Original Article

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

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Abstract

Background: Diabetes is a chronic disease characterized by glucose levels and results in impaired insulin secretion. This disorder has triggered oxidative stress and excess free radicals condition. *Smallanthus sonchifolius* is a traditional medicine that acts as a diabetic therapy. This research aims to bring out the antidiabetic and antioxidant potential of *S. sonchifolius* extract (SSE). **Materials and Methods:** This study was conducted to measure the qualitative phytochemical identification, antioxidant and anti-diabetic activity of SSE. The antioxidant assay was carried out using 2,2-diphenyl-1-picrylhydrazine (DPPH)-scavenging activity, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-scavenging and hydrogen peroxide (H_2O_2)-reducing activity assays, ferric reducing antioxidant power (FRAP) potency, while anti-diabetic activity of SSE assay was carried out using inhibitory of α-amylase, α-glucosidase, and Glucose-6-Phosphatase (G-6-Pase). **Results:** SSE contained phenols, flavonoids, steroids/triterpenoids, saponins, tannins, and alkaloids. The antioxidant and antidiabetic activities of samples were calculated based on median inhibitory concentration (IC_{50}). The IC_{50} values of SSE antioxidant, respectively, were DPPH (IC_{50} = 62.72 µg/mL), ABTS (IC_{50} = 61.03 µg/mL), H_2O_2 (IC_{50} = 438.36 µg/mL), the highest FRAP activity was 125.31 µM Fe(II)/µg extract at a concentration level of SSE 50 µg/mL. The IC_{50} values of SSE antidiabetic were α-amylase inhibition (IC_{50} = 37.86 µg/mL), α-glucosidase inhibition (IC_{50} = 90.41 µg/mL), and G-6-Pase inhibition (IC_{50} = 98.07 µg/mL), respectively. **Conclusions:** SSE has antidiabetic potential through antioxidant activities and α-glucosidase, α-amylase, and G-6-Pase inhibition activities.

Keywords: *Antioxidant, diabetes, Smallanthus sonchifolius, α-amylase, α-glucosidase*

Introduction

Diabetes mellitus (DM) occured when glucose levels in the blood are high because the insulin produced is not sufficient for the body's needs. This can be divided into three groups. In people with type 1 diabetes, pancreatic β cells produce less insulin. The most common condition is type 2, about 90–95% of all diabetic cases. Fat, muscles, and liver cells that undergo insulin insensitivity is one symptom of this disease.[1] The ability of the pancreas to produce insulin in response to food intake that has gradually decreased. Apart from that, pregnancy insulin insufficiency also causes high glucose levels in the blood. This is known as gestational diabetes.[2]

Insulin resistance caused dysfunction in β cells. Because the primary function of β-cells is to store and secrete insulin in response to glucose load, this dysfunction triggers β-cells lose the ability to adequately sense blood glucose concentration or to release sufficient insulin in response. Hence, this dysfunction leads to a condition called hyperglycemia.[3] Hyperglycemia could increase Reactive Oxygen Species (ROS) resulting in oxidative stress. Oxidative stress will result in various oxidative damage in the form of DM complications and could worsen the condition of DM patient; therefore, it is necessary to normalize ROS levels to prevent oxidative stress.[4]

Inhibiting the enzymes that hydrolyze carbohydrates could lower glucose absorption, which could help people with DM. The enzymes—amylase and glucosidase—play a crucial role in breaking down oligosaccharides and disaccharides into monosaccharides.[5]

The α-glucosidase found in the mucosal bulk of the small intestine is a biocatalyst for the digestion of starch and disaccharides. The α-glucosidase works by inhibiting carbohydrates

How to cite this article: Widowati W, Tjokropranoto R, Wahyudianingsih R, Tih F, Sadeli L, Kusuma HSW, *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;10:247-55.

Received: 05 Jan 2021
Accepted: 18 Apr 2021
Published: 17 Dec 2021

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Access this article online

Website: www.jrpsjournal.com

DOI: 10.4103/jrptps.JRPTPS_3_21

Quick Response Code:

and reducing postprandial blood glucose excretion. With this mechanism, glycosidase plays an important role in polysaccharide metabolism, glycoprotein processing, cellular interactions, and expanding prospects for creating new diabetes treatment, viral infections, obesity to metastatic cancer. The α-glucosidase functions selectively in hydrolyzing the terminal (1→4 residues) of α-glucose (starch or disaccharide) to produce a single α-glucose molecule. Thus, the various types of α-glucosidase inhibitors have been extensively developed. The enzyme inhibitors such as voglibose miglitol, acarbose have the ability to reduce postprandial blood glucose by interfering with the carbohydrate-digesting enzymes and delaying glucose absorption.[5] Inhibition of α-glucosidase and α-amylase enzymes can significantly decrease the postprandial glucose level after consuming carbohydrate diet and, therefore, can be used as an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients.[6]

