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Research Article

IN VITRO POTENTIAL OF A-AMYLASE ENZYME INHIBITION OF A COMBINATION OF ETHANOL EXTRACT OF YAKON POTATO LEAVES (SMALLANTHUS SONCHIFOLIUS) AND *KARAS TULANG* ROOTS (CHLORANTHUS ERECTUS) AND ITS PHYTOCHEMICAL SCREENING

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Abstract
Postprandial hyperglycemia in type 2 diabetes mellitus presents a severe health risk, necessitating safer management strategies compared to synthetic inhibitors like acarbose, which often cause gastrointestinal side effects. Although the antidiabetic potential of yacon leaves (Smallanthus sonchifolius) and *karas tulang* roots (Chloranthus erectus) has been validated individually, studies on the synergism of their combination remain remarkably limited. This study aimed to examine the phytochemical profile and α-amylase inhibitory activity of a combined ethanolic extract of both plants (1:1 ratio) as a novel polyherbal approach. Extraction was performed via maceration using 70% ethanol (yield: 14.2% for yacon leaves; 11.4% for *karas tulang* roots). Phytochemical screening using thin-layer chromatography revealed the presence of terpenoids, polyphenols, alkaloids, and flavonoids. The α-amylase inhibitory assay using the DNS method demonstrated that the combined extract exhibited concentration-dependent inhibition (34.53% at 20 ppm to 90.36% at 200 ppm) with an IC_{50} value of 63.30 $\pm$ 0.16 ppm. Although the IC_{50} value of the combined extract was higher than that of acarbose (43.18 $\pm$ 1.34 ppm), this significant inhibitory activity confirms that the combination of yacon leaf and *karas tulang* root extracts offers potential as a synergistic and promising natural antidiabetic candidate, providing a foundation for the development of safer polyherbal therapies.

Keywords: α-Amylase, Acarbose, Chloranthus Erectu, Smallanthus Sonchifolius, Type 2 Diabetes

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INTRODUCTION

Diabetes mellitus type 2 (T2DM) remains a critical global health challenge, with prevalence rates continuing to surge alarmingly, particularly in developing nations such as Indonesia, where a 2.1% increase was recently noted based on Basic Health Research data (Musayyaf, 2024). While the metabolic dysfunction of T2DM is well-documented in the literature, the current clinical urgency lies in managing postprandial hyperglycemia, which acts as a primary catalyst for severe microvascular and macrovascular complications. Prolonged spikes in blood glucose after meals significantly accelerate disease progression. Consequently, targeted therapeutic interventions specifically designed to delay carbohydrate digestion and glucose absorption in the gastrointestinal tract are no longer merely relevant, but an urgent necessity in modern antidiabetic management.

In the regulation of postprandial hyperglycemia, α-amylase and α-glucosidase enzymes serve as essential therapeutic targets due to their roles in hydrolyzing complex starches into monosaccharides (Dirir et al., 2022). Currently, synthetic inhibitors like acarbose represent the clinical standard for this mechanism. However, the clinical utility of these synthetic drugs is frequently hampered by their adverse side effect profile, such as flatulence, abdominal distention, and diarrhea, which arise from the fermentation of undigested carbohydrates in the large intestine (Dirir et al., 2022). These gastrointestinal side effects can lead to poor patient compliance. This clinical limitation drives the urgency to seek inhibitory compounds from natural plant sources that offer comparable efficacy with minimal toxicity and side effects (Dirir, Daou, Yousef, & Yousef, 2022).

Medicinal plants offer a promising reservoir of bioactive compounds capable of modulating carbohydrate-digesting enzymes. Recent studies indicate that secondary metabolites such as flavonoids, polyphenols, tannins, alkaloids, and terpenoids possess specific inhibitory actions against these enzymes. For instance, Lam et al. (2024) elucidated that flavonoids can significantly decrease the catalytic activity of α-amylase via the formation of hydrogen bonds, hydrophobic interactions, and binding to active or allosteric regions. Similarly, the phenolic profiles of various herbal plant infusions have been correlated with inhibitory activity against digestive enzymes relevant to diabetes (Wongsa et al., 2022). However, previous studies have predominantly profiled the activity of plants in isolation (single extracts), leaving the understanding of potential synergistic effects from combined different herbal metabolite profiles largely unexplored.

Among various potential herbs, yacon leaves (*Smallanthus sonchifolius*) and *karas tulang* roots (*Chloranthus erectus*) exhibit highly promising antidiabetic activities. Yacon leaf extract has been reported to possess hypoglycemic potential as well as inhibitory effects against the α-amylase enzyme (Rawal et al., 2025; Widowati et al., 2021). Concurrently, the ethanolic extract of *karas tulang* at doses of 50 and 100 mg/kgBW has been shown to effectively reduce blood glucose levels owing to its alkaloid, saponin, flavonoid, and tannin content (Musayyaf, 2024). While the efficacy of both plants has been validated, the reliance on single-extract assays remains a fundamental limitation. To date, no study has critically evaluated the complementary potential of combining yacon leaf and *karas tulang* root extracts. Therefore, this study aims to formulate and test the α-amylase inhibitory activity of the combined extract of these two plants, hypothesizing that the blend of different metabolites may yield superior antidiabetic activity.

In addition to yacon leaves, *karas tulang* (Chloranthus erectus) is also an interesting plant to investigate because it contains various secondary metabolites with biological activities. The antidiabetic action of Chloranthus erectus ethanolic extract may be due to the presence of alkaloids, saponins, flavonoids, and tannins (Musayyaf, 2024). Flavonoids and tannins may support the inhibition of carbohydrate-digesting enzymes because these compounds can interact with enzyme proteins and reduce their catalytic activity (Lam et al., 2024). Moreover, ethanolic extract of *karas tulang* has been shown to reduce blood glucose levels in alloxan-

induced diabetic animal models, indicating its potential as a natural antidiabetic candidate (Musayyaf, 2024).

