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Phytochemical Screening and α-Glucosidase Inhibition of Combined Yacon Leaf and Karas Tulang Root Extracts

Rizka Nur Annisa[1], Haryoto[1*]

[1] *Faculty of Pharmacy, Muhammadiyah Surakarta University, Jl. Garuda Mas 8 57169 Kartosuro, Central Java, Indonesia*

Corresponding Author. Email: har254@ums.ac.id

ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia and remains a major global health concern. Natural products containing secondary metabolites are being investigated as potential α-glucosidase inhibitors. This study evaluated the phytochemical profile and in vitro α-glucosidase inhibitory activity of a combined ethanol extract of yacon leaves (*Smallanthus sonchifolius*) and karas tulang roots (*Chloranthus erectus*). Phytochemical screening was performed by thin-layer chromatography, and enzyme inhibition was evaluated using p-nitrophenyl-α-D-glucopyranoside as the substrate with acarbose as the positive control. Both extracts contained flavonoids, saponins, tannins, and alkaloids. The combined extract produced concentration-dependent inhibition with an IC_{50} of 25.5 ± 0.82 µg/mL, whereas acarbose produced an IC_{50} of 15.5 ± 0.80 µg/mL. The observed activity may be associated with the combined presence of secondary metabolites, particularly phenolic compounds, but a synergistic interaction cannot be concluded because the individual extracts and multiple combination ratios were not tested. The findings support preliminary in vitro α-glucosidase inhibitory potential of the combined extract. Further studies should confirm the extract ratio, identify active compounds, assess toxicity, and evaluate activity in vivo.

Keywords:
α-Glucosidase; Yacon leaf extract; *Chloranthus erectus* root extract; Phytochemical screening; Antidiabetic activity

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion, insulin action, or both. In 2024, an estimated 589 million adults aged 20-79 years were living with diabetes worldwide, and this number is projected to reach 853 million by 2050 [1]. In Indonesia, national survey data also show an increase in diagnosed diabetes prevalence from 1.5% in 2013 to 2.0% in 2018 [2]. These trends highlight the need for complementary strategies that can help control postprandial blood glucose.

One therapeutic approach is inhibition of intestinal α-glucosidase, an enzyme that hydrolyses oligosaccharides and disaccharides into absorbable glucose. Delaying

this reaction can reduce the rapid postprandial increase in blood glucose. Plant-derived phenolic compounds, flavonoids, tannins, alkaloids, and related metabolites have therefore been investigated as natural α-glucosidase inhibitors [3].

Yacon leaves (*Smallanthus sonchifolius*) contain phenolic constituents and have demonstrated α-glucosidase inhibitory activity [4]. Karas tulang (*Chloranthus erectus*) has also been reported to contain flavonoids, tannins, saponins, and alkaloids, and its leaf extract has shown in vitro α-glucosidase inhibition [5]. Previous combination research involving yacon leaves and tea leaves showed that inhibitory activity depended strongly on the extract ratio [6]. However, the specific combination of yacon leaf extract and karas tulang root extract has not been evaluated in the studies cited here.

The novelty of the present study is the evaluation of this specific plant-part combination: yacon leaves and karas tulang roots. The study aimed to characterize the major phytochemical groups in each extract and to determine the preliminary in vitro α-glucosidase inhibitory activity of their combined ethanol extracts. Because the individual extracts and multiple mixture ratios were not evaluated, the study was designed to describe the activity of the tested combination rather than establish synergy.

2. Methods

Equipment and Materials

The equipment included filter paper, a metal spatula, tweezers, a porcelain dish, 0.5-mL microtubes, a 96-well microplate, volumetric glassware, micropipettes, an analytical balance, a drying cabinet, a grinder, a rotary evaporator, a water bath, a refrigerator, and an ELISA microplate reader. The materials were yacon leaves (*Smallanthus sonchifolius*), karas tulang roots (*Chloranthus erectus*), n-hexane, ethyl acetate, 70% ethanol, 96% ethanol, distilled water, α-glucosidase enzyme, p-nitrophenyl-α-D-glucopyranoside (pNPG; Sigma-Aldrich, USA), p-nitrophenol, dimethyl sulfoxide (DMSO; Merck), sodium carbonate (Na_2CO_3; Merck), acarbose 50-mg tablets (OGB Dexa), bovine serum albumin (BSA), sodium hydroxide, potassium dihydrogen phosphate, $AlCl_3$, $FeCl_3$, Dragendorff reagent, and Liebermann-Burchard reagent.

