inhibition with an IC50 value of 9.498 ± 0.224 µg/mL, the highest total flavonoid content (TFC) and total phenolic content (TPC) with value of 0.609 ± 0.003 mg QE/g extract and 327.088 ± 0.101 mg GAE/g extract, respectively. The study also proved that ethyl acetate fraction of freeze dried pulp, mainly type 3 exhibited the highest α-glucosidase inhibitory activity with an IC50 of 0.787 ± 0.018 µg/mL. The type possessed the highest TFC (9.592 ± 0.038 mg QE/g fraction) and TPC (840.869 ± 0.854 mg GAE/g fraction) value. Therefore, all types of C. cainito fruits could be suggested as natural sources with potential antioxidant and antidiabetic effects.

Key Words: *Chrysophyllum cainito, DPPH, Antioxidant, α-glucosidase inhibitor, Total flavonoid content, Total phenolic content*

Dört Chrysophyllum cainito L. Meyvesinin Antioksidan ve α-Glukozidaz İnhibitör Aktiviteleri

ÖZ

Endonezya'nın Doğu Java kentinde yetişen Chrysophyllum cainito L., diyabet tedavisinde geleneksel olarak kullanılmıştır. Çalışmada morfolojik olarak sınıflandırılmış dört C. cainito meyvesinin antioksidan ve antidiyabetik etkinliği araştırıldı. Antioksidan ve antidiyabetik aktivitelerin belirlenmesi için 1,1-difenil-2-pikril hidrazil (DPPH) radikal süpürme ve a-glukozidaz inhibisyonu deneyleri uygulandı. % 70 etanolik ekstrakt, diğer ekstraktlardan daha yüksek radikal süpürücü aktivite gösterdi. Tip 1, 24.469 ± 0.065 µg/mL IC50 ile en güçlü antioksidan kapasiteyi gösterdi. Bununla birlikte, tip 3, 9.508 ± 0.224 µg/mL IC50 değeri ile a-glukozidaz inhibisyonunda en yüksek etkinliğin yanında 0.609 ± 0.003 mg QE/g özü ve 327.088 ± 0.101 mg GAE/g özü değerleriyle sırasıyla en yüksek toplam flavonoid içeriğe (TFC) ve toplam fenolik içeriğe (TPC) sahip olduğunu göstermiştir. Çalışmada ayrıca esas olarak tip 3 olan dondurularak kurutulmuş pürenin etil asetat fraksiyonunun, 0.787 ± 0.018 µg/mL IC50 ile en yüksek a-glukosidaz inhibe edici aktivite sergilediği de kanıtlanmıştır. Bu tip, en yüksek TFC (9.592 ± 0.038 mg QE/g fraksiyonu) ve TPC (840.869 ± 0.854 mg GAE/g fraksiyonu) değerine sahipti. Bu sebeplerden ötürü, tüm C. cainito meyveleri, potansiyel antioksidan ve antidiyabetik etkilere sahip doğal kaynaklar olarak önerilebilir.

Anahtar Kelimeler: *Chrysophyllum cainito, DPPH, Antioksidan, α-glukosidaz inhibitörü, Total flavonoid içeriği, Toplam fenolik içeriği*

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INTRODUCTION

Recently, natural sources with antioxidant and α-glucosidase activity could be suggested in prevention or treatment of diabetes mellitus, particularly type 2 diabetes. Production of reactive oxygen species (ROS) increases in diabetes mellitus, mainly patients with poor glycemic control. (Choi & Ho, 2018). It was hypothesized that oxidative stress by free radicals is one of pathogenic factors in β-cell dysfunction, insulin resistance, and impaired glucose tolerance (Ceriello & Motz, 2004). Previous study showed beneficial effects in diabetic complications due to use of antioxidants, such as ascorbic acid, α-tocopherol, taurine, glutathione, coenzyme Q, and α–lipoic acid. Natural antioxidants prevent diabetic complications through several mechanisms comprising auto-oxidation of glucose, the polyol pathway activation, non-enzymatic glycation or glycosylation of proteins, NADPH oxidase activation, and electron transport system of mitochondria (Nishikawa & Araki, 2013). Moreover, it is important to prevent diabetes mellitus severity using α-glucosidase inhibitor to delay glucose release from carbohydrate. The use of enzyme inhibitor would retard hydrolysis of carbohydrate, and decrease postprandial blood glucose level in diabetic patients (Supasuteekul et al., 2016). Several α-glucosidase inhibitors frequently described in type 2 diabetes are acarbose, voglibose, and miglitol. Nevertheless, the drugs have unpleasant side effects, such as weight gain, and gastrointestinal disturbance (Sudhir & Mohan, 2002). Hence, a new α-glucosidase inhibitor and antioxidant with potent activity, and fewer side effects is demanded for treatment diabetes mellitus (Yin et al., 2014, Supasuteekul et al., 2016, Doan et al., 2018).

