J Herbmed Pharmacol. 2025; 14(1): 53-62.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2025.52551

Journal of Herbmed Pharmacology

Antioxidant and inhibitory activities of α-amylase, α-glucosidase, and G6Pase from *Smallanthus sonchifolius* tuber extract as a potential antidiabetic agent

Wahyu Widowati[1*], Rita Tjokropranoto[1], Roro Wahyudianingsih[1], Fen Tih[1], Penny Setyawati Martioso[1], Dani Dani[1], Vera Vera[1], Faradhina Salfa Nindya[2], RR. Annisaa Saraswati[1], Nadya Isabella Andretti[1], Nonny Oktaviany Putri[1], Fryda Cahya Saputra[3]

[1]Faculty of Medicine, Maranatha Christian University, Bandung 40164, Indonesia
[2]Aretha Medika Utama, Biomolecular and Biomedical Research Center, Bandung 40163, Indonesia
[3]Faculty of Technobiology, Universitas Katolik Indonesia Atma Jaya, South Jakarta 12930, Indonesia

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 24 April 2024
Accepted: 4 September 2024
epublished: 25 December 2024

Keywords:
Antidiabetic
Antioxidant
Diabetes mellitus
Smallanthus sonchifolius
Yacon tuber

ABSTRACT

Introduction: Diabetes mellitus (DM) is a chronic condition with free radicals and carbohydrate-hydrolyzing enzymes playing key roles in its progression. Yacon or *Smallanthus sonchifolius* (Poepp.) H.Rob, a low-sugar crop, has shown promising bioactivities. This research aimed to explore the antidiabetic and antioxidant potential of yacon tuber extract (YTE).

Methods: YTE's antioxidant and enzyme inhibitory activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) at concentrations of 6.25, 12.5, 25, 50, 100, 200 µg/mL, hydrogen peroxide (H_2O_2) scavenging activity at 12.5, 25, 50, 100, 200, 400 µg/mL, 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP) at 1.56, 3.13, 6.25, 12.5, 25, 50 µg/mL. The inhibition of α-amylase, α-glucosidase (6.25, 12.5, 25, 50, 100, 200 µg/mL), and glucose 6-phosphatase (G6Pase) (5.51, 11.03, 22.6, 44.12, 88.24, 176.47 µg/mL) was also assessed. Phytochemical analysis identified key bioactive compounds, and IC_{50} values were determined to quantify YTE's antioxidant and antidiabetic potentials.

Results: YTE contained flavonoids, terpenoids, triterpenoids, and phenolic compounds. It showed highest antioxidant activity ($P<0.05$) in DPPH, ABTS, H_2O_2, and FRAP assays at 200, 50, 400, and 50 µg/mL, respectively, compared to the other concentrations. In DPPH, H_2O_2, and ABTS assays, the IC_{50} values were 105.77 µg/mL, 726.64 µg/mL, and 61.03 µg/mL, respectively. FRAP activity was 338.68 µM/µg Fe (II) at 50 µg/mL. YTE also inhibited α-amylase, α-glucosidase, and G6Pase with IC_{50} values of 174.95 µg/mL, 222.17 µg/mL, and 112.51 µg/mL, respectively.

Conclusion: YTE exhibited significant antioxidant properties and inhibited carbohydrate-hydrolyzing enzymes, indicating its potential as an antidiabetic agent.

Implication for health policy/practice/research/medical education:
This study reported the potential for the anti-diabetic activity of yacon (*Smallanthus sonchifolius*) tuber extract through inhibiting corresponding enzymes. These results might serve as the foundation for further research and development of anti-diabetic medications.

Please cite this paper as: Widowati W, Tjokropranoto R, Wahyudianingsih R, Tih F, Martioso PS, Dani D, et al. Antioxidant and inhibitory activities of α-amylase, α-glucosidase, and G6Pase from *Smallanthus sonchifolius* tuber extract as a potential antidiabetic agent. J Herbmed Pharmacol. 2025;14(1):53-62. doi: 10.34172/jhp.2025.52551.

Introduction

Diabetes mellitus (DM) is a metabolic condition determined by hyperglycemic symptoms arising from secretory disorders (1,2). Diabetes is usually followed by a rise in oxidative stress, which can affect complications (3). In patients with hyperglycemia, increased free radical levels are responsible for oxidative stress (4). Reactive oxygen species (ROS) may contribute to type 2 diabetes mellitus (T2DM) by attacking healthy cells and destroying their functional integrity (5).

***Corresponding author**: Wahyu Widowati, Email: wahyu_w60@yahoo.com, wahyu.widowati@maranatha.edu

Carbohydrate-hydrolyzing enzymes, like α-glucosidase, glucose-6-phosphatase (G6Pase), and α-amylase aid in starch degradation, as well as the glycogenesis and gluconeogenesis pathways. In starch degradation, α-amylase and α-glucosidase enzymes catalyse the hydrolysis of 1,4-α-glycosidic carbohydrate bonds and release α-glucose. Moreover, a prior study reported that elevated activity of G6Pase leads to an accumulation of endogenous glucose production in type-2 diabetes subjects. The escalated activity of G6Pase initiates a rise in blood glucose concentration, enhancing long-term glycemic control (6). The activities of those enzymes can increase and alter blood glucose levels (5,7,8). Therefore, free radicals' scavengers and inhibition of carbohydrate-hydrolyzing enzymes are needed in DM treatment. In recent years, the capacity of enzymes to defend against free radicals has been improved by antioxidant compounds. These compounds may also minimize oxidative damage and prevent complications of diabetes from progressing. Many anti-diabetic drugs, like biguanides and sulfonylureas, are used for DM treatment. Despite that, these therapies have side effects (9,10).

Medicinal plants are expected to minimize the use of synthetic drugs, which have many side effects. Some medical plants like rambutan, rosella flowers, and white saffron have been used as alternative treatments for various diseases as they are suggested to be rich sources for controlling blood glucose. However, it is necessary to develop further research on the properties, mechanisms of action, and safety of using herbal extracts. In current years, considerable attention has been given to the quest for new compounds with hypoglycaemic and antioxidant properties from plant-based materials, which are supposed to act as antidiabetic agents (4,7,11).