Meanwhile, glucose-6-phosphatase (G-6-Pase) is the central hepatic gluconeogenic enzyme found in glucose homeostasis and type 2 diabetic patients. This enzyme activity is higher in diabetic animals and humans. Therefore, this enzyme could be an important key player in elevated glucose production.[7]

Because synthetic drugs have unpredictable and more severe side effects, there is a need to improve safer, more effective anti-diabetes mellitus potential compounds.[8] It is known that there are some plants that are considered anti-diabetes. This is proved and is expected to be a promising opportunity in the future.[9] Various species of herbal drugs like cinnamon, ginger, *Aloe vera*, okra, and yacon have been described in scientific and in popular literature as having antidiabetic activity. However, further studies on effectiveness and protection are needed for particular conflicts in herbal extracts. Therefore, in this study, yacon leaf (*Smallanthus sonchifolius*) will be mainly observed. The main compounds responsible for targeting health benefits are phenolics, terpenoids, flavonoids, and coumarins. At times, the reported clinical behavior was also linked to a group of phytochemicals exhibiting synergy. Hence, the combination of phytochemicals contained in an herbal anti-diabetic is thought to be useful also for many metabolic pathways.[10] Searching for novel and new herbal medicines that have anti-diabetic and antioxidant properties is of considerable attention in recently decades.[5]

This study investigates the bioactive compounds in *Smallanthus sonchifolius* for DM therapy and potential mechanisms of antioxidant activity including free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazine (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (H_2O_2) and potential of ferric reducing antioxidant power (FRAP), anti-diabetic activities namely α-glucosidase, α-amylase, and G-6-Pase inhibitory activity assay.

Subjects and Methods

Preparation of *S. sonchifolius* extract

Yacon plant was collected from Cibodas, Lembang, Bandung, west Java, Indonesia, and that has been classified as *Smallanthus sonchifolius* (Poepp.) H.Rob. or *Polymnia edulis* Wedd. by herbarium staff Mr. Djuandi, Biology Department, School of Life Science and Technology, Bandung Institute of Technology, west Java, Indonesia.

The extraction was performed in the form of maceration.[11-14] Dried *S. sonchifolius* leaves were soaked in 70% distilled ethanol. Filtration was taken every 24 h and the process was performed until the colorless filtrate was found. The filtrate was then evaporated with a rotatory evaporator to obtain *S. sonchifolius* extract (SSE), and then was stored at -20°C.[11-14]

Phytochemical screening

The modified Fransworth method is the basis for the SSE test because it can recognize phytochemical compounds such as phenols, saponins, steroids/triterpenoids, tannins, flavonoids, and alkaloids.[11-14]

Phenols identification

SSE as much as 10 mg was loaded on a drop plate and mixed with 1% $FeCl_3$ (Merck 1.03861.0250). Good results for the phenols test is green, red, purple, blue, and black.[11-14]

Saponins identification

A little water was added to the test tube which already contains 10 mg of SSE and boiled for 5 min. The foamy surface after shaking showed saponins.[11-14]

Steroids/triterpenoids identification

A drop plate containing 10 mg of SSE was added with acetic acid until coated. Then mixed with 1 drop of sulfuric acid (H_2SO_4) (Merck 109073) after 10–15 min. Positive results for the triterpenoids countermeasures in red and orange but green and blue for steroids.[11-14]

Terpenoid identification

Initially SSE was added to the dropping plate. Then vanillin and H_2SO_4 was added. The presence of terpenoids was suggested on the mixture by the purple color.[11-14]

Tannins identification

In a test tube, SSE as much as 10 mg and 2N HCl (Merck 1003171000) as much as 2 mL were mixed. Then the mixture was put in a water bath and cooled for 30 min. Further filtration was carried out with amyl alcohol (Merck 10979). The purple color that formed was an indicator of the tannins present in sample.[11-14]

Flavonoids identification

About 10 mg SSE, Mg (Merck EM105815), and 2N HCl were added to the test tube. The test tube was heated for 5–10 min, then filtered after cooling with the addition of amyl alcohol. A positive reaction is determined by red or orange color.[11-14]

Alkaloids identification

The test tube had 10 mg of SSE added, accompanied by 10% ammonia. Two layers of liquid were formed after chloroform

was applied to the mixture, and precipitate was formed at the bottom layer. HCl 1N was applied to the solution and two layers were formed. A shift in yellow color suggested that the sample contains alkaloids.[11-14]

DPPH scavenging activity assay

In a 96-well microplate, 50 mL of SSE with various concentrations (6.25; 25.00; 50.00; 100.00; and 200.00 µg/mL) was added to each well. Then, 2,2-Diphenyl-1-picrylhydrazil (DPPH) (Sigma Aldrich D9132) (0.077 mmol/L in methanol) as much as 200 µL was inserted. The samples were incubated at room temperature for 30 min in the darkroom. The microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific) translates the absorbance value at a wavelength of 517 nm. The following formula describes the calculation of the radical scavenging:[4,11-15]

% Scavenging = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance.