Chrysophyllum cainito L. (Sapotaceae), commonly known as star apple, is a traditional medicinal plant with potential antioxidant and α-glucosidase inhibitory activity. It grows mainly in several tropics and subtropics such as America, West Africa, Australia, and India. The ripe fruit was used to treat inflammation of pneumonia, laryngitis, and traditionally as antidiabetic agent (Shailajan & Gurjar, 2014). An ethnobotanical study performed by Koffi et al. (2009) showed that *C. cainito* was used as traditional medicine for treating diabetes in Aboude-Mandeke, Agboville. The aqueous decoction of *C. cainito* leaves had antidiabetic activity at doses greater than 10 g/L related to its alkaloids, sterols, and triterpenoids content. Doan et al. (2018) demonstrated that *C. cainito* stem bark extract had a powerful antioxidant capacity and α-glucosidase inhibition capacity with an IC_{50} value of 1.20 0.09 μg/mL.

There are several types of *C. cainito* fruit locally grown in Indonesia, i.e. type 1, a big fruit with round shape and green color; type 2, a small fruit with round shape and green color; type 3, a medium fruit with oval shape and green color; and type 4, a small fruit with round shape and red purplish color, as it was described in our previous work (Ningsih et al., 2016). Morphologically differences among those types could be seen in Figure 1. Water, methanolic, and ethyl acetate extracts of three types of *C. cainito* fruits had been observed for its DPPH scavenging activity (Hidayat and Umiyah, 2005; Hidayat and Ulfa, 2006; Amrun et al., 2007). Hence, we evaluated 96% ethanolic, 70% ethanolic, and water extracts of four types of *C. cainito* fruits for its antioxidant activity. The extract with higher antioxidant capacity was observed for its *in vitro* antidiabetic activity, total flavonoid content (TFC), total phenolic content (TPC), and phytochemical content. Different drying method, solvent selection, and treatment in plant material preparation may affect phytochemical contents and bioactivity of materials, as applied in the current work. Therefore, this study also determined α-glucosidase inhibitory activity, TFC, and TPC of ethyl acetate fractions from fresh pulp and freeze dried pulp.

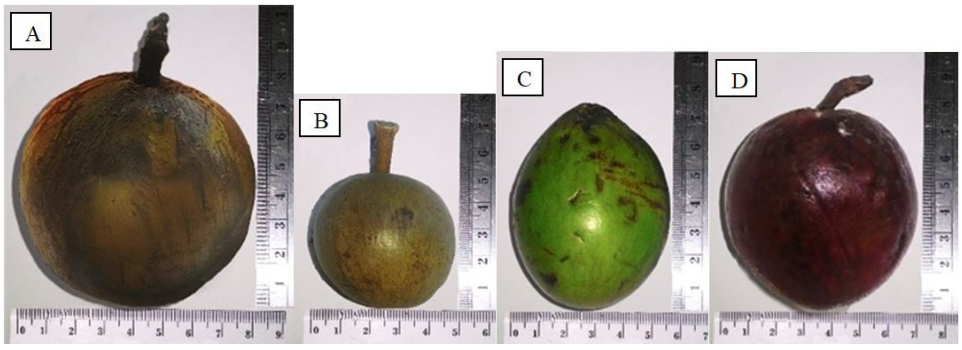

Figure 1. Several types of *C. cainito* fruit from Indonesia: A. Type 1, B. Type 2, C. Type 3, D. Type 4