Yacon or *Smallanthus sonchifolius* (Poepp.) H.Rob is a possible usable food origin. Yacon is included in the Asteraceae family and is a native plant to the Andes mountains. One of the benefits of the yacon plant is its antioxidant properties. Researchers are interested in yacon because of its properties and low sugar content (12). Several experiments on yacon have been conducted in search of new functional food, rich in chlorogenic, ferulic, and caffeic acids (13). Some previous research findings indicate that a notable number of phenolic components are found in yacon leaves and yacon tubers (14). Yacon tubers are also found to contain fructo-oligosaccharides (FOS), which are phenolic compounds low in sugar (bioactive compounds) (13). It has antioxidant and antidiabetic properties (15,16); however, its mechanism actions are not clear. Therefore, we evaluated the potential of yacon tuber extract's (YTE's) antidiabetic and antioxidant properties through inhibiting carbohydrate-hydrolyzing enzymes, namely α-glucosidase, α-amylase, and G6Pase, as well as antioxidant features by measuring free radical scavenging activities through 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), ferric reducing antioxidant power (FRAP), and 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays.

Materials and Methods

Yacon tuber was gathered from the grow site in Lembang, West Java, Indonesia, and authenticated by Mr. Djuandi a herbarium personnel from the Biology Department, School of Life Science and Technology, Bandung Institute of Technology as *Polymnia edulis* Wedd or *S sonchifolius* (Poepp.) H.Rob. A specimen was deposited there (0220419-A008). The extraction was processed using the maceration method. The tubers were dried, mashed, and extracted using 1500 mL ethanol 70% (Merck 480605). Filtering was repeated every day until the colorless filtrate was obtained. The filtrate was then evaporated to attain YTE. YTE was kept at -20 °C (8,16,17).

The altered Farnsworth procedure was used to classify steroids or triterpenoids, phenols, tannins, saponins, alkaloids, terpenoids, and other flavonoids as the basis for the phytochemical screening of YTE (5,8).

Phenolics identification

Approximately 0.01 g of sample filtrate was saved on the spotting plate, and 1% of $FeCl_3$ (Merck 103943) was subsequently added. The coloration in black/red/green/blue/purple showed the presence of phenolic materials (5,7).

Saponin identification

Approximately 0.01 g of the sample was inserted into a test tube with some water, subsequently simmered for around 5 minutes, and homogenized. If froth was on the surface, the solution was positive for saponins (5,8).

Steroid/triterpenoids identification

A sample of 0.01 g on the spotting plate was added with a drop of acetic acid and then soaked and closed for 10-15 minutes. A drop of sulfuric acid (H_2SO_4) (Merck 1007310510) was included in the sample. Sedimentation with a color of green or blue showed the presence of steroids, meanwhile, orange or red sedimentation marked the presence of triterpenoids (5,8).

Terpenoid identification

Approximately 0.01 g of sample, vanillin (Merck 108510), and H_2SO_4 (Merck 117048) were put into the spotting plate. The presence of terpenoids was shown by the formation of a purple mixture (5,8).

Tanin identification

A test tube containing 2 mL 2 N HCl (Merck 1003171000) and a 0.01 g sample was heated in a water bath for 30 minutes. After the mixture went through cooling and filtration steps, it was collected and added to n-amyl

alcohol (Merck 1009751000). The purple color specified a positive reaction with tannin (5,8).

Flavonoid identification

A sample of approximately 0.01 g Mg (Merck 1058151000) and HCl 2 N (Merck 1003171000) was inserted into the test tube. The solution was incubated (5 to 10 minutes), and then subjected to cooling and filtration. The filtrate was incorporated into amyl alcohol (Merck 1009751000). Red or orange color formation indicated the presence of flavonoid material (5,8).

Alkaloid identification

Approximately 0.01 g of the sample was added with 10% ammonia (Merck 109478) and then vaporized with chloroform (Merck 102431) in a water bath. The base layer was collected. Then, 3 drops of 1N HCl (Merck 109970) were added to the first tube after the formation of 2 layers of the solution. The second tube's residue was dissolved in 1N HCl and the residue was added 2 drops of Dragendorff (Merck 102035). The presence of alkaloids was confirmed if the color changed to yellow (5,8).

DPPH scavenging activity assay

Samples of 50 µL with different concentrations were injected into the wells on the 96-well microplate with 200 µL of DPPH solution (Sigma Aldrich D9132) (0.077 mmol/L in methanol). The samples were put into the incubation under dark conditions for 30 minutes at room temperature. The microplate reader was employed to read the absorbance at a wavelength of 517 nm. Methanol (250 µL) was used as a blank, and DPPH (250 µL) was employed for the negative control (8,17). The measurement of radical scavenging activity (%) was done by the following formula:

%Scavenging = (Ac – As) / Ac × 100
Ac = absorbance of the negative control (sample was not included).
As = absorbance of the sample

FRAP scavenging activity assay

Ten milliliters of supplemental acetic acid was combined with 10 mL of compatible acetate buffer (pH=3.6, 300 mM) to create the FRAP reagent (12). One milliliter of ferrous chloride hexahydrate (Merck 1.03943.0250) and 1 mL of 2,4,6-Tris (2-pyridyl) -s-triazine (TPTZ) (Sigma Aldrich 368235–7) were added to 1 mL of distilled water. With 142.5 mL of FRAP reagent, different sample rates of 7.5 µL (1000, 500, 250, 125, 62.5, 31.25 µg/mL) in the well were applied to 96 well plates. Incubation was done for 30 minutes at 37 °C. The value of the absorbance (Multiskan™ GO Spectrophotometer, Thermo Scientific) was calculated at 593 nm by the microplate reader. $FeSO_4$ was used for normal curve formation from the concentrations of 1.56-50 µg and $FeSO_4$ was used from the concentration of 1.56-50 µg/mL for the standard curve. The sample yield was expressed in Fe (II) µM/µg extract (8).

H_2O_2 scavenging activity assay

H_2O_2 scavenging activities were evaluated with this method. For each well on the 96-well plate, 3 µL 5mM H_2O_2 (Merck, 1.08597.1000), 60 µL sample, and 12 µL ferrous ammonium sulfate (1mM) (Merck, 1.03792.1000) were applied. Then, the mixture was incubated for 5 minutes at room temperature. The sample was incubated at room temperature for 10 minutes, after each well was filled with 75 µL of 1,10-phenanthroline (Merck, 1.07223.0010) (briefly). The microplate reader was utilized for the scavenging activity's absorbance measurement at a wavelength of 510 nm (5,8). The H_2O_2 scavenging activity (%) was quantified using the following formula:

%Scavenging = (Ac – As) / Ac × 100
Where Ac is the control absorbance and As is the sample absorbance.