FRAP activity assay

Preparation of the FRAP reagent was carried out by combining 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mM, Sigma Aldrich 368235–7) in HCl 40 mM and 300 mM acetate buffer (1 and 10 mL, respectively). Next, 7.5 µL of different SSE concentrations were combined with FRAP reagent as much as 142.5 µL in a 96-well microplate and then incubated at 37°C for 30 min. The absorbance value was determined at 593 nm. The standard curve was rendered using $FeSO_4$, ranging from 0.019 to 95 µg/mL $FeSO_4$. Sample findings were expressed in SSE of µM Fe(II)/µg.[4,11-14]

H_2O_2 scavenging activity assay

In each well, 60 µL of various concentration of SSE, ferrous ammonium sulfate 12 µL, 1 mM (Merck, 1.03792.1000), and H_2O_2 5 mM (3 µL) (Merck 1.08597.1000) were added. After that, the mixture was incubated in a darkroom for 5 min. The 1,10 Phenanthroline (Sigma Aldrich 131377) as much as 75 µL was put into the well. Then the mixture was incubated again for 10 min at room temperature. The absorbance was measured using a microplate reader at a wavelength of 510 nm.[13,14,16] The formula used to measure H_2O_2 scavenging activity is:

% Scavenging = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance.

ABTS scavenging activity assay

The antioxidant activity of SSE was measured with 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (Sigma Aldrich A1888). $ABTS^{\bullet+}$ was obtained by mixing 14 mM ABTS and 4.9 mM potassium persulfate (Merck EM105091) in a volume ratio of 1:1 for 16 h at room temperature in the dark condition. The mixture was dissolved with 5.5 mM Phosphate Buffer Saline (PBS) (pH 7.4) until the solution absorbance at a wavelength of 745 nm was 0.70 ± 0.02. In short, 2 µL of varying SSE concentration (1.56–50 µg/mL) were added to each well at 96-well microplate. Then 198 µL of $ABTS^{\bullet+}$ solution was added to the samples. The plates were incubated at 30°C for 6 min and then the absorption was measured at 745 nm. The percentage of ABTS radical resistance (percentage) was determined by the ratio of $ABTS^{\bullet+}$ between the decrease in absorbance in the presence of a sample compared with the absorbance without the sample.[4,11-14,17] The formula used to measure ABTS scavenging activity is:

% Scavenging = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance

α-amylase inhibitory activity assay

An updated procedure was used to conduct the α-amylase inhibitory activity assay. The SSE with various concentrations was put into the sample well, with dimethyl sulfoxide (DMSO) used as a blank. Furthermore, the α-amylase enzyme (Sigma Aldrich A7595) was added into each well, except for blank well. Then the mixture was incubated at 37°C for 10 min. After that a starch solution was added in each well, while control well was added with phosphate buffer. Another incubation for 15 min at 37°C was conducted. The addition of the acid iodine solution will stop the enzymatic reaction. The absorbance was observed at a wavelength of 565 nm.[12,18,19] Here is a formula that defines the percentage of α-amylase inhibition:

% Inhibition = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance.

α-glucosidase inhibitory activity assay

Various concentrations of SSE were mixed with 4-Nitrophenyl α-D-glucopyranoside (Sigma Aldrich N1377) and phosphate buffer (pH 7.0) in a 96-well microplate. After mixed well, α-glucosidase from *Saccharomyces cerevisiae* (Sigma Aldrich G5003-100UN) was added and then incubated at 37°C for 30 min. After that Na_2CO_3 (Merck 1.06392.0500) was added. The absorbance was measured at 400 nm wavelength.[12,18] Here is an equation for the percentage of α-glucosidase inhibition:

% Inhibition = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance.

G-6-Pase inhibitory activity assay

G-6-Pase from rabbit liver (Sigma Aldrich G5758-25UN) with the concentration of 0.09 U/mg was added as much as 10 µL. Then, 40-µL sodium acetate buffer solution (Sigma Aldrich S7899) was added to the solution. Lastly, 10 µL of various levels of SSE (5.51–176.47 µg/mL) was added to the solution .

The well plate was incubated for 20 mins at 37°C. Then, 20 µL glucose-6-phosphate disodium salt hydrate (Sigma Aldrich G7250) in sodium acetate buffer solution was added and incubated for 15 min at 37°C. Then, 1% ammonium molybdate tetrahydrate (Sigma Aldrich G8681) was added with 1% metol (Sigma Aldrich 69749) in 3% sodium bisulfite (Sigma Aldrich F2246). The blue color will appear positive and the absorbance is measured at 660 nm.[7,20] With this equation, the inhibitory activity of glucose-6-phosphatase was represented as a percentage:

% Inhibition = (Ac – As) / Ac × 100

Ac = negative control absorbance (without sample).

As = sample absorbance.