MATERIALS AND METHODS

Chemical materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), α-glucosidase from *Saccharomyces cerevisiae*, 4-nitrophenyl-α-D-glucopyranoside (pNPG), $NaHCO_3$, gallic acid, quercetin, dimethyl sulfoxide (DMSO), HCl, NaCl, $HgCl_2$, KI, NH_4OH, Dragendorff reagent, magnesium ribbon, glacial acetic acid, boric acid, citric acid, ferric chloride reagent, H_2SO_4, anisaldehyde solution, n-hexane, ethyl acetate, chloroform, butanol, methanol, and ethanol were supplied by Sigma-Aldrich (St. Louis, MO, USA), Na_2CO_3, Folin-Ciocalteau reagent, $AlCl_3.5H_2O$, KH2PO4, and silica gel 60 F_{254} plate were purchased from Merck (Darmstadt, Germany), and distilled water.

Plant materials

The four types of fresh and ripe *C. cainito* fruits were obtained from Lumajang, Jember, and Banyuwangi Districts, East Java, Indonesia. The plants were determined in Indonesian Institute of Sciences at Purwodadi Botanical Garden, East Java, Indonesia by Deden Mudiana, S.Hut., M.Si. with voucher number of 0694/IPH.06/HM/IV/2015 for type 1, 0695/IPH.06/HM/IV/2015 for type 2, 0696/IPH.06/HM/IV/2015 for type 3, and 0697/IPH.06/HM/IV/2015 for type 4.

Extraction and fractionation process

All samples were steamed for 10 minutes and the pulp was manually separated from the seed. To obtain freeze dried materials, the pulp was ground, and freeze dried for 12 hours. Powder of freeze dried pulp were sonicated in 90% ethanol, 70% ethanol, and distilled water for 4 h at 30°C. The mixture was filtered and concentrated to obtain dried extract. The extract with the highest antioxidant capacity would be evaluated for α-glucosidase inhibition assay, TFC, TPC, and phytochemical screening. To obtained fractions, fresh pulp and freeze dried pulp were independently extracted with methanol. Both extracts were fractionated by solvent to solvent partitioning following method described by Luo et al. (2002) using n-hexane and ethyl acetate, sequently. Ethyl acetate fractions from extract of fresh pulp and freeze dried pulp were concentrated and determined in α-glucosidase inhibition assay, TFC, TPC, and phytochemical screening.

Antioxidant capacity measurement

DPPH radical scavenging activity was assessed according to method described by Sánchez-Moreno et al. (1998) with minor modifications. A total of 0.3 ml sample was added into 1.2 ml of DPPH solution (0.004% w/v diluted with ethanol), and mixed thoroughly. The mixture was incubated in the dark for 30 min before measuring the absorbance using UV-Vis spectrophotometer (Hitachi U-1800, Japan) at 515 nm against the blank. Antioxidant activity (AA) was calculated using following equation (1).

$$AA\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} x100\% \ldots. (1)$$

Antioxidant capacity could be expressed as the half maximal inhibitory concentration (IC_{50}) value calculated using linear regression analysis.

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was performed according to chromogenic method as previously described with slight modifications (Moradi-Afrapoli et al., 2012). Twenty microliters of 10 mM pNPG in phosphate buffer was used as substrate and the experiment was conducted at pH 6.8. Moreover, 20 µL of α-glucosidase (0.5 unit/mL) mixed with 120 µL of phosphate buffer was used as enzyme solution. Five miligrams of samples were dissolved in DMSO to obtain serial concentrations and added into enzyme solution in 96 wells microplate. The mixed solutions were incubated for 15 minutes at 37°C, followed by

addition of the substrate and incubation for 90 minutes at 37°C. To stop the reaction, 80 µL of 0.2 M sodium carbonate was added into the mixed solution. Measurement of absorbance was carried out using UV-Vis microplate reader (Dialab Elx800, Austria) at 418 nm wavelength. The blank was prepared using the same procedures without enzyme addition. Meanwhile, negative control was prepared by replacing sample with solvent. The percentage inhibition of α-glucosidase was determined based on equation (2).

$$Inhibition(\%) = \frac{A_n - A_s}{A_n} x100 \ldots\ldots. (2)$$

A_n is absorbance difference between the blank and negative control. A_s is absorbance difference between the blank and sample. Inhibition percentage of serial concentrations was used to calculate IC_{50} value by probit analysis.