ABTS scavenging activity assay

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+) (Sigma A1888-2) diammonium salt-free radical test was used to calculate ABTS activities. ABTS+ solution was formed when 4.9 mM potassium persulfate (volume ratio 1:1) and 14 mM ABTS were reacted with 5.5 mM of PBS (pH 7.4) for 12 to 16 hours at room temperature in a dark environment. The diluted solution was then measured at a wavelength of 745 nm until the absorption of the solution was 0.70 ± 0.02. Different 2 µL sample levels (50, 25, 12.5, 6.25, 3.125, 1.5625 µg/mL) were added to the 96-well microplate. After that, the samples were added to 198 µL of $ABTS^{\bullet+}$ and the plates were put into incubation for 6 minutes at 37 °C. The absorbance was calculated at 745 nm (8,15). To measure the ABTS scavenging operation, the following equation was used:

%ABTS Reducing Activity = (1-As/Ac) × 100
Where Ac is the control absorbance and As is the sample absorbance.

α-Amylase inhibitory activity assay

A modified method was employed for the α-amylase inhibitory activity assay test (8,11,17). Thirty microliters of dimethyl sulfoxide (DMSO), 30 µL sample, and 30 µL blank were added to the wells on a 96-well plate. Then, each well (except empty wells) was added with the α-amylase enzyme (A7595, Sigma Aldrich). At 37 °C, the mixture was put into incubation for 10 minutes. Forty microliters of starch solution was added to each well, and 40 µL of sodium phosphate buffer was added to the control well. The prepared samples were put into incubation at 37 °C for 15 minutes. Then, 100 µL acid iodine solution was

administered to each well and the measurement was done using a spectrophotometer at a wavelength of 565 nm. The α-amylase inhibitory activity (%) was calculated using the formula below, with C as absorbance without starch and S as sample absorbance.

%α-Amylase inhibitory Activity=(C-S) ×-100/C

α-Glucosidase inhibitory activity assay

Briefly 1.0 mg α-glucosidase from *Saccharomyces cerevisiae* (Sigma Aldrich, G5003) was mixed with 100 mL phosphate buffer (pH 7.0) containing 200 mg bovine serum albumin (BSA) (Sigma, A7906) for enzymatic preparation. The mixture was diluted using 1/50 phosphate buffer. Then, 25 µL of 20 mM p-α-D-glucopyranoside p-nitrophenyl, 45 µL of phosphate buffer, and 5 µL sample were mixed to produce 5 µL DMSO and 25 µL enzyme mixture (negative and positive controls). At 37 °C, the microplate was incubated for 30 minutes and then added with 100 µL of 2 M Na_2CO_3. Measurement was done using the microplate reader (Multiskan Go Reader) at 400 nm (5,8,11). The activity of α-glucosidase inhibition was calculated using this equation with C as absorbance without sample and S as sample absorbance.

% α-Glucosidase Inhibitory Activity = (C-S) ×-100/C

Glucose-6-phosphatase inhibitory activity assay

Glucose-6-phosphatase (G6Pase) inhibitory activity assay was measured by a modified Barginsky method. Around 10 µL glucose-6-phosphatase 0.09 U/mg enzyme (Sigma Aldrich K7410) was added to the control, sample, and blank wells. Then, 40 µL of buffer (Sigma Aldrich S7899) was administered to the control well, 10 µL into the sample well, and 30 µL into the blank well. An amount of 30 µL sample was added to the blank and sample wells. The well plate was set into incubation for 20 minutes at 37 °C. An amount of 20 µL substrate G6Pase 0.1 M was added to the control and sample wells, then incubated again for 15 minutes at 37 °C. Ammonium molybdate 10% (0.4 g ammonium molybdate in 2.45 M H_2SO_4) was added. The enzymatic reaction was stopped by a reducing agent (1% menthol in 3% sodium bisulfite) in each well. The absorbance was quantified with a spectrophotometer at 660 nm wavelength (8). The G6Pase inhibitory activity (%) was calculated using the following equation, with C as absorbance without sample and S as sample absorbance.

% G6Pase Inhibitory Activity = (C-S) ×-100/C

Statistical analysis

SPSS program (version 20.0) was used to conduct statistical analyses. The results of DPPH scavenger, H_2O_2 scavenger, ABTS reduction, and FRAP assays, as well as the α-amylase, α-glucosidase, and G6Pase inhibition, were analyzed using linear regression analysis. The linear regression equation that was attained in this step was employed for the Median inhibitory concentration 50 (IC_{50}) calculation.

Results

Phytochemical screening in yacon tuber extract

The phytochemical screening of YTE detected flavonoids, phenols, triterpenoids, alkaloids, and terpenoids (+), while saponins, tannins, and steroids were undetected (-) (Table 1).

Antioxidant activity of yacon tuber extract

In this research, the antioxidant activities of YTE were quantified using DPPH, FRAP, H_2O_2, and ABTS assays. The results revealed that the scavenging activities of YTE were concentration-dependent or proportional to its concentrations (Figure 1). The results for YTE inhibition activities (IC_{50}) in the antioxidant activity test are shown in Table 2. The inhibitory activity (IC_{50}) of DPPH radical scavenging in YTE was 105.77 µg/mL (Figure 1A). Meanwhile, the FRAP test showed that the most effective YTE concentration for FRAP-reducing activity was 50 µg/mL with the scavenging activity of 338.68 µM Fe (II) (Figure 1B). The most active YTE concentration for H_2O_2 scavenging activity was 400 µg/mL, with scavenging activity of 28.43% (Figure 1C). Meanwhile, the H_2O_2 IC_{50} value was 726.64 µg/mL (Table 2). The highest ABTS scavenging activity from 50 µg/mL YTE was 42.29% (Figure 1D). The inhibitory activity (IC_{50}) of ABTS radical scavenging activity is shown in Table 2 with the IC_{50} value of 61.03 µg/mL for YTE.

Antidiabetic activity assay

The antidiabetic activity of YTE was investigated by testing the inhibitory activity of α-amylase, G6-Pase, and

Table 1. The result of qualitative phytochemical screening of *Smallanthus sonchifolius* extract

Number	Phytochemical test	Results
1	Flavonoid	+
2	Saponins	-
3	Terpenoid	+
4	Triterpenoids/Strenoids	+/-
5	Tannins	-
6	Phenols	+

+: Positive results; -: Negative results.