Table 1: The result of SSE qualitative phytochemical screening

Number	Phytochemical Test	Results (+/-)
1	Flavonoids	+
2	Saponins	+
3	Phenols	+
4	Tannins	+
5	Steroids/triterpenoids	+/+
6	Alkaloids	+

+ = detected, - = not detected

Results

Phytochemical screening of SSE

The screening result showed the presence of phenols, flavonoids, tannins, alkaloids, saponins, and terpenoids in SSE. The phytochemical screening results for SSE could be seen in Table 1. In Table 1, it is seen that the SSE phytochemical

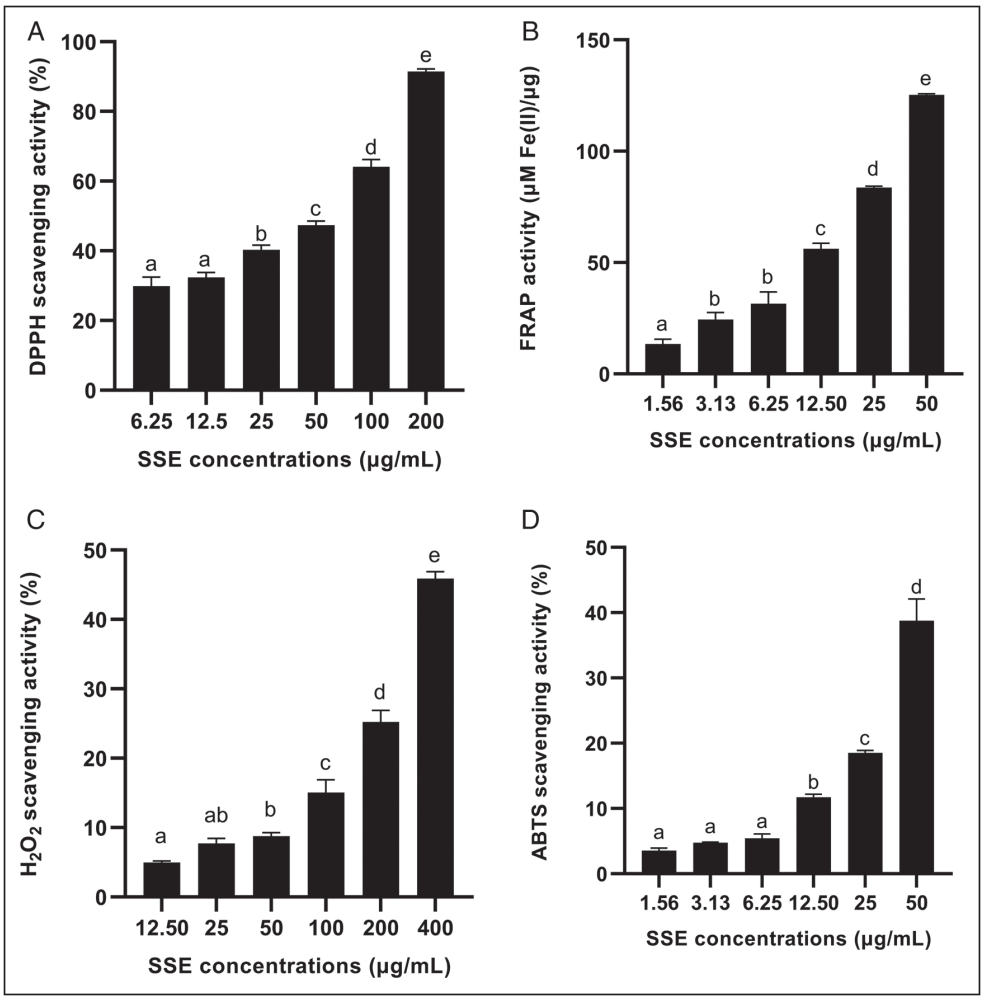

Figure 1: Effect variety concentrations of SSE toward antioxidant activities. (A) DPPH scavenging activity (%) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 6.25; 25.00; 50.00; 100.00; 200.00 (µg/mL). Different letter (a,b,c,d,e) shows significant differences among concentrations of DPPH scavenging activity based on Tukey's HSD *post-hoc* test ($P < 0.05$). (B) FRAP activity (µM Fe (II)/µg sample) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 1.56; 3.1.3; 6.25; 25.00; 50.00 (µg/mL). Different letter (a,b,c,d,e) shows significant differences among concentrations of FRAP activity based on Tukey's HSD *post-hoc* test ($P < 0.05$). (C) H_2O_2 scavenging activity (%) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 12.50; 25.00; 50.00; 100.00; 200.00; 400.00 (µg/mL). Different letter (a,ab,b,c,d,e) shows significantly differences among concentrations of H_2O_2 scavenging activity based on Tukey's HSD *post-hoc* test ($P < 0.05$). (D) ABTS scavenging activity (%) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 1.56; 3.13; 6.25; 25.00; 50.00 (µg/mL). Different letter (a,b,c,d) shows significant differences among concentrations of DPPH scavenging activity based on Tukey's HSD *post-hoc* test ($P < 0.05$)

assay of flavonoids, saponins, phenols, tannins, steroids, triterpenoids, and alkaloids shows positive results.