Total flavonoid and phenolic content determination

Analysis of total flavonoid concentration was conducted based on method described by Ordonez et al. (2006) with minor modifications. Quercetin was used as a standard of calibration curve. Briefly, absorbance of the mixture of sample and $AlCl_3$ solution was measured using UV-Vis spectrophotometer at 420 nm, after incubation at 25°C for 30 min. TFC was expressed as mg of quercetin equivalents (QE) in 1 g of samples. Meanwhile, the amount of phenolic compounds was carried out spectrophotometrically using Folin-Ciocalteau (FC) reagent and gallic acid as standard based on method described previously with slight modifications (Wolfe et al., 2003). Concisely, after incubation at 25°C for 30 min, absorbance of the mixture of sample and FC reagent was measured using UV-Vis spectrophotometer at 765 nm. TPC was expressed as mg of gallic acid equivalents (GAE) in 1 g of sample.

Preliminary phytochemical screening

70% ethanolic extract, ethyl acetate fractions of fresh pulp, and freeze dried pulp were assesed for its phytochemical content, i.e. alkaloids, flavonoids, polyphenols, triterpenoids, and (or) steroids. The tests were carried out according to methods described by Harborne (1998) and Trease & Evans (1989). The presence of chemical constituents was observed according to precipitate formation or color changes because of specific reagents.

Tests for alkaloids

0.3 g of sample was treated with 5 ml of HCl 2 N, heated for 2-3 min, while stirring. 0.3 g of NaCl was added to the mixture, stirred, and filtered. Then, 5 ml of HCl 2 N was added to the filtrate. Mayer's test: Mayer's reagent was added to the mixture. The presence of alkaloids was confirmed by the yellowish white colored precipitate. Mayer's reagent was prepared by mixing 13.5 g of $HgCl_2$ in 20 mL of water and 49.8 g of KI in 20 mL of water. The mixture was diluted in water to 1L.

TLC test: The mixture was added NH_4OH 28%, extracted in 5 mL of chloroform, and filtered. The filtrate was dried and dissolved in methanol for screening on silica gel plates. Development was performed using solvent system of ethyl acetate:methanol:water (9:2:2 v/v/v). The plate was sprayed with Dragendorff reagent. Discoloration of TLC spot to orange indicated the presence of alkaloids.

Test for flavonoids

To 0.3 g of sample, n-hexane was added, and shaked until the color was pale. Ethanol was added to the residue, and filtered.

Shinoda test: The filtrate was treated with few drops of concentrated HCl and magnesium ribbon. The pink color showed the entity of flavonoids.

TLC test: The filtrate was screened on silica gel plates and developed on solvent system of buthanol:glacial acetic acid:water (4:1:5 v/v/v). The plate was sprayed using boric-citric acid reagent and the yellow spot showed the presence of flavonoids.

Test for polyphenols

10 mL of hot distilled water was added to 0.3 g of sample, stirred, filtered, and cooled.

Ferric chloride test: The filtrate was added a few drops of ferric chloride reagent. A blackish green coloration indicated polyphenols' presence.

TLC test: The filtrate was screened on silica gel plate. The eluting solvents were chloroform:ethyl acetate (1:9 v/v). Ferric chloride reagent was sprayed on the plate. The black coloration showed the presence of polyphenols.

Test for triterpenoids and (or) steroids

Liebermann-Burchard test: 0.3 g of sample was dissolved in 15 ml of ethanol. To 5 ml of solution, 3 drops of glacial acetic acid and 1 drop of concentrated H_2SO_4 were added, slowly shaken, and observed for the discoloration. A bluish green color showed the presence steroidal saponins, and a purplish red color indicated the presence of steroidal triterpenoids.

TLC test: 5 mL of 2 N HCl was added to 0.5 g of sample. The mixture was heated for 2 hours, cooled, neutralized using NH_4OH, and extracted in 3 mL of

n-hexane. It was concentrated by evaporation the solvents, and screened on silica gel plate using eluting solvents of n-hexane:ethyl acetate (4:1 v/v). The plate was sprayed with anisaldehyde-sulfuric acid reagent. A purplish red spot showed the presence of triterpenoids and (or) steroids.

