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Standardization and α-glycosidase inhibition of extracts of *Vatica pauciflora* Blume stem barks and *Smallanthus sonchifolius* leaves

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Abstract
Objective: To investigate standardizations of extract of *V. pauciflora* and *S sonchifolius* and to conduct in vitro assay of α-glycosidase enzyme inhibitory effects of the extracts.
Methods: Extraction of leaf and stem bark powder was conducted with maceration by using 70% ethanol as solvent, followed by extract quality determination that involved extract specific parameters, such as extract organoleptic assay, ethanol soluble content, water soluble content, heavy metal residue as well as extract non-specific parameters, such as loss on drying, water content, total residue content, and solvent residue. The researchers used *in vivo* assay for α-glycosidase enzyme inhibitory effects.
Results: The study showed that ethanolic extracts of *V. pauciflora* and *S sonchifolius* could be standardized with specific and non-specific parameters and had α-glycosidase enzyme inhibitory effects, with IC_{50} values of 19.59 and 13,53 µg/mL.
Conclusions: Standardized extract of *V. pauciflora* and *S sonchifolius* have α-glycosidase enzyme inhibitory properties.

Keywords: standardization, alpha glicosidase inhibition, Vatica pauciflora Blume stem barks, Smallanthus sonchifolius leaves

1. Introduction
Diabetes Mellitus (DM) refers to a metabolic disorder, characterized by elevated blood glucose level, which is related to metabolic abnormality of carbohydrates, fats, and protein [1]. Indonesia ranks the fourth after India, China, and the United States in the number of diabetes cases throughout the world [2]. The number of diabetes cases was estimated to increase from 171 cases in 2000 to 366 cases in 2030 [3].
Medicinal plants are one of the alternatives of therapy, which is now under aggressive investigation, because it is relatively safer. One of the plans commonly used in the traditional medicine in Indonesia as anti-diabetic is yakon (*S sonchifolius*) and insulin plant (*V. pauciflora*).

Yakon plant (*S. sonchifolius)*

Insulin plant (*V. pauciflora*)

Fig 1: *S. sonchifolius* and *V. pauciflora* plants

Vatica pauciflora is a small medium-sized trees with yellowish dammar found in the branches. The stipules are oblong while the leaves occur in various shapes but usually elliptic and oval. The flowers are creamy white in color and sometimes a bit red at the bottom of the petals. The fruits are in various sizes with pale pinkish brown color [4]. Yakon [*Smallanthus sonchifolia* (Poepp. et Endl.)H. Robinson], syn. *Polymnia sonchifolia*, a native of the Andes closely related to the sunflower, is a vigorous, herbaceous perennial plant (family Compositae or Asteraceae – sunflower family). The plant produces large tuberous roots similar to sweet potatoes in appearance, but they have a much sweeter taste and crunchy flesh [5]. A study conducted by Valentová *et al*. (2003) observed that a yacon extract reduced the proportion of glucose in cultures of hepatocytes, acting in a manner similar to insulin. The study aims to find out bioactive compounds in the extract of *S. sonchifolia* and *V. pauciflora*. The study began with extraction process using 70% ethanolic solvent, followed by phytochemical screening test for the both extracts, by referring to extract standardization by using some quality parameters.

Standardization is the process of delivering product with a specified minimum level of one or more phytoconstituent (s), where we can make sure about the quality of the product; broadly, it covers the qualitative and quantitative part of analysis [6]. Standardization of extraction for herbal plants in Indonesia is an important phase in the development of native medicine in Indonesia. The herbal plant extracts can be used as basic ingredients, intermediary ingredients, and finished products. As a basic ingredient, the extract is analogous to raw materials of medicine, which process to a finished product by using phytopharmaceutical technology. Standardization is necessary to obtain various raw materials and eventually to ensure optimum pharmacological effects of the plant [7]. In the development of traditional medicine, particularly from herbal plants, which have been traditionally used as anti-diabetes, extraction of *raru* and *yakon* stem barks was conducted in reference to extract standardization, followed by the test for α-glycosidase enzyme-inhibitory activity.

2. Materials and methods

2.1 Sample collection

Samples of *S. sonchifolius* stem barks and *V. pauciflora* leaves were collected from the Balitro, Bogor, West Java. The samples were identified in Research Center of Biolog–Bogoriense Herbarium at Cibinong Bogor, West Java. Hot air-dried samples were processed for further analyses.

2.2 Preparation extracts

S. sonchifolius and *V. pauciflora* leaves and stem barks (1 kg of coarse powder) were extracted separately twice, with ethanol 70% (5 Lt) under maceration. The final extract was taken to dryness in a rotary evaporator to dryness on a boiling water bath to yield a maceration crude extract. Dry extracts were stored at 4 °C until analysis.

2.3 Specific test for extract parameter

2.3.1 Loss on drying determination

Loss on drying of each extract was determined according to the procedure described in Standard of ASEAN Herbal Medicine (1993). Each sample was done in triplicate and the average of weight lost on drying was reported [8].

2.3.2 Solubility extracts

The solubility of each extract in 95% ethanol and distilled water was investigated at room temperature. The level of solubility was recorded according to USP XXVI [9].