Table 2. The antioxidant activity (IC_{50}) values of *Smallanthus sonchifolius* extract

Assay	Linear equation	IC_{50} Value (µg/mL)
DPPH	Y =0.2631X + 22.173	105.77
H_2O_2	Y = 0.067X + 1.3154	726.64
ABTS	Y =0.7509X + 4.1718	61.03

DPPH: 2,2-diphenyl-1-picrylhydrazyl; H_2O_2: hydrogen peroxide; ABTS: 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

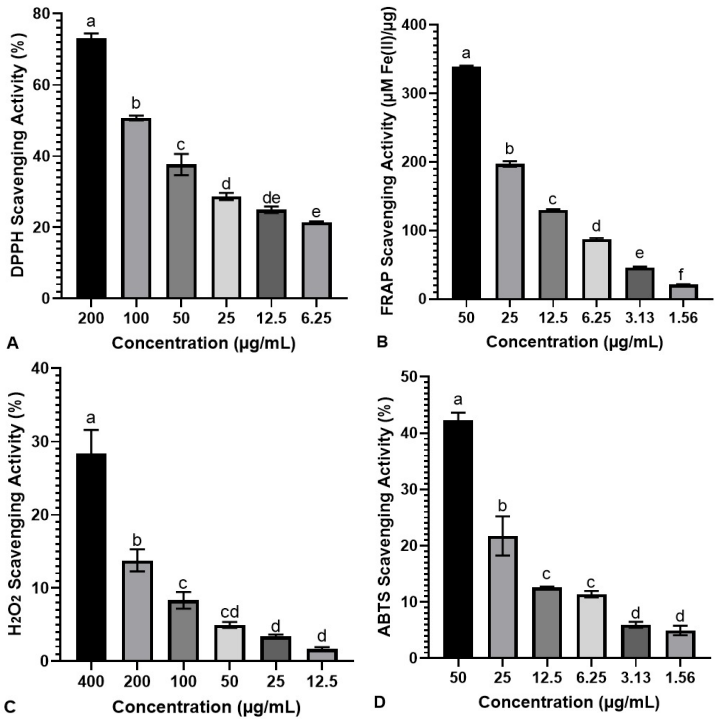

Figure 1. Effects of different *Smallanthus sonchifolius* extract concentrations on antioxidant activities. Data are presented as mean ± standard deviation. In each graph, distinct letters demonstrate significant differences among groups as determined by the Tukey HSD post hoc test ($P < 0.05$). A: DPPH (2,2-diphenyl-1-picrylhydrazyl), B: FRAP (ferric reducing antioxidant power), C: H_2O_2 (hydrogen peroxide), and D: ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity methods.

α-glucosidase (Figure 2). From the results of this study, it was found that the highest α-amylase inhibitory activity was achieved with 200 µg/mL YTE, with a result value of 52.7% (Figure 2A). The inhibitory activity (IC_{50}) on the α-amylase inhibitory activity of YTE was 174.95 µg/mL (Table 3). Meanwhile, the most notable α-glucosidase inhibitory activity was achieved with 200 ug/mL YTE, with a result of 46.84%. The IC_{50} of the α-glucosidase inhibitory activity of YTE was 222.17 µg/mL (Table 3). Moreover, the most significant G6-Pase inhibition activity was achieved from 176.47 µg/mL YTE (Figure 2C). The IC_{50} of α-glucosidase inhibitory activity is shown in Table 3. In this study, the obtained G6-Pase IC_{50} value of YTE was 112.51 µg/mL.

Discussion

Based on the study, YTE contained flavonoids. This output is consistent with previous studies that evaluated total flavonoid content in meat, skin, and all yacon tubers and cultivars (13). The total phenolic spectrum of different genotypes (New Zealand, Ecuador, Bolivia, and Germany) was 34.94-68.49 mg/g (18). Important phenolic acids are found in the tuber, which have the potential for antioxidant, anti-diabetes, and anti-cancer activities. In previous studies, fresh yacon tubers contained phenolic compounds, chlorogenic, and ferulic acid at 46.38, 21.22, and 7.28 mg/kg, respectively. These studies have also shown that the tubers contained caffeic acid. In comparison to the analysis performed by Pereira et al, ferulic acid and chlorogenic acid concentrations in yacon tubers were found to be very high (2.9 mg/kg and 39.1 mg/kg, respectively), with only 27.0 mg/kg for caffeic acid (19). Differences between yacon tubers' phenolic acid concentrations can be caused by variances between soil type, genotype diversity, climatic conditions, fertilizers, post-harvest time, and agronomic properties (19). Hydroxyl groups in the phenolic compounds contribute to their antioxidant activity and scavenging capacity. Phenolics can also suppress α-amylase, which will lower the amount of blood glucose (15). Thus, plant extracts with phenolic and flavonoid compounds have many beneficial effects on health such as antioxidant activities. Many bioactive compounds have different hypoglycemic effects as they can come from various plants to inhibit α-amylase (mostly phenolic, triterpenoids, and flavonoids) and also as antidiabetic agents (15).

Alkaloids can decline blood glucose levels by hindering the action of α-glucosidase in the duodenal mucosa. The

Table 3. The enzyme inhibitory (IC_{50}) value of *Smallanthus sonchifolius* extract

Assay	Linear equation	IC_{50} value (µg/mL)
α-glucosidase	y = 0.0868x + 34.814	174.95
α-amylase	y = 0.1822x + 9.5207	222.17
G6-Pase	y = 0.3566x + 9.8789	112.51

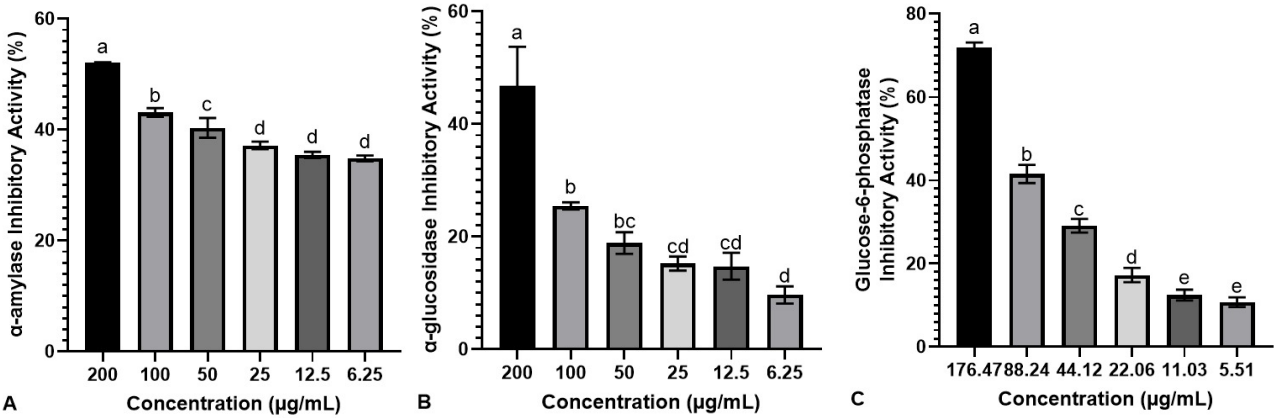

Figure 2. Effects of different *Smallanthus sonchifolius* extract concentrations on diabetic activities. A: α-amylase, B: α-glucosidase, and C: glucose 6-phosphatase inhibitory activity assays. Data are presented as mean ± standard deviation. In each graph, distinct letters demonstrate significant differences among groups as determined by the Tukey HSD post hoc test ($P < 0.05$).