Statistical analysis

The experiments were repeated at least three times. The result values were expressed as mean ± SD. The comparisons between means were determined using One-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) test. The data were considered significant different statistically, if p value less than 0.05. Several data of 70% ethanolic extract were also analyzed using simple linear regression to evaluate relationships of bioactivity, TFC, and TPC.

RESULTS AND DISCUSSION

Antioxidant capacity measurement

Free radical scavenging activity was carried out according to absorbance decline at 517 nm due to reduction of free radicals from DPPH which caused discoloration from purple to yellow. After interaction with DPPH, antioxidant compounds transfer an electron from a hydrogen atom to free radical of DPPH in order to neutralize the radical activity (Huang et al., 2005). As presented in Table 1, fruit extracts of all types showed significantly different DPPH scavenging activity (p<0.05). DPPH radical scavenging activity for extracts with the same solvent were in order of type 1 > type 4 > type 3 > type 2. Type 1 demonstrated higher antioxidant capacity, whereas type 2 revealed lower antioxidant capacity than the other types (p<0.05). This might indicate that there was possible effect of fruit type differences to free-radical scavenging activity. The result was in agreement with previous study of Ningsih et al. (2016) informing that type 1 had the highest DPPH radical scavenging activity.

Table 1. Antioxidant activity (IC_{50}) of *C. cainito* fruit extracts using different solvent (µg/mL)

Sample	Type 1	Type 2	Type 3	Type 4
96% ethanolic extract	43.490 ± 0.031^{a1}	50.610 ± 0.550^{a2}	48.749 ± 0.519^{a3}	45.806 ± 0.071^{a4}
70% ethanolic extract	24.469 ± 0.065^{b1}	39.440 ± 0.230^{b2}	27.426 ± 0.146^{b3}	26.950 ± 0.081^{b4}
Water extract	53.116 ± 0.953^{c1}	65.454 ± 1.303^{c2}	60.080 ± 1.0202^{c3}	57.137 ± 0.855^{c3}

Data are average of samples (mean) ± SD (n=3). The first superscript letter was used for comparison antioxidant activity of the same type with different extraction solvents in the same column. The second superscript letter was used for comparison antioxidant activity of extracts with the same extraction solvent in the same row. A significant differences was indicated by different superscript letters according to LSD test (p<0.05).

The study showed that 70% ethanolic extract had higher DPPH scavenging activity with IC_{50} value of 24.469 to 39.440 µg/mL than another extracts (p<0.05). According to Blois (1958), antioxidant activity of the extract was categorized as very powerful. In decreasing order of scavenging activity, this included: 70% ethanolic extract > 96% ethanolic extract > water extract. It indicated that combination of high polarity solvents (ethanol and water) was the more effective solvent in extraction of antioxidant compounds. The results agree with previous study reported that 70% ethanolic extract of four types of *C. cainito* leaves revealed the highest DPPH scavenging activity compared to another solvents (Ningsih et al., 2016). Aqueous ethanolic (70:30) extract of *Merremia boeneensis* showed stronger antioxidant activity through DPPH method than that of other extracts (Hossain & Shah, 2015). Different compounds extracted using different solvents having different solubility. This might cause different bioactivity among different extracts. Hence, 70% ethanolic extract was evaluated for its α-glucosidase inhibition capacity, TFC, and TPC.

α-glucosidase inhibition assay

Inhibitory effect against α-glucosidase of 70% ethanolic extract and ethyl acetate fractions is presented in Table 2. The *in vitro* α-glucosidase inhibition activity in descending order was: type 3 > type 4 > type 2 > type 1. Type 3 was the most potent inhibitor of α-glucosidase among all types (p<0.05). It was expected that type 3 contained antidiabetic compounds in higher level than the other types. Type difference in plants may cause difference in bioactivity which suggested caused by quantity difference of chemical contents (Poovitha & Parani, 2016, Rohaeti et al., 2017).