Plant Determination

Samples of yacon leaves (*Smallanthus sonchifolius*) and karas tulang roots (*Chloranthus erectus*) were authenticated at the Biology Laboratory, Setia Budi University. The determination document numbers were 208E/DET/UPT-LAB/2.12.2025 for yacon and 209E/DET/UPT-LAB/3.12.2025 for karas tulang. No voucher specimens were deposited in a herbarium or institutional collection. The original determination documents are retained by the research team and are available from the corresponding author upon reasonable request.

Extraction

Approximately 500 g of fresh karas tulang roots and 2,000 g of fresh yacon leaves were processed. The materials were dried in a drying cabinet at 50 °C for 24 h and ground into powder. A 10 g portion of each dried powder was used for extraction. Karas tulang root powder was macerated with 100 mL of 70% ethanol, whereas yacon leaf powder was macerated with 100 mL of 96% ethanol at a material-to-solvent ratio of 1:10 for 3 days with occasional stirring. The extraction was conducted in two cycles, consisting of an initial maceration followed by one remaceration using fresh solvent. The use of 70% ethanol for karas tulang was intended to extract a broad range of polar and semipolar metabolites, whereas 96% ethanol for yacon leaves followed the established extraction procedure for obtaining phenolic and flavonoid compounds. The combined

filtrates were concentrated using a rotary evaporator and further evaporated in a water bath to obtain thick extracts. The extraction produced 0.912 g of yacon leaf extract with a yield of 9.12% and 0.972 g of karas tulang extract with a yield of 9.72%.

Phytochemical Screening by Thin-Layer Chromatography

Phytochemical screening was performed on 10 × 2 cm silica gel GF_{254} plates using n-hexane and ethyl acetate (6:4, v/v) as the mobile phase. The chromatography chamber was saturated with the mobile-phase vapor for 15 min before development. Each extract was prepared at a concentration of 1 mg/mL, and 5 µL of the solution was applied 1 cm above the lower edge of the plate. The plate was developed until the solvent front reached a distance of 8 cm from the spotting line. After development, the plates were dried and observed under visible light and UV light at 254 and 366 nm, followed by spraying with the relevant detection reagents. The migration distance of each spot and the solvent-front distance were recorded to determine the Rf values. Each TLC analysis was performed in triplicate to confirm the consistency of the detected spots.

Detection of Flavonoids, Tannins, Saponins, and Alkaloids

Flavonoids were detected by spraying the developed plate with 1% $AlCl_3$ and observing yellow to yellowish-green fluorescence under UV 366 nm. Tannins were detected using $FeCl_3$, with blue-black to green-black coloration interpreted as positive [15]. Saponins were detected with Liebermann-Burchard reagent, producing green to blue-green spots, whereas alkaloids were detected using Dragendorff reagent, producing orange to reddish-brown spots. The observed colors and Rf values were documented for each extract.

Preparation of the α-Glucosidase Inhibition Assay

α-Glucosidase Enzyme Solution

α-Glucosidase from *Saccharomyces cerevisiae* was obtained from Sigma-Aldrich (catalogue no. G5003; activity ≥10 U/mg protein). The enzyme stock solution was prepared at 40 U/mL. An aliquot of 50 µL of the stock solution was diluted with BSA solution to a final volume of 10 mL, producing a working enzyme solution of 0.2 U/mL. A volume of 25 µL of this working solution was added to each well, corresponding to 0.005 U per well or a final enzyme concentration of 0.05 U/mL in the 100 µL enzymatic reaction mixture.