release of glucose is slowed and its absorption rate into the blood is reduced, preventing peak blood sugar levels (18). Terpenoids can lower blood glucose levels by inducing insulin release and by inducing glucose transporter protein type-4 (GLUT-4) in cells for glucose absorption (20). Meanwhile, flavonoids reduce blood glucose levels by triggering insulin secretion and restoring insulin receptor sensitivity in cells (20). Previous research findings showed that yacon tuber contains derivatives such as coumaric acid, ferulic acid, caffeic acid, and chlorogenic acid (13). This is consistent with previous reports suggesting plant foods containing phenolic compounds might be used as antibacterial, antidiabetic, anti-carcinogenic, and anti-lipidemic agents (21).

In the pathogenesis of DM, several studies have shown the antioxidant and free radical scavenging activities of phenolic compounds. Polyphenols and FOS are found in yacon tubers. These substances affect blood sugar by reducing its levels. As studies have stated, oxidative stress also contributes to blood sugar levels. In yacon tuber, the antioxidant activities reduce oxidative stress, resulting in a decrease in blood sugar levels (1).

DPPH is a stable free radical reagent employed to investigate the antioxidant activity of plant extracts and fractions (22). Antioxidants usually destroy free radicals in DPPH through hydrogen donation (13). The reduction in the conjugated double bonds in DPPH causes the colour to change from violet to yellow (22). The change is measured using a spectrophotometer. According to Figure 1A, the scavenging activity of DPPH radicals was dependent on concentration. As shown by another study, flavonoids can scavenge radicals (23). Antioxidant activity is also calculated by the percentage of inhibition or the amount of antioxidant activity that can capture DPPH free radicals. Higher antioxidant activity is distinct by a lower IC_{50} value. The IC_{50} values are categorized into several groups. $IC_{50} < 50$ µg/mL falls into the "very strong" antioxidant category, IC_{50} at values in the array of 50-100 µg/mL fall into the "strong" category, IC_{50} at values in the range of 101-250 µg/mL fall into the "moderate" category, and IC_{50} values in the array of 250-500 µg/mL fall into the "weak" category (24). In this study, the IC_{50} value (105.77 µg/mL) of YTE is categorized as a moderate antioxidant with an IC_{50} value of 101-250 µg/mL.

Based on a previous study, the DPPH result of yacon's ethanolic extract was revealed to be 466.92 µg/mL (25). Moreover, studies regarding *S. sonchifolius* with numerous solvents such as n-hexane, chloroform, chloroform/methanol, and methanol reported IC_{50} values of DPPH scavenging activity within the range of 2.08 and 4.39 µg/mL (15). In the tuber of yacon, chlorogenic acid is the main antioxidant compound. Chlorogenic acid and ferulic acid in yacon tubers are polyphenols, highly related to DPPH radical scavenging (26). Yacon tuber contains polyphenols (2030 mg/kg) with chlorogenic acid predominating at 48.5 ± 12.9 mg/kg (27).

The FRAP procedure is hinged on the decline of the ferroin analog, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to the strongly blue coloured Fe^{2+} complex $Fe(TPTZ)_2^{+}$ by antioxidants reduction in an acidic medium. In this method, the antioxidant strength is measured based on the absorbance at 593 nm (28). The FRAP test results are equivalent to the iron ion concentration (mM) which can form a complex with the caffeic acid in yacon tubers. This study found that FRAP activity was proportional to the concentration (28). The highest YTE concentration for FRAP-reducing activity was 50 ug/mL, equivalent to 338.68 µM Fe(II). A prior study reported that a yacon's dried extract demonstrated FRAP activity within 31.55 and 66.80 mg TE/g (29).

Another factor that can induce oxidative stress is the presence of H_2O_2. This compound is a highly reactive oxidizing agent in the presence of metal ions in the human body, leading to sequential oxidations. H_2O_2 can directly

inactivate some enzymes via oxidation of the essential thiol groups (-SH). H_2O_2 can respond to the presence of Cu^{2+} and Fe^{2+} ions and form hydroxyl radicals (OH-) while passing through the cell membrane. The scavenging of H_2O_2 by phenolic compounds is an important antioxidant defense mechanism. This is caused by their electron-donating ability (30).

Phenolic compounds such as flavonoids can decrease the quantity of ROS and increase the breakdown of H_2O_2 into oxygen (O_2) and water (H_2O) by the catalase enzyme. Thus, damage to cells and their growth are prevented (31). Based on Table 2, the IC_{50} of YTE's H_2O_2 scavenging activities in this study is categorized as a frail activity (24). H_2O_2 scavenging activity studies are still limited; however, a previous study showed that yacon extract could significantly increase superoxide dismutase (SOD) antioxidant activity (32). Studies have shown that the extract of yacon with various solvents yielded SOD activity with IC_{50} values of 0.81-3.81 mg/mL (15). SOD is an enzymatic antioxidant that catalytically converts the superoxide anion (O_2^-) into H_2O_2 and produces OH^- free radicals (30). Studies have shown that yacon extracted with various solvents has SOD activity with IC_{50} values ranging from 0.81 to 3.81 mg/mL (15).

ABTS is a radical compound containing nitrogen atoms. YTE contains tryptophan, caffeic acid, and chlorogenic acid, which are highly sensitive or reactive to ABTS (26). An ABTS activity test will assess the relative capacity of an antioxidant to catch the resulting ABTS. The blue-green colour is an antioxidant activity marker; hence, ABTS will become colorless (33,34). The blue-green colour reduction is measured at 734 nm (34). This study found that the scavenging activity of ABTS was concentration-dependent. In this study, the IC_{50} value of YTE (61.03 µg/mL) can be classified as a powerful antioxidant (50-100 µg/mL) (24). This is supported by previous research that found numerous sections of yacon had ABTS values of 10.38-8,456.2 µmol/g TE (19).