The use of combined plant extracts is an interesting approach in herbal drug development because each plant may contain different groups of bioactive compounds. A combined extract may produce a complementary effect through the contribution of various secondary metabolites acting on the same biological target or through different but related mechanisms. In polyherbal research, combinations of several medicinal plants have been reported to exhibit α-amylase inhibitory activity comparable to positive controls at certain concentrations (Quazi et al., 2022). However, synergistic claims require further confirmation through comparisons between single extracts and combined extracts in different ratios; therefore, the term "potential combined effect" is more appropriate at the preliminary stage of this study.

Based on these considerations, the combination of ethanolic extracts of yacon leaves and *karas tulang* roots may have potential as a natural antidiabetic candidate through α-amylase enzyme inhibition. There has been some research that has examined the α-amylase inhibitory action of these two plants together, especially when it comes to ethanolic extract combinations with a specific ratio. Hence, the purpose of this research was to assess the phytochemical screening results for secondary metabolites and to find out how well the combined ethanolic extracts of yacon leaves and karas bone roots inhibited α-amylase in vitro. Herbal antidiabetic medicines generated from a combination of medicinal plant extracts are anticipated to be developed on the basis of this study's results.

RESEARCH METHOD

Research Design

This study employed an *in vitro* experimental design to evaluate the antidiabetic potential of the combined ethanolic extracts of yacon leaves (*Smallanthus sonchifolius*) and *karas tulang* roots (*Chloranthus erectus*). The α-amylase inhibition assay was specifically selected as the sole parameter because this enzyme acts as the primary catalyst in the initial phase of starch hydrolysis; thus, inhibiting its activity serves as a crucial first-line biochemical indicator for predicting the efficacy of postprandial hyperglycemia management. This research is designed as an initial proof-of-concept study to evaluate the effectiveness of the combined extract, which was directly compared with acarbose as a positive control, prior to future advanced studies involving single-extract comparisons. Furthermore, to identify the secondary metabolites that potentially contribute to this inhibitory action, thin-layer chromatography (TLC) was utilized for phytochemical screening. The research was conducted at the Laboratory of the Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. Plant determination was previously carried out at UPT-Laboratorium Universitas Setia Budi. The overall research activities encompassed plant sample preparation, extraction, phytochemical screening, preparation of test solutions, α-amylase inhibitory assay, and data analysis.

Research Target/Subject

The research subjects were yacon leaves (*Smallanthus sonchifolius*) and *karas tulang* roots (*Chloranthus erectus*). The inclusion criteria for the samples required healthy plants, free from pests and diseases, harvested at a mature stage (mature leaves for yacon and fully developed roots for *karas tulang*) to ensure optimal accumulation of secondary metabolites. Yacon leaves were obtained from Wonosobo, Central Java, while *karas tulang* roots were sourced from Bandung, West Java. The test sample used in the α-amylase inhibitory assay was the combined ethanolic extract of yacon leaves and *karas tulang* roots at a ratio of 1:1 (w/w). This equivalent 1:1 ratio was specifically selected as an ideal baseline to evaluate the fundamental additive or synergistic interactions of both phytochemical profiles without the bias of one material dominating the extract. Acarbose was used as the positive control, while

phosphate buffer and DMSO were used in the reaction system according to their respective functions.

Research Procedure

Plant Determination

Plant determination was carried out to confirm the identity of the plant materials used in this study. The yacon leaf sample was obtained from Wonosobo, Central Java, while the *karas tulang* root sample was obtained from Bandung, West Java. The plant samples were determined at UPT-Laboratorium Universitas Setia Budi. Based on the determination results, the samples were confirmed as yacon leaves (*Smallanthus sonchifolius*) and *karas tulang* roots (*Chloranthus erectus*). Plant determination was conducted to ensure the authenticity of the plant materials before extraction and biological activity testing.

Preparation of Ethanolic Extracts

To achieve a consistent weight, the yacon leaves and karas bone roots were washed and dried in a drying cabinet at temperatures ranging from 40 to 50°C. A grinder was used to grind the dried simplicia, and then a 60-mesh screen was used to achieve a consistent powder particle size. Maceration, employing 70% ethanol as the solvent, was used to carry out the extraction procedure. Filtration was carried out every 24 hours after macerating the powdered simplicia for three days. After collecting the filtrates, they were concentrated at 50°C in a rotary evaporator. Extracts of yacon leaves and karas bone roots were thickened by drying them in a water bath at 50-60°C. This process was repeated until the concentrated contents were produced.

Preparation of Solution Test

Prior to conducting the α-amylase inhibitory experiment, the test solutions were prepared. A phosphate buffer with a pH of 6.9 was made by combining solutions of Na_2HPO_4 and NaH_2PO_4, and the pH was adjusted with the help of a pH meter. For the preparation of the α-amylase enzyme solution, the enzyme was dissolved in phosphate buffer with a pH of 6.9. To make a 1% starch solution, the starch was dissolved in a phosphate buffer with a pH of 6.9 during the course of 5-10 minutes of boiling. The yacon leaf and karas bone root ethanolic extracts were mixed in a 1:1 (w/w) ratio to create the combined extract solution. To make a stock solution, the combined extract was dissolved in dimethyl sulfoxide (DMSO). To get the concentration series needed, it was diluted in phosphate buffer pH 6.9. To make acarbose, we dissolved the powder in phosphate buffer with a pH of 6.9 and diluted it to the same concentrations as the test sample. This was done to create the positive control. An enzymatic process was halted and reducing sugars generated by starch hydrolysis were detected with the use of the DNS reagent, which was made from 3,5-dinitrosalicylic acid, NaOH, and sodium potassium tartrate tetrahydrate.