α-glucosidase inhibition activity of ethyl acetate fractions, notably fraction of freeze dried pulp was

stronger than 70% ethanolic extract. Several studies had proved that ethyl acetate fraction had higher activity against α-glucosidase than 70% ethanolic extract (Dewi & Maryani, 2015, Hyun et al., 2018). It suggested that solvent polarity affect α-glucosidase inhibitory activity, particularly quantity of active compounds dissolved into the solvent. This assay also indicated that ethyl acetate fraction from freeze dried pulp exhibited higher α-glucosidase inhibition than fresh pulp fraction. Difference preparation of both samples caused distinction in chemical contents. Freeze drying method produced more concentrated extract than sample without drying. Hence, fraction of ethyl acetate of freeze dried pulp had concentrated chemical contents and higher bioactivity.

Table 2. α-glucosidase inhibition activity of 70% ethanolic extract and ethyl acetate fractions

Sample	Type 1	Type 2	Type 3	Type 4
70% ethanolic extract	16.367 ± 0.006^{a1}	14.549 ± 0.015^{a2}	9.498 ± 0.224^{a3}	12.913 ± 0.038^{a4}
Ethyl acetate fraction of fresh pulp	3.767 ± 0.007^{b1}	2.865 ± 0.031^{b2}	1.998 ± 0.013^{b3}	2.384 ± 0.021^{b4}
Ethyl acetate fraction of freeze dried pulp	3.457 ± 0.059^{c1}	2.564 ± 0.024^{c2}	0.787 ± 0.018^{c3}	1.130 ± 0.019^{c4}

Data are average of samples (mean) ± SD (n=3). The first superscript letter was used for comparison α-glucosidase activity of different sample from the same type in the same column. The second superscript letter was used for comparison α-glucosidase activity of sample from different type in the same row. A significant differences was indicated by different superscript letters according to LSD test (p<0.05).

Total flavonoid and phenolic content determination

From Table 3 and 4, it was known that there was the same order for all samples. The quantity of TFC and TPC was ranked as follows: type 3 > type 4 > type 2 > type 1. Type 3 of ethyl acetate fraction of freeze dried pulp had the highest TFC and TPC value of 9.592 ± 0.038 mg QE/g extract and 840.869 ± 0.854 mg GAE/g extract, respectively (p<0.05). Whereas, type 1 contained the lowest flavonoid and phenolic contents.

Table 3. TFC of 70% ethanolic extract and ethyl acetate fractions (mg QE/g sample)

Sample	Type 1	Type 2	Type 3	Type 4
70% ethanolic extract	0.362 ± 0.002^{a1}	0.443 ± 0.005^{a2}	0.609 ± 0.003^{a3}	0.521 ± 0.001^{a4}
Ethyl acetate fraction of fresh pulp	1.333 ± 0.035^{b1}	3.794 ± 0.007^{b2}	5.953 ± 0.010^{b3}	4.493 ± 0.025^{b4}
Ethyl acetate fraction of freeze dried pulp	1.670 ± 0.056^{c1}	4.269 ± 0.043^{c2}	9.592 ± 0.038^{c3}	5.618 ± 0.126^{c4}

Data are average of samples (mean) ± SD (n=3). The first superscript letter was used for comparison TFC value of different sample from the same type in the same column. The second superscript letter was used for comparison TFC value of sample from different type in the same row. A significant differences was indicated by different superscript letters according to LSD test (p<0.05).

The same order of TFC and TPC value with α-glucosidase inhibitory capacity for all types indicated that chemical compound difference related to bioactivity difference. Extract of green tea significantly showed higher α-glucosidase inhibition than white tea extract and acarbose due to its cathechins content, mainly EGCG (p<0.05) (Yilmazer-Musa et al., 2012). In addition, it might suggest that ethyl acetate fraction of freeze dried pulp demonstrated the most potent inhibition activity against α-glucosidase because of the highest TFC and TPC. This experiment indicated that more soluble active constituents in the fraction than the other samples, such as semi polar compounds of flavonoid and phenolic compounds.