Antioxidant activity of SSE

Figure 1A shows the percentage of DPPH scavenging activity of SSE. There were decolorization and a decrease in absorbance value as a result of the free radical scavenger. In this research, SSE has a DPPH scavenging activity with IC_{50} = 62.72 µg/mL [Table 2]; it was categorized as an active antioxidant which has an IC_{50} value of 50–100 µg/mL.[15]

FRAP activity of SSE could be seen in Figure 1B. The outcome of this study showed concentration-dependent activity of FRAP, in which higher concentrations increased FRAP activity. The highest FRAP activity was 125.31 µM Fe(II)/µg extract at a concentration level of SSE 50 µg/mL.

Hydrogen peroxide acts as both an oxidizer and a direct function, usually by oxidizing the essential thiol group (-SH). From Figure 1C, it could be observed that H_2O_2 scavenging activity is influenced by SSE concentration. In Table 2, it could be seen that SSE has low H_2O_2 scavenging activity with IC_{50} = 438.36 µg/mL, it was categorized weak antioxidant activity with an IC_{50} value 200–500 µg/mL.[15]

The long-wave spectrum absorption method can calculate the reduction of blue-green radical ABTS solutions by hydrogen-donating antioxidants. Figure 1D shows the ABTS-reducing action of SSE as a result. As demonstrated in Table 2, SSE has an ABTS scavenging activity of 61.03 µg/mL, and it was categorized as an active antioxidant with an IC_{50} value of 50–100 µg/mL.[15]

Antidiabetic activity assay

The activity of α-amylase inhibition showed concentration-dependent activity, in which increased concentrations produced higher inhibitory activity [Figure 2A]. Inhibitory activity of α-amylase with IC_{50} = 37.86 µg/mL, it was the most active compared with α-glucosidase inhibition, G-6-Pase inhibition activities [Table 3]. SSE had highly active α-amylase inhibition with IC_{50} value <50 µg/mL.[15]

Table 2: IC_{50} value of DPPH, H_2O_2, ABTS radical scavenging of SSE

Assay	Highest activity of scavenging activity (%)	Linear equation	R^2	IC_{50} (µg/mL)
DPPH	91.43	y = 0.3136x + 30.33	0.99	62.72
H_2O_2	45.90	y = 0.1044x + 4.2364	0.99	438.36
ABTS	42.29	y = 0.7244x + 1.9042	0.99	61.03

The IC_{50} values, linear equation, R^2 are presented based on the average value of triplicate experiment. The IC_{50} value of each sample was calculated based on linear regression with R^2 value 0.99

As shown in Figure 2B, SSE had significant α-glucosidase inhibition activity differences at each concentration; higher concentration increased the α-glucosidase inhibition activity. Inhibition of α-glucosidase. In Table 3, SSE had α-glucosidase inhibition properties with an IC_{50} value of 90.41 µg/mL, it was categorized active α-glucosidase inhibition activity.[15]

G-6-Pase as a carbohydrate hydrolyzing enzyme is closely related to DM. The G-6-Pase inhibitory of SSE could be seen in Figure 2C, higher concentration increased the G-6-Pase inhibition activity. In Table 3, SSE also showed G-6-Pase inhibitory properties with IC_{50} 98.07 µg/mL, it was categorized active G-6-Pase inhibition activity.[15]

Discussion

Yacon leaves extract consists of various chemical compounds.[2] This result was in line with previous study that tuberous roots, leaves, and rhizome of *S. sonchifolius* from various genotypes (New Zealand, Ecuador, Bolivia, Germany) contained various total phenol 34.94–68.49 mg/g.[21] SSE also contained flavonoids, and this result data were validated with previous research that total flavonoid content in peel, flesh, whole yacon tubers has been significantly affected by cultivar and tuber part.[22] Butanol extract on yacon leaves showed the presence of three dicaffeoilquinic, caffeic, and chlorogenic acids.[2,23] Previous research also stated the presence of chlorogenic acid, gallic acid, ferulic acid, and caffeic as phenolic compounds from the hydroethanolic extract in yacon.[24] Five races of *S. sonchifolius* were tested, the ethanol extract and the decoction extract were proved to produce a higher number of flavonoids, like luteolin 7-O-glucoside and luteolin 3′,7O-diglucoside together with luteolin and apigenin.[2,25] Yacon extract with various solvent contained tannins 6.38–14.58 mg tannic acid equivalent/g (mg TAE/g).[26]