Phytochemical Screening by Thin-Layer Chromatography

Phytochemical screening was performed using thin-layer chromatography on silica gel GF254 plates. The extract solution was spotted on the TLC plate and developed using an appropriate mobile phase. After the elution process, the plate was dried and observed under UV light at 254 nm and 366 nm. Specific spray reagents were used to identify secondary metabolites. Flavonoids were detected using $AlCl_3$ reagent, alkaloids using Dragendorff reagent, polyphenols using $FeCl_3$ reagent, and terpenoids using Liebermann–Burchard reagent. Positive results were indicated by the appearance of characteristic colored spots on the TLC plate. The Rf value was calculated by comparing the distance traveled by the compound spot with the distance traveled by the solvent front.

α-Amylase Inhibitory Assay

Using the DNS technique, the inhibitory activity against α-amylase was assessed. The α-amylase enzyme solution, phosphate buffer pH 6.9, 1% starch solution, and sample or acarbose solution are made up the reaction combination. The combination was pre-chilled for 5 minutes at 37°C. The α-amylase enzyme solution was added after pre-incubation, and the mixture was incubated at 37°C for another 15 minutes. When DNS reagent was added, the enzymatic process ceased. A 540 nm absorbance was recorded by means of an ELISA reader.

The assay consisted of four reaction systems: blank, control, S0, and S1. The blank contained DMSO, starch substrate, and phosphate buffer without enzyme. The control contained DMSO, starch substrate, phosphate buffer, and α-amylase enzyme. S0 contained the sample or acarbose without enzyme, while S1 contained the sample or acarbose with enzyme. The S0 system was used to correct the absorbance of the sample so that the color of the extract did not interfere with the measurement. The percentage of α-amylase inhibition was calculated from the corrected absorbance values, and the IC_{50} value was determined from the relationship between concentration and percentage inhibition.

Instruments, and Data Collection Techniques

The instruments used included an analytical balance, drying oven, grinder, sieve, rotary evaporator, water bath, pH meter, micropipette, microplate 96 well, TLC chamber, UV lamp, and ELISA reader. Data were collected from extraction yield, phytochemical screening results, absorbance values, percentage inhibition, and IC_{50} values.

Data Analysis Technique

Extraction yield was calculated by comparing the extract weight with the initial simplicia weight. The Rf value was calculated by comparing the distance traveled by the compound spot with the distance traveled by the solvent front. The percentage of α-amylase inhibition was calculated using corrected absorbance values. The corrected sample absorbance was obtained from S1 minus S0, while the corrected control absorbance was obtained from control minus blank. The IC_{50} value was determined from the relationship between concentration and percentage inhibition. Data were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Plant Determination

Plant determination was carried out to confirm the botanical identity of the plant materials used in this study. The yacon leaf sample was obtained from Wonosobo, Central Java, while the *karas tulang* root sample was obtained from Bandung, West Java. Based on the determination results conducted at UPT-Laboratorium Universitas Setia Budi, the plant materials were confirmed as yacon leaves (Smallanthus sonchifolius) and *karas tulang* roots (Chloranthus erectus (Buch.-Ham.) Verdc.). This step was essential to ensure the authenticity of the plant species before extraction, phytochemical screening, and biological activity testing. The determination result showed that yacon belongs to the family Asteraceae and was identified as *Smallanthus sonchifolius*. The *karas tulang* sample was identified as *Chloranthus erectus* (Buch.-Ham.) Verdc., a member of the family Chloranthaceae. The determination results confirmed that the plant materials used in this study were appropriate and valid for further extraction and α-amylase inhibitory activity testing.

Extraction Yield

Extraction was carried out using the maceration method with 70% ethanol as the solvent. Maceration was selected because it is a simple extraction technique that minimizes direct

exposure to high temperatures, thereby reducing the risk of degradation of thermolabile compounds. Hydroethanolic solvents are suitable for extracting polar to semi-polar bioactive compounds, including phenolics, flavonoids, tannins, and several alkaloids, which are commonly associated with digestive enzyme inhibition (Wongsa et al., 2022).

Table 1. Extraction Yield Results

Sample	Simplicia Weight (g)	Extract Weight (g)	Yield (%)
Yacon Leaves	300	42.6	14.2
Karang Tulang Roots	200	22.8	11.4

Table 1 shows that 70% ethanol extraction produced yields of 14.2% for yacon leaves and 11.4% for *karas tulang* roots. The difference in yield may be influenced by variations in plant organs, tissue structure, and the distribution of ethanol-soluble metabolites. Although yield indicates extraction efficiency, it does not directly represent biological potency because it reflects the total extracted compounds. Therefore, the yield results should be interpreted together with phytochemical profiles and α-amylase inhibitory activity to evaluate the contribution of bioactive constituents.

The extraction results showed that 300 g of yacon leaf simplicia produced 42.6 g of extract, corresponding to a yield of 14.2%, whereas 200 g of *karas tulang* root simplicia produced 22.8 g of extract, corresponding to a yield of 11.4% . The higher yield of yacon leaves may be related to differences in plant organs, tissue structure, solvent penetration, and the amount of ethanol-soluble compounds. Yacon leaves are known to contain various polar and semi-polar metabolites, including phenolic and flavonoid compounds, which may be efficiently extracted using hydroethanolic solvents (Widowati et al., 2021). However, extraction yield does not directly indicate biological potency because an extract with a lower yield may still contain highly active compounds that contribute to pharmacological activity (Febriyanti et al., 2025).