The α-amylase enzyme catalyses the initial step of starch hydrolysis in the digestive system. Inhibiting this enzyme decreases glucose absorbance and can delay starch digestion (7,31). It also participates in lowering post-prandial blood glucose levels. The activity of α-amylase indicates that the inhibitory activity can increase with rising concentration. This inhibitory activity is affected by phenolic and flavonoid compounds (9). In this study, the IC_{50} value of YTE α-amylase inhibitory activity can be categorized as moderate activity (in the range between 101 and 250 µg/mL) (24). This finding is supported by a previous study that evaluated the α-amylase inhibitory activity of yacon that was put into extraction with various solvents (15).

Similar to α-amylase, the α-glucosidase enzyme catalyses starch hydrolysis and plays a role in the digestive system. This enzyme cleaves the glycosidic bonds and the hydrolysis substrate to produce yellow p-nitrophenyl and α-D-glucose. The α-glucosidase inhibitor is adequately elevated in samples (35). α-Glucosidase inhibitor can decrease postprandial plasma glucose levels and modulate post-prandial hyperglycaemia (PPHG) by decelerating the release of D-glucose from complex carbohydrates and detaining glucose absorption (36). This study found that YTE α-glucosidase inhibitory activity was proportional to concentration and might normalize blood glucose levels. In the current study, α-glucosidase inhibitory activity had an IC_{50} value that could be categorized as a moderate antioxidant (24). This inhibition was brought by smaditerpenic acid, which was reported to be similar to acarbose and capable of inhibiting α-glucosidase. Yacon extract with various solvents was stated to possess α-glucosidase inhibitory activity with IC_{50} of 1.00-6.50 mg/mL and concentrations of smallanthaditerpenic acids A (1.43 µM), smallanthaditerpenic acids B (1.76 µM), and smallanthaditerpenic acids C (1.86 µM) (11,37).

The G6Pase enzyme plays a role in regulating glucose homeostasis by hydrolyzing G6P. This hydrolysis produces glucose, which is released into the blood. Inhibiting this enzyme decreases glucose concentration in the blood of DM patients. The G6Pase inhibitory activity assay determines phosphate as the catalytic substrate of the enzyme. Phosphate binds with ammonium molybdate, which in turn binds to the reducing agent (38).

Free phosphates, such as blue phosphomolybdate, determine the level of enzyme activity. YTE glucose-6-phosphate inhibition had an IC_{50} value of YTE that could be categorized as a moderate activity with an IC_{50} value of 101-250 µg/mL (24). Previous studies have shown that supplementation with fermented yacon leaf tea water extract reduces G6Pase in DM model mice. DM mice had 80.58 nmol/min/mg protein, and the extracts decreased it to 70.39 when supplemented at a low dose and 56.79 nmol/minute/mg when supplemented at a high dose (39).

Yacon tubers contain chlorogenic acid that acts as a competitive inhibitor of glucose 6-phosphate, decreasing its activity (40). The results revealed that the polyphenols in YTE are closely correlated with DPPH, ABTS, H_2O_2, and FRAP free radical scavenging activities. These correlations highlight their potential antioxidant properties, which play a significant role in anti-diabetes mellitus effects (19,21). Based on these results and the study literature, we proposed a pathway for YTE as an antidiabetic agent (Figure 3).

YTE contains phytochemicals, including phenolics, flavonoids, terpenoids, and alkaloids. Those compounds showed antioxidant activities, including scavenging many oxidants (DPPH, ABTS, H_2O_2, and FRAP) and inhibiting many glucose-regulating enzymes, such as α-amylase, G6PAse, and α-glucosidase. Thus, YTE has the potential to be used as an antidiabetic remedy.

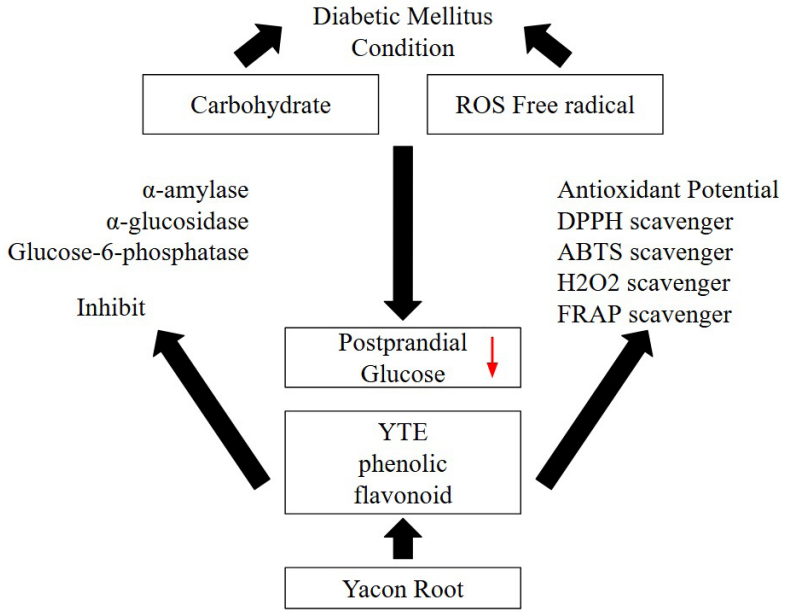

Figure 3. Proposed mechanism of *Smallanthus sonchifolius* extract (YTE) as an antidiabetic agent. ROS: Reactive oxygen species.

Conclusion

YTE was found to have a promising prospect of being used as an antioxidant and antidiabetic agent, probably due to its DPPH, H_2O_2, ABTS reducing, and FRAP activities, and carbohydrate-hydrolyzing enzymes (α-amylase, α-glucosidase, and G6Pase) inhibitory activities.

Acknowledgments

This research was supported by the Grants-in-Aid from Maranatha Christian University (Skema Hibah Internal Khusus). The authors are also thankful to Fadhilah Haifa Zahiroh, Annisa Firdaus Sutendi, Adilah Hafizha Nur Sabrina, Vini Ayuni, Nindia Salsabila Mia Dewi, and Dwi Nur Triharsiwi from Aretha Medika Utama, Bandung, Indonesia, for their treasured assistance.