One of the reagents that could be used in the compound free-radical scavenging activity test was DPPH. The final outcome of the solution is yellow because SSE could reduce stable DPPH radicals to diphenylpicrylhydrazine (DPPH-H).[4,11,12] Based on Figure 1A, DPPH was a concentration-dependent operation in which higher concentrations increased DPPH scavenging activity. In the study, the DPPH scavenging activity of SSE was in the range of 50–100 µg/mL, indicating that it was classified as an active antioxidant.[15,27] This result was approved with a previous study that DPPH had radical scavenging activity in some parts of yacon including whole tuber, peel, flesh from a variety of cultivar.[22] This result data were also supported by previous research that yacon compounds, namely caffeic acid and chlorogenic acid had DPPH scavenging activity with IC_{50} value 0.86 and 2.56 µg/mL, respectively.[28] *S. sonchifolius* (yacon) landraces with *n*-hexane, chloroform, chloroform/methanol, and methanol resulted IC_{50} value of DPPH scavenging activity 2.08–4.39 µg/mL.[26]

FRAP method is based on the reduction by antioxidants in acidic media of a ferroin analog, the Fe_3^+ complex of tripyridyltriazine Fe $(TPTZ)_3^+$ to the deep blue color Fe_2^+

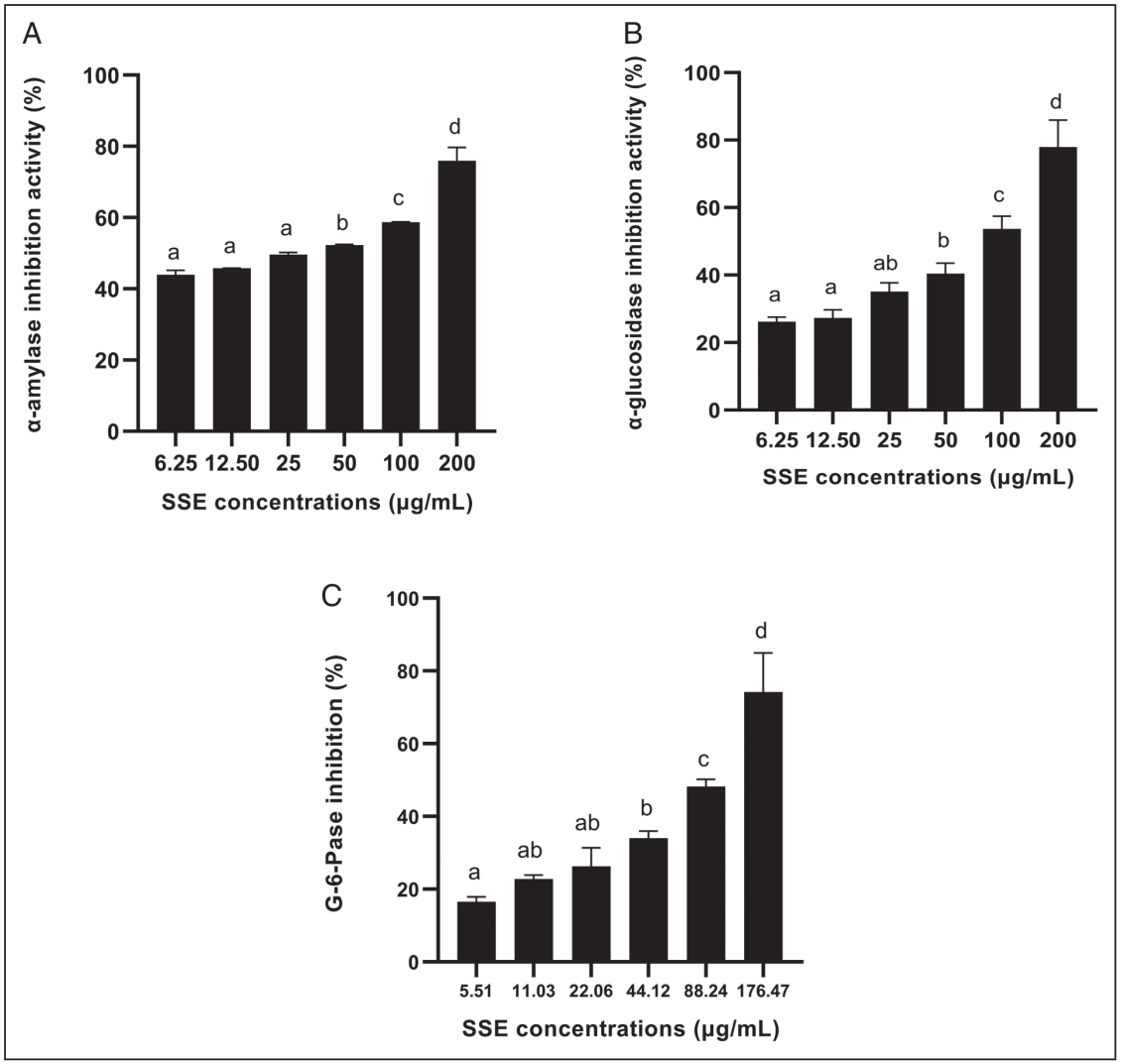

Figure 2: Effect variety concentrations of SSE toward antidiabetes mellitus activities. (A) α-amylase inhibition activity (%) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 6.25; 25.00; 50.00; 100.00; 200.00 (µg/mL). Different letter (a,b,c,d) shows significant differences among concentrations of α-amylase inhibition activity based on Tukey's HSD *post-hoc* test ($P < 0.05$). (B) α-amylase inhibition activity of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 6.25; 25.00; 50.00; 100.00; 200.00 (µg/mL). Different letter (a,b,c,d) shows significant differences among concentrations of α-amylase inhibition activity based on Tukey's HSD *post-hoc* test ($P < 0.05$). (C) G-6-Pase inhibition activity (%) of SSE. SSE were diluted in DMSO 1% to reach the final concentration of 5.51; 11.03; 22.06; 44.12; 88.24; 176.47 (µg/mL). Different letter (a,ab,b,c,d) shows significant differences among concentrations of G-6-Pase inhibition activity based on Tukey's HSD *post-hoc* test ($P < 0.05$)

Table 3: IC_{50} value of α-glucosidase, α-amylase, G-6-Pase inhibition of SSE

Assay	Highest activity of scavenging activity (%)	Linear equation	R^2	IC_{50} (µg/mL)
α-glucosidase	77.95	y = 0.2641x + 26.122	0.99	90.41
α-amylase	75.93	y = 0.1582x + 44.01	0.99	37.86
G-6-Pase	74.24	y = 0.3233x + 18.295	0.99	98.07

The IC_{50} values, linear equation, R^2 are presented based on the average value of triplicate experiment. The IC_{50} value of each sample were calculated based on linear regression with R^2 value 0.99

complex Fe $(TPTZ)_2^+$. Absorption of Fe(II) complex at 593 nm by antioxidant reduction of the corresponding tripyridyltriazine Fe(III) complex.[4,11,12,25] This data result was in line with previous research that hot-water extract of yacon herbal tea had FRAP activity of 21.8–46.1 µg TE/mL according time range and temperature range.[29] Yacon extract using various solvent for extraction had FRAP activity with range of 31.55–66.80 mg TE/g.[25]