Phytochemical Screening

Extraction using 70% ethanol produced yields of 14.2% for yacon leaves (Smallanthus sonchifolius) and 11.4% for *karas tulang* roots (Chloranthus erectus). The difference in yield may be related to variations in plant organs, tissue structure, and the distribution of ethanol-soluble metabolites. The higher yield of yacon leaves may be associated with the abundance of polar and semi-polar secondary metabolites, such as phenolics and flavonoids, which are efficiently extracted using hydroethanolic solvents with (Ariska Dewi & Haryoto, 2025). The obtained yields are within the expected range for medicinal plant extraction and indicate that 70% ethanol was effective in recovering bioactive constituents. However, extraction yield alone cannot be used as a direct indicator of pharmacological potency because it represents the total extracted material, including both active and inactive compounds. Therefore, the biological activity of the extract depends more on the composition and concentration of bioactive metabolites rather than the total extract mass. In this study, the presence of flavonoids, polyphenols, alkaloids, and terpenoids identified through phytochemical screening suggests that the obtained yield contributed to the recovery of compounds with potential antioxidant activity (Khaisya Sabila, Bangkit Riska Permata, & Siwi Hastuti, 2026). These metabolites have been reported to inhibit carbohydrate-digesting enzymes through interactions with catalytic residues, which may explain the α-amylase inhibitory activity observed in the combined extract.

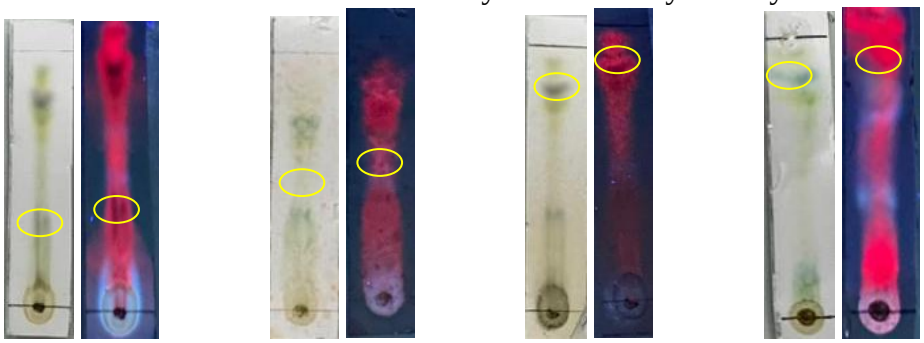
The extraction process yielded 14.2% for yacon leaves and 11.4% for *karas tulang* roots. These yield percentages are considered high and optimal when compared to extractions using non-polar solvents in previous studies, indicating that the hydroethanolic solvent (70% ethanol) was highly efficient in facilitating the mass extraction of polar and semi-polar metabolite compounds. This optimal extraction yield strongly correlates with a substantial content of

bioactive compounds possessing potent antidiabetic properties. To validate the specific compound classes contributing to this yield, phytochemical screening using thin-layer chromatography (TLC) was conducted. The characteristic color changes on the TLC plate following the application of specific reagents confirmed that the combined extract is highly rich in flavonoids, alkaloids, polyphenols, and terpenoids. These findings comprehensively substantiate the initial yield data; the presence of these compound groups not only dominates the extract's weight but is also empirically responsible for the α-amylase inhibitory activity. This result aligns with the study by (Ariska Dewi & Haryoto, 2025), which reported that ethanolic extract fractions of yacon leaves contained alkaloids, flavonoids, tannins, and triterpenoids based on TLC screening. It is also strongly supported by (Khaisya Sabila, Bangkit Riska Permata, & Siwi Hastuti, 2026), who reported that the ethanolic extract of yacon leaves from Wonosobo exhibited abundant positive results for phenolics and flavonoids while displaying very strong antioxidant activity.

Table 2. Phytochemical Screening Results

Secondary Metabolie	Positive Result	Interpretation
Flavonoid	Yellowish-brown spot appeared	Positive (+)
Alkaloid	Orange/red color appeared	Positive (+)
Polyphenol	Dark green color appeared	Positive (+)
Terpenoid	Dark blue color appeared	Positive (+)

Table 2 presents the results of phytochemical screening of the combined ethanolic extract of yacon leaves and *karas tulang* roots using thin-layer chromatography (TLC). The extract showed positive results for flavonoids, alkaloids, polyphenols, and terpenoids, as indicated by the appearance of characteristic color changes after spraying with specific detection reagents. The presence of these secondary metabolites suggests that the extraction process successfully recovered various bioactive compounds with potential biological activities. These phytochemical groups have been reported to contribute to carbohydrate-digesting enzyme inhibition through different mechanisms, although further quantitative analysis is required to determine their individual contribution to α-amylase inhibitory activity.

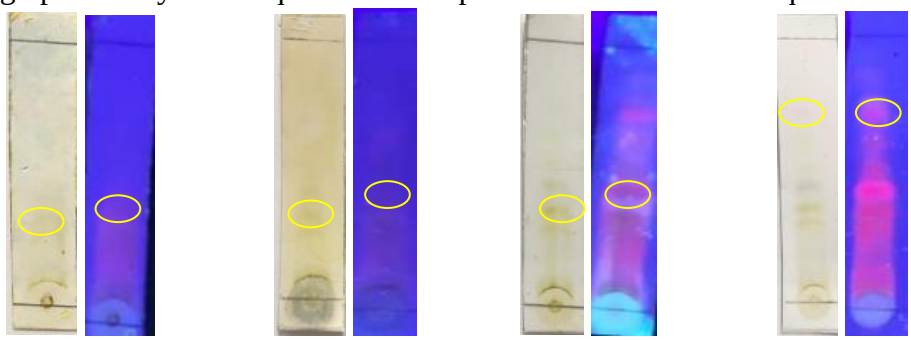


(a) (b) (c) (d)

Figure 1. Phytochemical Screening Results of Yacon Leaf Ethanol Extract for Compound Groups (a) Flavonoids, (b) Alkaloids, (c) Polyphenols, and (d) Terpenoids.