Authors' contribution

Conceptualization: Wahyu Widowati, Rita Tjokropranoto, Roro Wahyudianingsih.
Data curation: Fen Tih, Penny Setyawati Martioso.
Formal analysis: Dani Dani, Vera Vera.
Funding acquisition: Wahyu Widowati.
Investigation: RR. Annisaa Saraswati, Nadya Isabella Andretti.
Methodology: Nonny Oktaviany Putri, Fryda Cahya Saputra.
Project administration: Rita Tjokropranoto.
Resources: Roro Wahyudianingsih.
Software: Dani Dani.
Supervision: Wahyu Widowati.
Validation: Dani Dani, Vera Vera.
Visualization: Faradhina Salfa Nindya.
Writing–original draft: RR. Annisaa Saraswati, Nadya Isabella Andretti, Nonny Oktaviany Putri, Fryda Cahya Saputra, Wahyu Widowati.
Writing–review & editing: Faradhina Salfa Nindya, Wahyu Widowati.

Conflict of interests

The authors declared no conflict of interest.

Ethical considerations

This study does not involve in vivo experiments, and therefore ethical approval is not applicable.

Funding/Support

This study was funded by Hibah Internal from Maranatha Christian University (243/LPPM/UKM/VII/2022).

References

1. de Andrade EF, de Souza Leone R, Ellendersen LN, Masson ML. Phenolic profile and antioxidant activity of extracts of leaves and flowers of yacon (*Smallanthus sonchifolius*). Ind Crops Prod. 2014;62:499-506. doi: 10.1016/j.indcrop.2014.09.025.
2. Baynes HW. Classification, pathophysiology, diagnosis and management of diabetes mellitus. J Diabetes Metab. 2015;6(5):1-9. doi: 10.4172/2155-6156.1000541.
3. Pasupuleti VR, Arigela CS, Gan SH, Salam SKN, Krishnan KT, Rahman NA, et al. A review on oxidative stress, diabetic complications, and the roles of honey polyphenols. Oxid Med Cell Longev. 2020;2020:8878172. doi: 10.1155/2020/8878172.
4. Dludla PV, Joubert E, Muller CJ, Louw J, Johnson R. Hyperglycemia-induced oxidative stress and heart disease-cardioprotective effects of rooibos flavonoids and phenylpyruvic acid-2-O-β-D-glucoside. Nutr Metab (Lond). 2017;14:45. doi: 10.1186/s12986-017-0200-8.

5. Pujimulyani D, Yulianto WA, Setyowati A, Arumwardana S, Amalia A, Kusuma HS. Amylase inhibition and free radical scavenging activities of white turmeric extract and fractions. J Teknol Ind Pangan. 2018;29(1):10-8. doi: 10.6066/jtip.2018.29.1.10.
6. Jawad AH, Ibrahim AE, Alsayed R, Hallab ZS, Al-Qaisi Z, Al-Amery A, et al. Study the impact of glucose-6-phosphatase activity in type 2 diabetic patients and non-diabetic counterparts. Preprints. October 31, 2016. Available from: https://www.preprints.org/manuscript/201610.0137.
7. Ferreira BM, Dagostin JL, de Andrade EF, Takashina TA, de Souza Neves Ellendersen L, Masson ML. Relationship between parameters of development and functional compounds of yacon leaves. Braz Arch Biol Technol. 2019;62(2):e19180439. doi:10.1590/1678-4324-2019180439.
8. Widowati W, Tjokropranoto R, Wahyudianingsih R, Tih F, Sadeli L, Kusuma HS, et al. Antidiabetic potential yacon (*Smallanthus sonchifolius* (Poepp.) H. Rob.) leaf extract via antioxidant activities, inhibition of α-glucosidase, α-amylase, G-6-Pase by in vitro assay. J Rep Pharm Sci. 2021;10(2):247-55. doi: 10.4103/jrptps.JRPTPS_3_21.
9. Rajendiran D, Packirisamy S, Gunasekaran K. A review on role of antioxidants in diabetes. Asian J Pharm Clin Res. 2018;11(2):48-53. doi: 10.22159/ajpcr.2018.v11i2.23241.
10. Zaman MS, Hassan PH, Islam SS, Ullah MA, Rabbani MG. Comparative efficacy of insulin, biguanides and sulfonylureas in the glycemic control of newly diagnosed diabetes mellitus patients in Rajshahi, Bangladesh. J Biosci. 2023;31(1):39-49. doi: 10.3329/jbs.v31i1.69533.
11. Gondokesumo ME, Kusuma HS, Widowati W. α-/β-glucosidase and α-amylase inhibitory activities of roselle (*Hibiscus sabdariffa* L.) ethanol extract. Mol Cell Biomed Sci. 2017;1(1):34-40. doi: 10.21705/mcbs.v1i1.3.
12. de Sousa RS, de Andrade AS, Masson ML. Extraction and characterization of nanofibrillated cellulose from yacon plant (*Smallanthus sonchifolius*) stems. Polímeros. 2021;31(2):e2021016. doi: 10.1590/0104-1428.09620.
13. Kolawole OT, Akinji MA. Effects of extract of leaves of *Newbouldia laevis* on the activities of some enzymes of hepatic glucose metabolism in diabetic rats. Afr J Biotechnol. 2014;13(22):2273-81. doi: 10.5897/ajb2014.13667.
14. Khajehei F, Merkt N, Claupein W, Graeff-Hoenninger S. Yacon (*Smallanthus sonchifolius* Poepp. & Endl.) as a novel source of health promoting compounds: antioxidant activity, phytochemicals and sugar content in flesh, peel, and whole tubers of seven cultivars. Molecules. 2018;23(2):278. doi: 10.3390/molecules23020278.
15. Sakamoto T, Imai H. Hydrogen peroxide produced by superoxide dismutase SOD-2 activates sperm in *Caenorhabditis elegans*. J Biol Chem. 2017;292(36):14804-13. doi: 10.1074/jbc.M117.788901.
16. Sundhani E, Syarifah DC, Zumrohani LR, Nurulita NA. The effect of adam hawa (*Rhoeo discolour*) and red pucuk (*Syzygium campanulatum* Korth.) leaves ethanolic extract to decrease blood glucose level on rats male strain Wistar induced with glucose. Pharmacy. 2016;13(2):137-49.
17. Pujimulyani D, Yulianto WA, Setyowati A, Arumwardana S, Rizal R. Antidiabetic and antioxidant potential of *Curcuma mangga* Val extract and fractions. Asian J Agric Biol. 2018;6(2):162-8.
18. Liana L, Rizal R, Widowati W, Fioni F, Akbar K, Fachrial E, et al. Antioxidant and anti-hyaluronidase activities of dragon fruit peel extract and kaempferol-3-o-rutinoside. Jurnal Kedokteran Brawijaya. 2019;30(4):247-52. doi: 10.21776/ub.jkb.2019.030.04.3.
19. Prahastuti S, Hidayat M, Hasiana ST, Widowati W, Widodo WS, Handayani RA, et al. The ethanol extract of the bastard cedar (*Guazuma ulmifolia* L.) as antioxidants. Pharmaciana. 2020;10(1):77-88. doi: 10.12928/pharmaciana.v10i1.13636.
20. Tonisi S, Okaiyeto K, Hoppe H, Mabinya LV, Nwodo UU, Okoh AI. Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. 3 Biotech. 2020;10(3):141. doi: 10.1007/s13205-020-2126-5.
21. Heleno SA, Martins A, Queiroz MJ, Ferreira IC. Bioactivity of phenolic acids: metabolites *versus* parent compounds: a review. Food Chem. 2015;173:501-13. doi: 10.1016/j.foodchem.2014.10.057.
22. Kumara P, Sunil K, Arun Kumar B. Determination of DPPH free radical scavenging activity by RP-HPLC, rapid sensitive method for the screening of berry fruit juice freeze dried extract. Nat Prod Chem Res. 2018;6(5):1-7.
23. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022;27(4):1326. doi: 10.3390/molecules27041326.
24. Kusumawati AH, Farhamzah F, Alkandahri MY, Sadino A, Agustina LS, Apriana SD. Antioxidant activity and sun protection factor of black glutinous rice (*Oryza sativa* var. *glutinosa*). Trop J Nat Prod Res. 2021;5(11):1958-61. doi: 10.26538/tjnpr/v5i11.11.
25. Myint T, Myint PP, Than NN. Biochemical studies and hepatoprotective potentiality of *Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson (yacon) leaves. J Myanmar Acad Arts Sci. 2021;19(1A):21-8.
26. Cao Y, Ma ZF, Zhang H, Jin Y, Zhang Y, Hayford F. Phytochemical properties and nutrigenomic implications of yacon as a potential source of prebiotic: current evidence and future directions. Foods. 2018;7(4):59. doi: 10.3390/foods7040059.
27. Pereira JA, Teixeira MC, Saczk AA, de Fátima Piccolo Barcelos M, de Oliveira MF, de Abreu WC. Total antioxidant activity of yacon tubers cultivated in Brazil. Ciênc Agrotec. 2016;40(5):596-605. doi: 10.1590/1413-70542016405009416.
28. Widowati W, Wargasetia TL, Afifah E, Mozef T, Kusuma HS, Nufus H, et al. Antioxidant and antidiabetic potential of *Curcuma longa* and its compounds. Asian J Agric Biol. 2018;6(2):149-61.
29. Russo D, Valentão P, Andrade PB, Fernandez EC, Milella L. Evaluation of antioxidant, antidiabetic and anticholinesterase activities of *Smallanthus sonchifolius* landraces and correlation with their phytochemical profiles. Int J Mol Sci. 2015;16(8):17696-718. doi: 10.3390/ijms160817696.
30. Sundaram V, Sadhasivam S, Chandrasekaran S, Nanjian R, Pandian A. *Strobilanthes heyneanus* root extract as a potential source for antioxidant and antimicrobial activity. Futur J Pharm Sci. 2021;7(1):91. doi: 10.1186/s43094-021-