p-Nitrophenyl-α-D-Glucopyranoside Solution

pNPG (60 mg) was dissolved in 10 mL of water for injection to prepare a 20 mM substrate solution. With 25 µL substrate in a 100 µL reaction volume before addition of the stopping solution, the final pNPG concentration was 5 mM.

p-Nitrophenol Standard Curve

p-Nitrophenol (14 mg) was dissolved in 10 mL of pH 7.0 phosphate buffer to prepare a 10 mM stock solution. Six working-standard concentrations of 30, 60, 90, 120, 150, and 180 µM were prepared by diluting the stock solution with pH 7.0 phosphate buffer. The absorbance of each standard solution was measured at 405 nm to construct the p-nitrophenol calibration curve.

Acarbose Solution

Four acarbose tablets, each containing 50 mg of acarbose, were weighed and pulverized into a homogeneous powder. The total weight of the four tablets was approximately 800 mg, corresponding to an average tablet weight of approximately 200 mg. Based on the label claim, the entire powdered sample was equivalent to 200 mg of active acarbose. The powder was dispersed in a 1:1 mixture of pH 7.0 phosphate buffer and 2 N HCl to a final volume of 5 mL. The mixture was homogenized and centrifuged

at 3,000 rpm for 10 min. The supernatant was subsequently filtered through a 0.45 µm membrane filter to remove insoluble tablet excipients, and the clear filtrate was used as the acarbose stock solution.

BSA Solution

BSA (100 mg) was dissolved in 50 mL of pH 7.0 phosphate buffer.

Phosphate Buffer (pH 7.0)

Potassium dihydrogen phosphate (1.5 g) was dissolved in 50 mL of distilled water. The pH was adjusted to 7.0 with 0.1 N NaOH, and CO_2-free water was added to a final volume of 200 mL.

Combined Extract Working Solutions

A stock solution of the combined extract was prepared by dissolving 100 mg of extract in 5 mL of DMSO, producing a concentration of 20,000 µg/mL. The previous designation of 40,000 µg/mL was corrected because it was inconsistent with the extract mass and solvent volume used. Working solutions of 15,000, 10,000, 5,000, and 2,500 µg/mL were prepared by diluting the stock solution with DMSO. For a final volume of 5 mL, the respective volumes of stock solution and DMSO were 3.75 and 1.25 mL; 2.50 and 2.50 mL; 1.25 and 3.75 mL; and 0.625 and 4.375 mL. In the assay, 1 µL of each solution was added to a 100 µL reaction mixture, producing final extract concentrations of 200, 150, 100, 50, and 25 µg/mL. The final DMSO concentration in the reaction mixture was 1% (v/v).

α-Glucosidase Inhibition Assay

Control Reaction

The control reaction contained 25 µL pNPG solution, 49 µL pH 7.0 phosphate buffer, and 1 µL DMSO. After pre-incubation at 37 °C for 5 min, 25 µL enzyme solution was added, and the mixture was incubated at 37 °C for 15 min. The reaction was stopped with 100 µL of 200 mM Na_2CO_3, and absorbance was measured at 405 nm [5].

Sample and Sample-Blank Reactions

The sample reaction contained 1 µL combined extract solution, 49 µL pH 7.0 phosphate buffer, and 25 µL pNPG solution. After pre-incubation at 37 °C for 5 min, 25 µL enzyme solution was added. The mixture was incubated at 37 °C for 15 min, stopped with 100 µL of 200 mM Na_2CO_3, and measured at 405 nm.

The sample-blank reaction contained 1 µL combined extract solution, 49 µL pH 7.0 phosphate buffer, and 25 µL pNPG solution. After pre-incubation, 25 µL phosphate buffer was added instead of enzyme. The mixture was incubated for 15 min, stopped with 100 µL of 200 mM Na_2CO_3, and measured at 405 nm [5].