Figure 1 shows the TLC profile of ethanolic extract of yacon leaves for the identification of flavonoids, alkaloids, polyphenols, and terpenoids. The appearance of specific colored spots after visualization with selective reagents confirmed the presence of these metabolite groups. The detection of flavonoids and polyphenols in yacon leaves supports previous findings that this plant contains bioactive compounds associated with antioxidant and

antidiabetic activities. However, TLC analysis provides only qualitative evidence and further chromatographic analysis is required for compound identification and quantification.



(a) (b) (c) (d)

Figure 2. Phytochemical Screening Results of Ethanol Extract of *Karas tulang* Root Compounds of the Groups (a) Flavonoids, (b) Alkaloids, (c) Polyphenols, and (d) Terpenoids.

Figure 2 illustrates the TLC profile of ethanolic extract of *karas tulang* roots, demonstrating the presence of flavonoids, alkaloids, polyphenols, and terpenoids based on the characteristic color reactions obtained after derivatization. The detection of these metabolites indicates that *karas tulang* roots contain various secondary compounds that may contribute to their biological activity. The presence of multiple metabolite classes in the extract may provide a complementary effect when combined with yacon leaf extract; however, further quantitative and structural characterization is needed to confirm their role in α-amylase inhibition.

The positive result for flavonoids in this study was indicated by the appearance of yellow-brown spots after treatment with specific reagents . The presence of flavonoids in yacon leaf extract is supported by (Ariska Dewi & Haryoto, 2025), who reported that the ethyl acetate fraction of ethanolic yacon leaf extract contained flavonoids and demonstrated antidiabetic activity in alloxan-induced Wistar rats. (Khaisya Sabila et al., 2026) also reported that ethanolic yacon leaf extract was positive for flavonoids and had an antioxidant IC_{50} value of 28.08 ppm, indicating that flavonoids in yacon leaves may contribute to the biological activity of this plant. In *karas tulang*, (Hasan et al., 2023) reported that *Chloranthus erectus* extract contained flavonoids, while (Zemry et al., 2023) also detected flavonoids in leaf and twig extracts of *Chloranthus erectus* using various solvents.

The positive result for polyphenols was indicated by the appearance of a dark green color after spraying with $FeCl_3$, suggesting the presence of phenolic compounds in the combined ethanolic extract of yacon leaves and *karas tulang* roots. In this study, the detection of polyphenols through TLC screening provides preliminary evidence that these compounds may contribute to the observed α-amylase inhibitory activity; however, the exact contribution of each metabolite group cannot be confirmed because the total phenolic and flavonoid contents were not quantitatively determined. Previous studies have reported the presence of phenolic compounds in yacon leaves, supporting the findings of the current study, where ethanolic yacon leaf extract showed positive phenolic identification (Khaisya Sabila et al., 2026). Furthermore, Serra-Barcellona et al. (2025) reported that yacon leaf decoction contained total phenolic compounds of 77.47 ± 1.23 mg GAE/g, with caffeic acid and chlorogenic acid identified using TLC and HPLC, indicating that phenolic constituents are important components of S. sonchifolius. In addition, Chloranthus erectus has also been reported to contain phenolic compounds, with methanolic leaf extract showing the highest phenolic content of 9.64 ± 0.15 µg GAE/g, while hexane twig extract contained 7.39 ± 0.27 µg GAE/g (Zemry et al., 2023). Therefore, the presence of polyphenols in the combined extract may support its biological activity, but further quantitative analysis and compound profiling are required to establish the direct relationship between phenolic content and α-amylase inhibition.

The positive result for alkaloids in this study was indicated by the appearance of orange to red coloration after treatment with Dragendorff reagent . This result is consistent with (Ariska Dewi & Haryoto, 2025), who reported that fractions of ethanolic yacon leaf extract contained alkaloids based on TLC analysis. In *Chloranthus erectus*, (Hasan et al., 2023) reported that methanolic and aqueous extracts of *Chloranthus erectus* contained alkaloids, flavonoids, terpenoids, saponins, quinones, glycosides, and steroids. (Musayyaf, 2024) also reported that ethanolic extract of *karas tulang* contained alkaloids, saponins, flavonoids, and tannins and showed antidiabetic activity in alloxan-induced Wistar rats.

The positive result for terpenoids was indicated by the appearance of dark blue spots after spraying with Liebermann–Burchard reagent, confirming the presence of terpenoid compounds in the combined ethanolic extract of yacon leaves and *karas tulang* roots. In the present study, the detection of terpenoids through TLC analysis suggests that these compounds may contribute to the biological activity of the combined extract; however, their specific role in α-amylase inhibition remains unclear because individual compound activity and quantitative terpenoid content were not evaluated. Previous studies have reported the presence of terpenoid compounds in Smallanthus sonchifolius, where ethanolic extract contained polyphenols, flavonoids, tannins, glycosides, and terpenoids (Indriyanti et al., 2025),. Moreover, yacon extracts have been reported to exhibit inhibitory activity against carbohydrate-digesting enzymes, including α-amylase, α-glucosidase, and G6Pase, which may be associated with the combined action of various secondary metabolites, including terpenoid compounds (Widowati et al., 2021). In Chloranthus erectus, the presence of terpenoids has also been reported in different solvent extracts, supporting the findings of the current study (Hasan et al., 2023; Zemry et al., 2023). Therefore, the detected terpenoids may act as complementary contributors within the phytochemical profile of the combined extract, while further studies using quantitative analysis and compound identification are needed to clarify their contribution to α-amylase inhibitory activity.