H_2O_2 serves as an oxidizer, although H_2O_2 could protect the cell membranes, it could be toxic inside the membranes. This was thought to derive from the hydroxyl radicals (OH*) formed when H_2O_2 was found with Fe^{2+} or Cu^{2+}. The value H_2O_2 scavenging activity of SSE categorized as weak with IC_{50} value in the range of 250–500 µg/mL.[15,26,27] Based on the previous research, it has not been conducted yet for H_2O_2 scavenging activity. Previous study

stated that hot-water extract on yacon herbal tea exhibited have antioxidant activity namely superoxide dismutase activity (SOD) 512-1.400 µg TE/mL according to time range and temperature range.[28] Yacon extract with various solvent had SOD activity with IC_{50} value 0.81–3.81 mg/mL.[25] SOD is enzymatic antioxidant convert catalytically anion superoxide (O_2^{*-}) to H_2O_2 free radical which H_2O_2 will produce OH* free radical.[30]

ABTS-reducing behavior monitoring calculates the antioxidant relative capacity to scavenge the ABTS it created. Strong oxidizers such as the ABTS salt (potassium permanganate/potassium persulfate) when reacting could produce ABTS. Based on Figure 1D, the scavenging activity of ABTS was directly proportional to the concentration. When the concentration was high, the scavenging activity of ABTS increases. ABTS scavenging activity of SSE categorized as an active antioxidant with IC_{50} value 50–100 µg/mL.[15,27] This data was in line with the previous research that various parts of yacon (pulp flour of yacon, peel flour of yacon, yacon pulp, yacon peel) have ABTS value in range of 10.38-8.456.2 µmol TE/g.[31]

The α-glucosidase activity of SSE occurred in a concentration-dependent manner in which higher α-glucosidase inhibition present in a higher concentration of the sample. Inhibition of SSE to α-glucosidase was in the range of IC_{50} = 50–100 µg/mL, indicating that it was classified as an active antioxidant.[15,27] This inhibition was caused by smaditerpenic acid-type compounds that reported could inhibit α-glucosidase strongly and similar to acarbose.[32] Yacon extract using various solvent had α-glucosidase inhibition activity with IC_{50} in range of 1.00–6.50 mg/mL.[26] The IC_{50} activity of α-glucosidase smallanthaditerpenic acids A (1.43 µM), smallanthaditerpenic acids B (1.76 µM), smallanthaditerpenic acids C (1.86 µM), and smallanthaditerpenic acid D (1.86 µM).[33]

The α-amylase functions as a catalyst at the beginning of starch hydrolysis and was present in the digestive system. If the enzyme was inhibited, the breakdown of starch and oligosaccharides will be retained, so that postprandial blood glucose levels will decrease.[12,18] Inhibition activity of SSE toward α-amylase was categorized as highly active when the IC_{50} value was <50 µg/mL.[15,27] This result was supported with previous data that α-amylase inhibition activity of yacon extract using various solvent had IC_{50} < 1–<2 mg/mL.[25]