Table 4. TPC of 70% ethanol extracts and ethyl acetate fractions (mg GAE/g sample)

Sample	Type 1	Type 2	Type 3	Type 4
70% ethanolic extract	79.615 ± 0.031^{a1}	90.731 ± 0.174^{a2}	327.088 ± 0.101^{a3}	132.362 ± 0.094^{a4}
Ethyl acetate fraction of fresh pulp	250.418 ± 0.621^{b1}	381.452 ± 1.392^{b2}	790.716 ± 0.184^{b3}	551.026 ± 1.104^{b4}
Ethyl acetate fraction of freeze dried pulp	279.085 ± 0.910^{c13}	428.467 ± 0.484^{c14}	840.869 ± 0.854^{c2}	614.608 ± 0.639^{c34}

Data are average of samples (mean) ± SD (n=3). The first superscript letter was used for comparison TFC value of different sample from the same type in the same column. The second superscript letter was used for comparison TFC value of sample from different type in the same row. A significant differences was indicated by different superscript letters according to LSD test (p<0.05).

Preliminary phytochemical screening

Antioxidant and antidiabetic effect of 70% ethanolic extract and ethyl acetate fractions from *C. cainito* fruits could be caused by the chemical compositions. Preliminary phytochemical study performed using color reaction or precipitate formation test and TLC test was showed similar results (Table 5 and Table 6).

Table 5. Phytochemical analysis of 70% ethanol extracts and ethyl acetate fractions using various reagents

Samples	Type	Alkaloids	Flavonoids	Polyphenols	Triterpenoids and (or) steroids
70% ethanolic extract	1	-	++	++	+ (bluish green color)
	2	-	++	++	+ (bluish green color)
	3	-	++	++	+ (bluish green color)
	4	-	+	++	+ (bluish green color)
Ethyl acetate fraction of fresh pulp	1	+	++	++	+ (bluish green color)
	2	+	++	++	+ (bluish green color)
	3	+	++	++	+ (bluish green color)
	4	+	+	++	+ (bluish green color)
Ethyl acetate fraction of freeze dried pulp	1	+	++	++	+ (bluish green color)
	2	+	++	++	+ (bluish green color)
	3	+	++	++	+ (bluish green color)
	4	+	+	++	+ (bluish green color)

++ = positive result; + = moderately positive result;
- = negative result. Result based on precipitate or color formation after addition specific reagents.

The study exhibited that 70% ethanolic extract from various *C. cainito* fruits contained flavonoids, polyphenols, triterpenoids, and steroids. Nevertheless, alkaloids were not found in the extracts. Besides containing higher quantity of flavonoid and phenolic compounds, ethyl acetate fractions also had alkaloids. These compounds were known possessing α-glucosidase inhibitory activity (Yin et al., 2014). Phenolic compounds, including flavonoid are multifunctional dietary components having capability as free radical savenging, antiinflammatory, and antidiabetic agent (Patel et al., 2012, Gökbulut et al., 2017). The glicemic response would decline as the result of inhibition digestive enzymes and protein digestion (Griffiths & Moseley, 1980). *C. cainito* fruit contained several antioxidant phenolic compounds, i.e. (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin, quercetin, quercitrin, isoquercitrin, myricitrin, and gallic acid (Luo et al., 2002). Eindbond et al. (2004) found an antioxidant anthocyanin from *C. cainito* fruits, namely cyanidin-3-O-β-glucopyranoside. Moreover, another compounds of *C. cainito* fruits were several phenolic acids such as chlorogenic, syringic, ferulic, benzoic, p-coumaric, vanilic, caffeic, and protocatechuic acids (Fujuki et al., 2014).

Table 6. Preliminary phytochemical screening of 70% ethanol extracts and ethyl acetate fractions using TLC

Samples	Type	Alkaloids	Flavonoids	Polyphenols	Triterpenoids and (or) steroids
70% ethanolic extract	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
Ethyl acetate fraction of fresh pulp	1	+	+	+	+
	2	+	+	++	+
	3	+	+	++	+
	4	+	+	++	+
Ethyl acetate fraction of freeze dried pulp	1	+	+	+	+
	2	+	+	++	+
	3	+	+	++	+
	4	+	+	++	+

++ = high color intensity (positive result); + = low color intensity (positive result); - = completely absent (negative result). Result based on color formation on spots after spraying specific reagents.