Based on the TLC results in this study, *karas tulang* root extract showed Rf values of 0.50, 0.42, 0.47, and 0.60, while yacon leaf extract showed Rf values of 0.70, 0.73, 0.63, and 0.68. The differences in Rf values indicate variations in the chemical characteristics and polarity of compounds present in each extract, which may be influenced by plant species, plant organs, metabolite composition, stationary phase, mobile phase, and elution conditions. These variations suggest that the combination of both extracts may provide a broader phytochemical profile that could contribute to the observed α-amylase inhibitory activity. Previous research also used TLC analysis to confirm the presence of alkaloids, flavonoids, tannins, and triterpenoids in fractions of ethanolic yacon leaf extract (Ariska Dewi & Haryoto, 2025). However, TLC analysis only provides preliminary qualitative information and cannot determine the concentration or individual contribution of each compound to enzyme inhibition. Therefore, although the combined extract demonstrated α-amylase inhibitory activity in vitro, the relationship between the detected metabolites, IC_{50} differences compared with acarbose, and biological effectiveness cannot be fully established without further quantitative analysis and statistical evaluation. Advanced techniques such as HPLC, LC-MS/MS, or GC-MS are required to identify active compounds and clarify their contribution to the inhibitory mechanism.

Overall, the phytochemical screening results showed that the combined ethanolic extracts of yacon leaves and *karas tulang* roots contained flavonoids, alkaloids, polyphenols, and terpenoids. These findings support the assumption that the α-amylase inhibitory activity observed in this study may be related to the presence of these secondary metabolites. Thus, the phytochemical screening results in this study are consistent with previous studies and support the potential of the combined extract as a source of bioactive antidiabetic compounds.

α-Amylase Inhibitory Assay

Table 3. The α-Amylase Inhibitory Assay

Sampel	IC_{50} (µg/ml)
Akarbose	43,183 ± 1,335
Ekstrak	63,298 ± 0,162

Table 3 shows the inhibitory activity of acarbose and the combined ethanolic extract of yacon leaves and *karas tulang* roots against α-amylase expressed as IC_{50} values. Acarbose exhibited stronger inhibitory activity with an IC_{50} value of 43.183 ± 1.335 µg/mL compared with the combined extract, which showed an IC_{50} value of 63.298 ± 0.162 µg/mL. The lower IC_{50} value of acarbose indicates a higher enzyme inhibitory potency; however, the combined extract still demonstrated considerable α-amylase inhibitory activity, suggesting the presence of bioactive secondary metabolites that may contribute to enzyme inhibition.

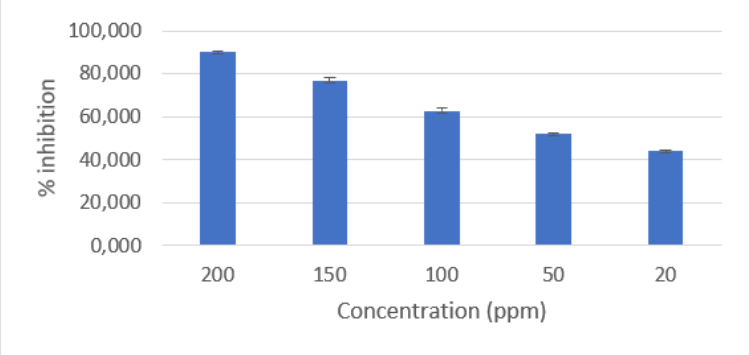


Figure 3. Inhibition of Acarbose

Figure 3 illustrates the percentage inhibition of α-amylase by acarbose at different concentrations (20–200 ppm). The results demonstrate a concentration-dependent inhibitory pattern, where increasing acarbose concentration resulted in higher enzyme inhibition. The highest inhibition was observed at 200 ppm, indicating the strong capacity of acarbose as a reference α-amylase inhibitor.

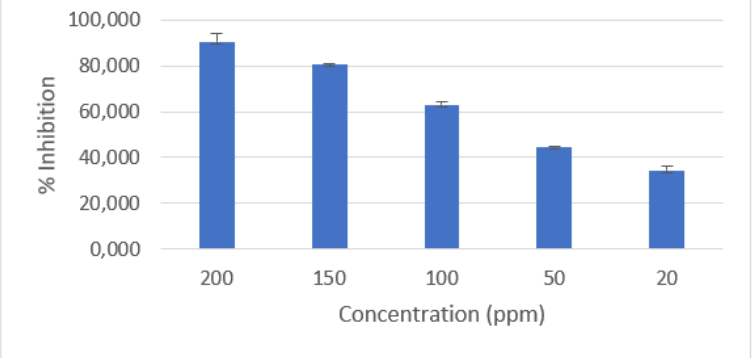


Figure 4. The Results of the Inhibition of the Combination of Ethanol Extract of Yacon Leaves and *Karas tulang* Root

Figure 4 shows the α-amylase inhibitory activity of the combined ethanolic extract of yacon leaves and *karas tulang* roots at concentrations ranging from 20 to 200 ppm. The extract exhibited a concentration-dependent increase in inhibition, with the highest inhibitory activity observed at 200 ppm. This pattern indicates that increasing extract concentration enhances the interaction between bioactive compounds and α-amylase, resulting in greater inhibition of starch hydrolysis.

The α-amylase inhibitory assay demonstrated that both acarbose and the combined ethanolic extract of yacon leaves and *karas tulang* roots exhibited concentration-dependent

inhibitory activity. The percentage of inhibition increased from 44.262% to 90.152% for acarbose and from 34.528% to 90.359% for the combined extract at concentrations ranging from 20 to 200 ppm, indicating that higher concentrations resulted in greater inhibition of α-amylase activity. This concentration-dependent response suggests that the bioactive constituents present in the combined extract may interact with the enzyme and reduce starch hydrolysis; however, the similarity of inhibition percentages at certain concentrations should be interpreted cautiously because statistical analysis comparing the significance of differences between groups was not performed. Therefore, the observed inhibitory effect does not necessarily indicate that the extract has equivalent potency to acarbose, particularly because acarbose is a purified synthetic inhibitor with a specific mechanism of action, whereas plant extracts contain complex mixtures of active and inactive compounds. α-Amylase inhibition remains an important pharmacological approach for controlling postprandial hyperglycemia by delaying carbohydrate digestion and reducing glucose release after meals (Hidayati, Shinta Mayasari, Setyaningrum, Wardani, & Aini, 2022). Acarbose was used as a positive control because it inhibits carbohydrate-digesting enzymes and has been clinically applied to reduce postprandial blood glucose elevation (Dirir et al., 2022). Nevertheless, further studies involving statistical comparison, enzyme kinetics, compound profiling, and in vivo evaluation are required to confirm the antidiabetic potential of the combined extract.