Reagent Blank

The reagent blank contained 25 µL pNPG solution, 1 µL DMSO, and 49 µL pH 7.0 phosphate buffer. After pre-incubation at 37 °C for 5 min, 25 µL phosphate buffer was added in place of enzyme. After a further 15 min at 37 °C, the reaction was stopped with 100 µL of 200 mM Na_2CO_3, and absorbance was measured at 405 nm [5].

Data and Statistical Analysis

Rf values, percentage inhibition, and IC_{50} values were calculated using the equations provided below. The α-glucosidase inhibition assay was conducted in three independent experiments, with each concentration tested in triplicate. IC_{50} values were determined by linear regression of percentage inhibition (y) against the final assay concentration (x, µg/mL) using Microsoft Excel 2019. The results were expressed as mean ± standard deviation. No inferential statistical test was performed to compare the

combined extract with acarbose; therefore, their inhibitory activities were compared descriptively based on the percentage inhibition and IC_{50} values.

$$Rf\ value = \frac{\text{Distance travelled by the analyte}}{\text{Distance travelled by the eluent}}$$

The intended final extract concentrations used for regression were 25, 50, 100, 150, and 200 µg/mL. A value expressed as 1 ppm is numerically equivalent to 1 µg/mL for these dilute solutions; therefore, one unit system (µg/mL) is used throughout the revised manuscript.

3. Results and Discussion

Phytochemical Screening Results

Thin-layer chromatography (TLC) was performed to identify the major classes of secondary metabolites present in the ethanol extracts of karas tulang roots and yacon leaves[14]. This preliminary phytochemical screening provides qualitative information on the chemical constituents that may contribute to the α-glucosidase inhibitory activity of the combined extract. The detected phytochemical groups together with their corresponding Rf values are summarized in **Tables 1** and **2**. Representative TLC chromatograms of both extracts are presented in **Figures 1** and **2**, respectively.

Table 1. TLC phytochemical screening of karas tulang root extract

Phytochemical group	Detection reagent	Result	Rf value
Flavonoids	1% $AlCl_3$	+	0.47
Saponins	Liebermann-Burchard	+	0.45
Tannins	$FeCl_3$	+	0.67
Alkaloids	Dragendorff	+	0.35

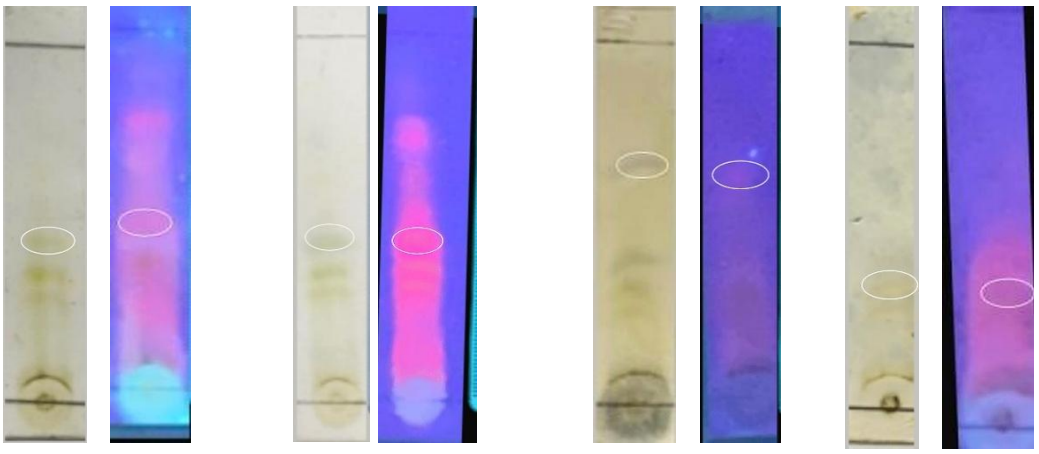
TLC screening showed flavonoids, saponins, tannins, and alkaloids in the karas tulang root extract. The respective Rf values were 0.47, 0.45, 0.67, and 0.35. After derivatization, flavonoids produced yellowish-green fluorescence under UV 366 nm, tannins produced blue-black to green-black spots, saponins produced green spots, and alkaloids produced orange-brown spots [13]. These responses were consistent with the selected detection reagents.