2.3.3 Organoleptics

Consistency and color of the ethanolic extract was visually observed; while the smell was determined using sensory organs, like nose and tongue.

1) Determination of water soluble compound

Five grams of extract were macerated for 24 hours in 100 ml of chloroform using a capped flask, stirred for the first 6 hours, and let for the remaining 18 hours. Then, filter and air 20.0 ml of the filtrate to dryness in a shallow and flat pan, which has been pre-weighed, and heat the residue in an oven under a temperature of 105 ^{0}C to reach a constant weight. Calculate the concentration in percent water-soluble compounds, compared to the baseline extract [8].

2) Determination of ethanol-soluble compound

Five grams of extract were macerated for 24 hours in 100 ml of ethanol (96%). The maceration used a capped flask; stir the extract for the first 6 hours and let it for the remaining 18 hours. Do the filtering in an accurate way to prevent ethanolic evaporation; evaporate 20.0 ml of the filtrate to dryness in a shallow and flat pan, which has been weighed first. Heat the residue in an oven under a temperature of 105 ^{0}C to reach a constant weight. Calculate the concentration in percent 96% ethanol-soluble compounds, compared to the baseline extract [8].

2.4 Non-specific test for the extract parameter

2.4.1 Loss on drying

One grams of the extract was weighed in a careful way. Put the extract in a shallow capped weighing bottle, which has been heated under a temperature of 105 ^{o}C for 30 minutes, and make it even. Put in to drying room, open the cap, and dry the extract under a temperature of 105 ^{o}C to reach a constant weight. Before the drying phase, have the bottle capped and put it in an exicator. If the extract is hard to dry and solve heat, add 1 gram of drying silica, which has been carefully weighed, dried, and stored in an exicator under room temperature. Mix the silica in an even way into the hot extract, then dry the mixture again under a temperature of 105 ^{o}C to reach a constant weight [8].

2.4.2 Water content

Water content was determined using Karl Fischer reactor, to which 50 mg of extract was introduced. The water content would be detected by Karl Fischer reactor.

2.4.3 Total residues content

About two to three grams of the extract, which had been carefully sheared and weighted, were introduced into the silicate crucible that had been centrifuged and weighed. If the resulting charcoal could not remove, add some hot water, filter with a dust-free paper filter. Centrifuge the residue and paper filter in the same crucible tool. Add the filtrate into the crucible, evaporate, and centrifuge the mixture into a constant weight; calculate the dust content in comparison with the air dried extract [8].

2.4.4 Acid insoluble dust content
Boil the dust that had been obtained in the measurement of total dust content by adding 2.5 ml of chloride acid for 5 minutes. Collect the acid-insoluble dust, filter using a dust-free paper filter, wash with hot water, and centrifuge the residue and paper filter under a temperature of 400-600 °C to produce a constant weight. Acid-insoluble dust content was determined in comparison with the air-dried extract [8].

2.5 Phytochemical Screening
The phytochemical analysis of *S. sonchifolius* bark and *V. pauciflora* leaf extract has been performed to find the presence of major secondary metabolites like flavonoids, tannins, saponins, steroid, glycosides, coumarins, anthraquinones and alkaloids. Steroidal rings analysis was performed following the method described by Sofowora [11].

2.6 α-glycosidase Inhibitory Activity Assay
The alpha-glycosidase inhibitory effects of the ethanolic extracts of *S. sonchifolius* and *V. pauciflora* were assayed according to the procedure described previously by Matsui *et al*. (2001) with minor modifications. Briefly, the enzyme reaction was performed using p-Nitrophenyl-alpha-D-glucopyranoside (PNP-glycoside) as a substrate in 0.1 M piperazine- N, N´-bis (2-ethanesulfonic acid) (PIPES) buffer, pH 6.8. The PNP-glycoside (2.0 mM) was premixed with samples at various concentrations. Each mixture was added to an enzyme solution (0.01 unit) to make 0.5 ml of final volume. The reaction was terminated by adding 1ml of 0.64% N-(1-naphthyl) ethylenediamine solution (pH 10.7). Enzymatic activity was quantified by measuring the p-nitrophenol released from PNP-glycoside at 405 nm wavelength [12]. All reactions were carried out at 37 °C for 30 min with three replications. Acarbose and quercetine were used as a positive control [12]. α-glycosidase Inhibitory activity assay was calculated using the equation as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs 410(control)} - \text{Abs 410 (extract)}}{\text{Abs 410 (control)}} \times 100$$

The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by nonlinear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

3. Results
3.1 Yield of extracts
Extraction was conducted by using 70% ethanol solvent since it is a polar solvent. Therefore, it is expected that most of the chemical components are soluble in it. Table 1 presents yield of each extract, namely 11,02% for S. *sonchifolius* extract and 10.02% for *V. pauciflora* extract. Parameters of the extract quality are presented in Table 1.