In the combined extract group, the increase in inhibition percentage from low to high concentrations indicates that the extract was able to inhibit α-amylase in a concentration-dependent manner. This finding is consistent with (Rawal et al., 2025), who reported that ethanolic extract of yacon leaves showed dose-dependent α-amylase inhibitory activity. (Widowati et al., 2021) also reported that yacon leaf extract has antidiabetic potential through antioxidant activity and inhibition of α-amylase, α-glucosidase, and glucose-6-phosphatase. At 100 ppm, the combined extract produced 62.903% inhibition, which was nearly comparable to acarbose at 62.735%. At 150 and 200 ppm, the combined extract even showed slightly higher inhibition than acarbose, with values of 80.676% and 90.359%, respectively. This phenomenon suggests that at moderate to high concentrations, the bioactive compounds in the combined extract were able to strongly inhibit α-amylase activity. (Quazi et al., 2022) also reported that polyherbal extracts could exhibit α-amylase inhibitory activity approaching that of acarbose at certain concentrations.

The IC_{50} value of acarbose was 43.183 ± 1.335 ppm, while the combined extract had an IC_{50} value of 63.298 ± 0.162 ppm. IC_{50} represents the concentration of a sample required to inhibit 50% of enzyme activity; therefore, the lower the IC_{50} value, the stronger the inhibitory activity. Based on these values, acarbose showed stronger α-amylase inhibitory potential than the combined extract because it required a lower concentration to achieve 50% inhibition. This comparison is commonly observed in natural product studies because plant extracts are complex mixtures consisting of both active and inactive compounds, whereas acarbose is a drug compound that works more specifically on carbohydrate-digesting enzymes (Hidayati et al., 2022). (Hidayati et al., 2022) also reported that the extract and fractions of Peperomia pellucida showed α-amylase inhibitory activity, but their activity was still lower than acarbose. Nevertheless, the IC_{50} value of the combined extract still indicates good activity because, at concentrations of 100–200 ppm, the extract produced inhibition of more than 60% up to 90%.

The α-amylase inhibitory activity of the combined extract is likely related to its secondary metabolite content, such as flavonoids, polyphenols, tannins, alkaloids, and terpenoids. (Lam et al., 2024) explained that flavonoids can act as α-amylase and α-glucosidase inhibitors through interactions with carbohydrate-digesting enzymes. Flavonoid and phenolic compounds may interact with amino acid residues in enzymes through hydrogen bonding, hydrophobic interactions, and possible binding to active or allosteric sites (Lam et al., 2024). (Wongsa et al., 2022) also reported that phenolic profiles in various herbal plants are associated with inhibitory activity against digestive enzymes relevant to type 2 diabetes mellitus. Yacon

leaves may provide an important contribution because Smallanthus sonchifolius has been reported to contain phenolic and flavonoid compounds associated with antidiabetic activity. (Rawal et al., 2025) reported that Smallanthus sonchifolius leaf extract exhibited antioxidant activity, α-amylase inhibition, and hypoglycemic effects in an in vivo model. Rawal et al. (2024) also showed that polymatin B and chlorogenic acid had binding affinity toward α-amylase based on an in silico approach. Widowati et al. (2021) the presence of α-amylase inhibitory activity-associated phenols, flavonoids, steroids/triterpenoids, saponins, tannins, and alkaloids in yacon leaf extract was found. Given that Chloranthus erectus is known to include tannins, alkaloids, saponins, and flavonoids not to mention that it lowers blood glucose levels in alloxan-induced Wistar rats the combined extract may also benefit from the action of Karas bone roots.(Musayyaf, 2024).

CONCLUSION

The combined ethanolic extracts of yacon leaves (*Smallanthus sonchifolius*) and *karas tulang* roots (*Chloranthus erectus*) demonstrated in vitro α-amylase inhibitory activity. Phytochemical screening showed that the extract combination contained flavonoids, alkaloids, polyphenols, and terpenoids, which may contribute to its inhibitory activity against α-amylase. The extraction yield of yacon leaves was 14.2%, while *karas tulang* roots produced a yield of 11.4%. The α-amylase inhibitory assay showed a concentration-dependent response, with inhibition of the combined extract increasing from 34.528% at 20 ppm to 90.359% at 200 ppm. The IC_{50} value of the combined extract was 63.298 ± 0.162 ppm, while acarbose as the positive control showed an IC_{50} value of 43.183 ± 1.335 ppm. Although the combined extract was less potent than acarbose based on IC_{50} value, it still showed promising inhibitory activity and may be developed as a natural antidiabetic candidate through inhibition of carbohydrate-digesting enzymes. Further studies are recommended to evaluate the activity of each single extract, different combination ratios, total phenolic and flavonoid contents, α-glucosidase inhibition, active compound profiling, and in vivo antidiabetic activity.

DECLARATION OF AI AND AI ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The writers used AI to aid with language editing, translation, document organization, and academic writing enhancement while they were preparing this paper. The authors accept all responsibility for the final manuscript's correctness, originality, and integrity after utilizing this tool and reviewing, editing, and verifying the material as necessary.