The yacon leaf extract showed the same four phytochemical groups, with Rf values of 0.70 for flavonoids, 0.93 for saponins, 0.87 for tannins, and 0.60 for alkaloids. Differences in Rf values between the extracts may reflect differences in metabolite composition, concentration, polarity, and interaction with the stationary and mobile phases.

Table 2. TLC phytochemical screening of yacon leaf extract

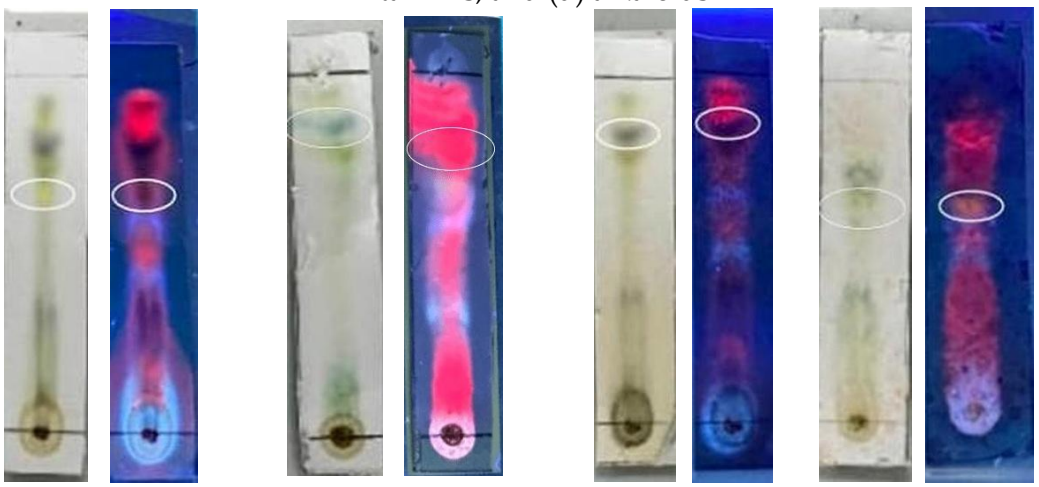
Phytochemical group	Detection reagent	Result	Rf value
Flavonoids	1% $AlCl_3$	+	0.70
Saponins	Liebermann-Burchard	+	0.93
Tannins	$FeCl_3$	+	0.87
Alkaloids	Dragendorff	+	0.60

Previous studies also reported flavonoids, saponins, tannins, and alkaloids in karas tulang leaf extract [5], [9]. The present results extend the phytochemical description to the root material used in this study. However, TLC screening identifies chemical groups rather than individual compounds; confirmatory chromatographic and spectrometric analyses are required to identify the specific constituents responsible for enzyme inhibition. Representative chromatographic profiles illustrating the detected phytochemical groups are shown in **Figures 1** and **2**.



a) Flavonoids (b) Saponins (c) Tannins (d) Alkaloids

Figure 1. TLC profiles of karas tulang root extract: (a) flavonoids, (b) saponins, (c) tannins, and (d) alkaloids



(a) Flavonoids (b) Saponins (c) Tannins (d) Alkaloids

Figure 2. TLC profiles of yacon leaf extract: (a) flavonoids, (b) saponins, (c) tannins, and (d) alkaloids

α-Glucosidase Inhibition Results

The α-glucosidase inhibitory activity of the combined yacon leaf and karas tulang root extracts was evaluated using an in vitro enzymatic assay with acarbose as the positive control[12]. The IC_{50} values obtained from the assay are presented in **Table 3**,

whereas the concentration-dependent inhibition percentages together with the regression equations used for IC_{50} determination are summarized in **Table 4**.