Correlation analysis

Analyzing the correlation coefficient (R) among DPPH scavenging activity, α-glucosidase inhibition capacity, TFC, and TPC from 70% ethanolic extracts was carried out using simple linear regression. According to the result in Table 7, it was known that DPPH scavenging activity, TFC, and TPC for type 1, 2, and 4 of *C. cainito* fruit extract had good and moderate correlation. It was assumed that flavonoid and phenolic compounds were major contributors to antioxidant capacity. Nevertheless, there was weak and moderate correlation among DPPH scavenging activity, TFC, and TPC for types 3 of *C. cainito* fruit extract. This might be caused by morphological differences compared to another types. Type 3 of *C. cainito* fruit had a green color, medium size, and oval shape. Whereas, the other types were green or red, small or big with round shape. The morphological differences could cause phytochemical content and activity differences. Hachani et al. (2018) reported that characteristics differences of date fruits resulted in TPC, TFC, condensed tannin content, and *in vitro* antioxidant activities differences.

Table 7. Correlation analysis of several parameters determined from 70% ethanolic extracts of *C. cainito* fruits

Type of *C. cainito* fruit	Parameters comparison	Correlation coefficient (R)
Type 1 (n=3)	DPPH scavenging activity vs TFC	0.8496
	DPPH scavenging activity vs TPC	0.9575
	α-glucosidase inhibition activity vs TFC	0.6056
	α-glucosidase inhibition activity vs TPC	0.3780
	DPPH scavenging activity vs α-glucosidase inhibition activity	0.0949
Type 2 (n=3)	DPPH scavenging activity vs TFC	0.9437
	DPPH scavenging activity vs TPC	0.7741
	α-glucosidase inhibition activity vs TFC	0.6372
	α-glucosidase inhibition activity vs TPC	0.9898
	DPPH scavenging activity vs α-glucosidase inhibition activity	0.8563
Type 3 (n=3)	DPPH scavenging activity vs TFC	0.2917
	DPPH scavenging activity vs TPC	0.7255
	α-glucosidase inhibition activity vs TFC	0.6075
	α-glucosidase inhibition activity vs TPC	0.4393
	DPPH scavenging activity vs α-glucosidase inhibition activity	0.9370
Type 4 (n=3)	DPPH scavenging activity vs TFC	0.9226
	DPPH scavenging activity vs TPC	0.8946
	α-glucosidase inhibition activity vs TFC	0.3734
	α-glucosidase inhibition activity vs TPC	0.4350
	DPPH scavenging activity vs α-glucosidase inhibition activity	0.0141

Furthermore, there were moderate and weak correlation among α-glucosidase inhibitory activity, TFC, and TPC for all types of *C. cainito* fruit extract. It was suggested that inhibition activity against α-glucosidase of 70% ethanolic extract could be due to the presence of another compounds, such as triterpenoids and (or) steroids. Moreover, polar solvent like 70% ethanol could dissolve some polar compounds, mainly glycosides flavonoid which exhibited weaker α-glucosidase inhibition activity than its aglycones (Dewi & Maryani, 2015). Therefore, there was presumption that flavonoid and phenolic compounds gave less contribution to α-glucosidase inhibition activity of 70% ethanolic extract.

DPPH scavenging activity and α-glucosidase inhibition activity of type 3 showed a strong correlation (R = 0.9370). It was indicated that there was antioxidant capacity contribution to α-glucosidase inhibition activity of the extract (Vinholes & Vizotto, 2017). Meanwhile, moderate and weak correlation between DPPH scavenging activity and α-glucosidase inhibition activity of the other types might be caused by another mechanisms which also supported antidiabetic capacity of 70% ethanolic extract from *C. cainito* fruits, besides its antioxidant capacity.

CONCLUSION

70% ethanolic extract of *C. cainito* fruits, mainly type 1 showed the highest DPPH scavenging activity. Type 3 had greater inhibition effect of α-glucosidase, TFC and TPC value than the other types. Ethyl acetate fraction of freeze dried pulp, particularly type 3 exhibited higher α-glucosidase inhibitory capacity, TFC and TPC value than fraction of fresh pulp. Chemical compounds that expected contributing to the activity was alkaloids, flavonoids, polyphenols, triterpenoids, and (or) steroids. Thus, *C. cainito* fruit can be considered as a source of natural antioxidant and antidiabetic agent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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