Hyperglycemia and hyperglucose could be overcome by inhibiting G-6-Pase as enzyme hydrolysis using a therapeutic approach. Inhibitory properties of SSE to G-6-Pase is categorized as active when the IC_{50} value was 50–100 µg/mL.[15,27] Supplementation of fermented yacon leaves tea water extract decrease G-6-Pase in the DM mice model. Low-dose yacon decreased G-6-Pase to 70.39 nmol/min/mg protein and high-dose yacon decreased G-6-Pase to 56.79 nmol/min/mg protein compared with G-6-Pase in DM mice 80.58 nmol/min/mg protein.[34]

Polyphenols are closely linked to radical scavenging activities free of DPPH, ABTS, H_2O_2, FRAP, which means that these substances have the potential to act as antioxidants and anti-diabetic mellitus. Leaves produce large quantities of phenolic compounds (ferrulic acids, chlorogenic acids, and caffeic acids), flavonoids, and sesquiterpene lactone (SLs), as a source of biofunctional compounds.[35] The following pathway could be proposed based on the potential of yacon leaves as an antidiabetic agent [Figure 3].

Conclusions

SSE has antidiabetic potential through antioxidant activities and α-glucosidase, α-amylase, and G-6-Pase inhibition activities.

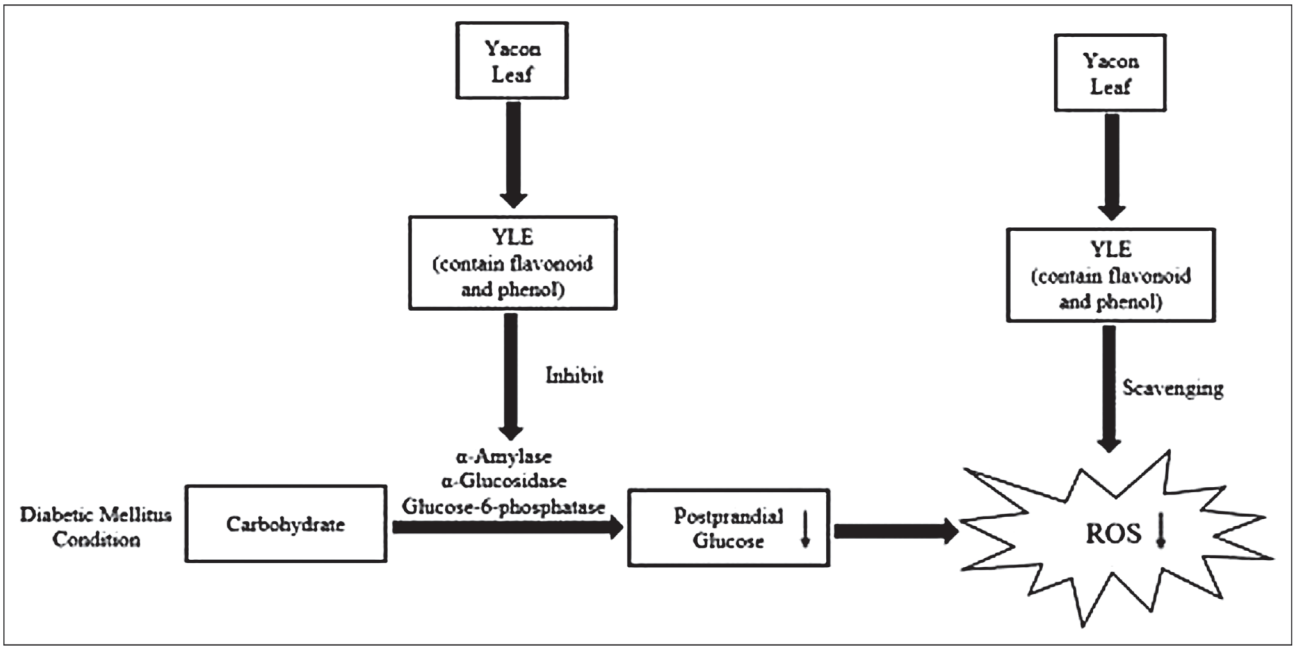

Figure 3: Proposed mechanism of yacon leaf extract as antidiabetic and antioxidant agents

It is important to continue this research on DM animal model for proving antioxidant and anti-diabetic activities of SSE.

Acknowledgements

This study was funded, facilitated, and supported by Aretha Medika Utama Biomolecular and Biomedical Research Center, Bandung, Indonesia. The author is also thankful to Seila Arumwardana, Muhammad Aldi Maulana, Aditya Rinaldy, Cintani Dewi Wahyuni, Cahyaning Riski Wijayanti, and Ervi Afifah from Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia for their valuable assistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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