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AUTHOR CONTRIBUTIONS

Author 1: Conceptualization, investigation, methodology, data collection, formal analysis, data interpretation, and writing the original draft.

Author 2: Supervision, validation, methodology review, project administration, resources.

Author 3: Writingreview and editing.

All authors have read and approved the final version of the manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Ariska Dewi, T., & Haryoto, H. (2025). Aktivitas Antidiabetes Fraksi Dari Ekstrak Etanol Daun Yakon (Smallanthus sonchifolius) Pada Tikus Galur Wistar Yang Diinduksi Aloksan Secara In Vivo. *CERATA Jurnal Ilmu Farmasi*, *16*(2), 174–184. https://doi.org/10.61902/cerata.v16i2.2015

Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, *21*(4), 1049–1079. https://doi.org/10.1007/s11101-021-09773-1

Febriyanti, R. M., Indradi, R. B., Maisyarah, I. T., Iskandar, Y., Susanti, R. D., & Lestari, D. (2025). Alpha-amylase and Alpha-glucosidase enzymes inhibition and antioxidant potential of selected medicinal plants used as anti-diabetes by Sundanese community in West Java, Indonesia. *BMC Complementary Medicine and Therapies*, *25*(1), 426. https://doi.org/10.1186/s12906-025-05144-x

Hasan, N., Ariffin, S., Azzeme, A. M., Hasbullah, N. I., Nawahwi, M. Z., & Bin Zemry, I. H. (2023). Preliminary phytochemical screening of medicinal herb, SAMBAU PAYA (Chloranthus erectus). *Materials Today: Proceedings*, *88*, 6–9. https://doi.org/10.1016/j.matpr.2023.01.365

Hidayati, S., Shinta Mayasari, S., Setyaningrum, L., Wardani, A. D., & Aini, Q. (2022). In vitro antidiabetic activity of Peperomia pellucida extract and fraction by alpha-amylase inhibition pathway. *Pharmaciana*, *12*(2), 156. https://doi.org/10.12928/pharmaciana.v12i2.21874

Indriyanti, R. A., Kharisma, Y., Damayanti, M. M., Yuniarti, L., Trusda, S. A. D., & Fatmawati, S. A. (2025). The Phytochemical Screening and Antioxidant Activity of Ethanol Extract Yacon Tubers (Smallanthus sonchifolius). *Bandung Conference Series: Medical Science*, 1559–1566. https://doi.org/10.29313/bcsms.v5i3.21660

Khaisya Sabila, Bangkit Riska Permata, & Siwi Hastuti. (2026). Antioxidant Body Lotion Formulation From Yakon Leaf Extract (Smallanthus Sonchifolius). *International Journal of Health Engineering and Technology*, *4*(6). https://doi.org/10.55227/ijhet.v4i6.659

Lam, T.-P., Tran, N.-V. N., Pham, L.-H. D., Lai, N. V.-T., Dang, B.-T. N., Truong, N.-L. N., … Tran, T.-D. (2024). Flavonoids as dual-target inhibitors against α-glucosidase and α-amylase: a systematic review of in vitro studies. *Natural Products and Bioprospecting*, *14*(1), 4. https://doi.org/10.1007/s13659-023-00424-w

Musayyaf, A. F. (2024). Determination of Antidiabetic Activity of Ethanolic Extract of *Karas tulang* Leaves (Chloranthus Erectus) In Alloxan-Induced Wistar Rats. *International Journal of Medical Science and Clinical Research Studies*, *04*(12). https://doi.org/10.47191/ijmscrs/v4-i12-39

Quazi, A., Patwekar, M., Patwekar, F., Alghamdi, S., Rajab, B. S., Babalghith, A. O., & Islam, F. (2022). In Vitro Alpha-Amylase Enzyme Assay of Hydroalcoholic Polyherbal Extract: Proof of Concept for the Development of Polyherbal Teabag Formulation for the Treatment of Diabetes. *Evidence-Based Complementary and Alternative Medicine*, *2022*, 1–7. https://doi.org/10.1155/2022/1577957

Rawal, P., Pandey, B., Yadav, R. K., & Panta, S. (2025). Antioxidant, Alpha-Amylase Inhibitory and Hypoglycemic Activity of *Smallanthus sonchifolius* Leaves from Nepal:

An Integrated In Vitro, In Vivo, and In Silico Approach. *Food Science & Nutrition*, *13*(1). https://doi.org/10.1002/fsn3.4672

Serra-Barcellona, C., Habib, N. C., Mercado, M. I., Sánchez, S. S., & Genta, S. B. (2025). Yacon leaf aqueous extract: Effects on diabetic oxidative stress and its therapeutic implications. *Journal of Phytomoleculs and Pharmacology*, *4*(1), 61–73. https://doi.org/10.56717/jpp.2025.v04i01.038

Widowati, W., Tjokropranoto, R., Wahyudianingsih, R., Tih, F., Sadeli, L., Kusuma, H. S. W., … Agatha, F. A. (2021). 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. *Journal of Reports in Pharmaceutical Sciences*, *10*(2), 247–255. https://doi.org/10.4103/jrptps.JRPTPS_3_21

Wongsa, P., Phatikulrungsun, P., & Prathumthong, S. (2022). FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. *Scientific Reports*, *12*(1), 6631. https://doi.org/10.1038/s41598-022-10669-z

Zemry, I. H., Hasan, N. A., Hasbullah, N. I., Nawahwi, M. Z., Mohamad Azzeme, A., Ahmad, S. N. D., & Ariffin, S. (2023). Antioxidant Potential of Chloranthus erectus (Chloranthaceae) from various solvents extract. *Journal of Experimental Biology and Agricultural Sciences*, *11*(1), 75–80. https://doi.org/10.18006/2023.11(1).75.80