Table 3. IC_{50} values for the combined extract and acarbose

Sample	IC_{50} (µg/mL)	Interpretation
Combined yacon leaf and karas tulang root extracts	25.5 ± 0.82	Active in vitro inhibition
Acarbose	15.5 ± 0.80	Positive control

The combined extract produced an IC_{50} of 25.5 ± 0.82 µg/mL, while acarbose produced an IC_{50} of 15.5 ± 0.80 µg/mL. A lower IC_{50} indicates greater inhibitory potency; therefore, the positive control was more potent under the reported assay conditions. Because no inferential comparison is currently documented, this difference should be interpreted descriptively rather than as statistically significant. The concentration-specific inhibition values, regression equations, R^2 values, and replicate number must be inserted in Table 4 before submission.

The current IC_{50} value lies between previously reported values for the relevant plant extracts. A 96% ethanol extract of yacon leaves was reported to have an IC_{50} of 19.70 µg/mL [6], whereas an ethanol extract of karas tulang leaves showed an IC_{50} of 30.541 µg/mL [5]. In the yacon-tea combination study, IC_{50} values varied markedly with the ratio: 64.73 µg/mL for 1:1, 7.34 µg/mL for 1:3, and 11.03 µg/mL for 3:1 [6]. These findings reinforce the reviewer's concern that the exact yacon-to-karas tulang ratio must be reported and that activity of a mixture cannot be interpreted as synergistic without testing the individual extracts and multiple ratios.

Table 4. Percentage inhibition and regression data required for reproducible IC_{50} calculation

Sample	Test concentrations (µg/mL)	Inhibition at each concentration, mean ± SD (%)	Regression equation and R^2
Combined extract	25, 50, 100, 150, and 200	25 µg/mL: 50.18 ± 0.82; 50 µg/mL: 55.63 ± 0.76; 100 µg/mL: 69.03 ± 0.91; 150 µg/mL: 80.78 ± 0.88; 200 µg/mL: 93.78 ± 0.95	y = 0.2500x + 43.625; R^2 = 0.9995
Acarbose	5, 10, 15, 20, and 25	5 µg/mL: 27.10 ± 0.65; 10 µg/mL: 37.50 ± 0.70; 15 µg/mL: 49.30 ± 0.80; 20 µg/mL: 59.50 ± 0.75; 25 µg/mL: 71.10 ± 0.85	y = 2.2000x + 15.900; R^2 = 0.9995

Acarbose IC_{50} values also vary across published assays. Values of 17.73 µg/mL and 3.873 µg/mL have been reported in studies using tablet-derived acarbose controls [6], [5], respectively. Such variation may arise from differences in enzyme source and activity, substrate concentration, incubation conditions, active-content calculation, excipient removal, and regression procedures. The present acarbose result of 15.5 ± 0.80 µg/mL should therefore be interpreted within the specific assay conditions after all missing methodological details are confirmed. The combined presence of flavonoids, tannins, saponins, and alkaloids may contribute to inhibition through enzyme-binding and substrate-interaction mechanisms [3], [7], but the responsible compounds and their interactions were not established in this study.

Study Limitations

This study has five principal limitations. First, the assay was conducted only in vitro and does not represent absorption, metabolism, safety, or efficacy in vivo. Second, the individual yacon leaf and karas tulang root extracts were not tested alongside the combination, so additivity, antagonism, or synergy cannot be determined. Third, the active constituents were not isolated or identified. Fourth, toxicity testing was not performed. Fifth, the combination ratio, full extraction parameters, replicate structure, raw inhibition data, and regression outputs require confirmation from the original laboratory records.

4. Conclusion

The combined ethanol extracts of yacon leaves and karas tulang roots showed preliminary in vitro α-glucosidase inhibitory activity, with an IC_{50} of 25.5 ± 0.82 µg/mL compared with 15.5 ± 0.80 µg/mL for acarbose. TLC screening detected flavonoids, saponins, tannins, and alkaloids in both extracts. These findings support further investigation but do not establish synergy or therapeutic efficacy. Before the results can be interpreted conclusively, the combination ratio and missing assay details must be verified. Future work should compare the individual extracts and multiple ratios, identify active compounds, evaluate toxicity, and confirm activity in vivo.

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Conflicts of Interest:

The authors declare no conflicts of interest related to this study or its publication.

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