Table 1: Parameters of extract quality

Quality parameter	*S. sonchifolius*	*V. pauciflora*
Yield of extract	11,02%	10,02%
Measurement of soluble compound content		
- Ethanol soluble compound content	13,66	15,46
- Water soluble compound content	72,46	69,24
Loss on drying	13,84	8,09
Water content	7,34	7,27
Total dust content	5,63	5,46
Acid insoluble dust content	0,46	0,15

All tests were performed in triplicate

Table 2: Phytochemical Screening Identification

Group of Chemical Compound	*S. sonchifolius*	*V. pauciflora*
Alkaloid	-	-
Flavonoid	++	+++
Saponin	+	+++
Kuinon	-	-
Tannin gallate/katekuat	-	+++/+++
Steroid/triterpenoid	+/+	-/++
Essential oils	-	-
Coumarin	-	-

- : negative, ++ : Strongly positive, + : positive, +++ : Very strongly positive

Table 3: IC_{50} Values of the Extract, in comparison with acarbose and quercetine

Chemical/extract compound	IC_{50} Value (μg/mL)
Acarbose	17,15 ± 1,23
S. sonchifolius extract	19,59 ± 2,13
V. pauciflora extract	13,53 ± 1,98

All tests were performed in triplicate.

3.2 Specific test for extract parameter
Extract specific parameters include organoleptic assay of the extract, ethanol soluble extract, water soluble extract, and heavy metal residue. Ethanol soluble and water soluble contents of *S. sonchifolius* extract was 13,66% and 72,46%, respectively, compared to 15,46% and 69,24%, respectively for those of V. *pauciflora* extracts (Table 1).

3.3 Non-specific test for the extract parameter
Extract non-specific parameters showed that loss on drying assay of the ethanolic extract of *S. sonchifolius* was 13,84%, compared to 8,09% for the ethanolic extract of *V. pauciflora*. Water soluble content of *S. sonchifolius* extract was 7,34%, compared to 7,27% for that of *V. pauciflora* extract; total dust content of *S. sonchifolius* extract was 5,63%, compared to 5,46% for that of *V. pauciflora* extract; and acid insoluble dust content of *S. sonchifolius* extract was 0,46%, compared to 0,15% for that of *V. pauciflora* extract (Table 1).

3.4 α-glycosidase Inhibitory Activity
Assay for α-glycosidase enzyme inhibitory effect of the *S. sonchifolius* extract indicated an IC_{50} value of 19,59 μg/mL compared to 13,53 μg/mL for that of *V. pauciflora* extract. IC_{50} value for both extracts was comparable to that of positive control of acarbose, namely 17,15 μg/mL.

4. Discussion
Loss on drying was determined to find out the water content and evaporating compound in the extract after a gravimetric drying process in an oven under a temperature of 105 ^{0}C. Loss on drying was found to be 13.84%. Water content measurement was conducted to find out the water contained in the extract. The lower the water content is, the more stable the extract will be for a longer term. Water content was found to be 7.34%.

Measurement of total dust content and acid-insoluble dust content in the extract aimed at finding out mineral elements in the extract; this is known as inorganic substance or dust. In the heating process in an oven under a temperature of 450 ^{0}C, it was found that organic matters in the extract could be burnt while the inorganic matters, like dust, could not. Measurement of total dust content aimed at finding out mineral compounds, both physiological compound like K, Mg, and non-physiological compounds, like pollutant, dust, and soil in the extract.

α-glycosidase enzyme hydrolyzed nitrophenyl-α-D-glucopyranoside substrate into yellow p-nitrophenyl and glucose. The extract showed α-glycosidase inhibitory effects, which were determined from p-nitrophenyl absorption that had been formed and measured using UV-VIS Spectrophotometer with a wavelength of 400 nm. The IC_{50} value showed the extract concentration that could inhibit 50% of the α-glycosidase enzyme. The α-glucosidase inhibitor effectiveness of extracts of the different plant species were compared on the basis of their resulting IC_{50} values. Table 3 presents that *S. sonchifolius* and *V pauciflora* extracts had α-glycosidase enzyme inhibitory effects of 19.59 and 13,53 μg/mL, respectively. These are comparable with the positive controls, namely 17,15 μg/mL for acarbose. The antihyperglycemic action of acarbose results from a competitive, reversible inhibition of pancreatic alpha-amylase and membrane-bound intestinal alpha-glycoside hydrolase enzymes. Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, while the membrane-bound intestinal alpha-glycosidase hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharide in the brush border of the small intestine. In diabetic patients, this enzyme inhibition results in a delayed glucose absorption and a lowering of postprandial hyperglycemia [13].

The results of the present study indicate that out of the two plant extracts, the ethanolic extracts of *S. sonchifolius* and *V pauciflora* showed the maximum alpha glucosidase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. The present study was restricted to the preliminary screening of enzyme inhibitory activities of the selected plant extracts.

5. Conclusion
The study found that both extracts met the standard criteria as the basic extract ingredient to use as anti-diabetes since they did not contain pollutants. α-glycosidase enzyme inhibitory effects showed that both extracts had α-glycosidase enzyme inhibitory effects with IC_{50} values of that *S. sonchifolius* and *V pauciflora* extracts had α-glycosidase enzyme inhibitory effects of 19.59 and 13,53 μg/mL.

6. Conflict of interest statement
We declare that we have no conflict of interest.

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