00242-2.
31. Ueda Y, Apiphuwasukcharoen N, Tsutsumi S, Matsuda Y, Areekul V, Yasuda S. Optimization of hot-water extraction of dried yacon herbal tea leaves: enhanced antioxidant activities and total phenolic content by response surface methodology. Food Sci Technol Res. 2019;25(1):131-9. doi: 10.3136/fstr.25.131.
32. Oliveira PM, Coelho RP, Pilar BC, Golke AM, Güllich AA, Maurer P, et al. Antioxidative properties of 14-day supplementation with yacon leaf extract in a hypercholesterolemic rat model. Revista Brasileira de Biociências. 2017;15(4):178-86.
33. Sousa S, Pinto J, Rodrigues C, Gião M, Pereira C, Tavaria F, et al. Antioxidant properties of sterilized yacon (*Smallanthus sonchifolius*) tuber flour. Food Chem. 2015;188:504-9. doi: 10.1016/j.foodchem.2015.04.047.
34. Imrawati MS, Gani SA, Bubua KI. Uji aktivitas antioksidan fraksi etil asetat daun kersen (*Muntingia calabura* L.) menggunakan metode ABTS. J Pharm Med Sci. 2017;2(2):59-62.
35. Kashtoh H, Baek KH. Recent updates on phytoconstituent alpha-glucosidase inhibitors: an approach towards the treatment of type two diabetes. Plants (Basel). 2022;11(20):2722. doi: 10.3390/plants11202722.
36. Widowati W, Herlina T, Ratnawati H, Constantia G, Deva ID, Maesaroh M. Antioxidant potential of black, green and oolong tea methanol extracts. Biol Med Nat Prod Chem. 2015;4(2):35-9. doi: 10.14421/biomedich.2015.42.35-39.
37. Widowati W, Fauziah N, Herdiman H, Afni M, Afifah E, Kusuma HS, et al. Antioxidant and anti-aging assays of *Oryza sativa* extracts, vanillin and coumaric acid. Journal of Natural Remedies. 2016;16(3):88-99. doi: 10.18311/jnr/2016/7220.
38. Rajas F, Gautier-Stein A, Mithieux G. Glucose-6 phosphate, a central hub for liver carbohydrate metabolism. Metabolites. 2019;9(12):282. doi: 10.3390/metabo9120282.
39. Kim IS, Lee J, Lee JS, Shin DY, Kim MJ, Lee MK. Effect of fermented yacon (*Smallanthus sonchifolius*) leaves tea on blood glucose levels and glucose metabolism in high-fat diet and streptozotocin-induced type 2 diabetic mice. Korean J Nutr. 2010;43(4):333-41. doi: 10.4163/kjn.2010.43.4.333.
40. Kavimani S, Saminathan K, Senthil Kumar R. Antioxidant and free radical scavenging activities of *Dolichandrone atrovirens* using various in vitro assay models. Int J Phytopharmacol. 2014;5(4):293-300.