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# Dipeptidyl peptidase 4 (DPP-4) inhibitory activity of leaf extract and sesquiterpene lactones isolated from *Smallanthus sonchifolius*

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**Abstract:** The Sesquiterpene lactones, enhydrin and uvedalin, are the chemotype and subtype compounds of the yacon leaf. They are known to have antidiabetic activity, but their mechanisms are not precise. One mechanism of antidiabetic agents is to inhibit the activity of the dipeptidyl peptidase-4 enzyme (DPP-4). The research aimed to determine the inhibitory effects of the extract, enhydrin, and uvedalin isolated from the yacon leaf on DPP-4 enzyme activity. Yakon leaves were extracted using 70% ethanol by the maceration method. Enhydrin and uvedalin were obtained from previous research. The inhibitory activity of the DPP-4 enzyme was then determined by the fluorescence assay method using a multi-well plate reader. Sitagliptin was used as a standard inhibitor of the DPP-4 enzyme. The ethanol extract of yacon leaves inhibits the DPP-4 enzyme with an $IC_{50}$ of 856.9 ppm. The percentage inhibition of the DPP-4 enzyme by the enhydrin and uvedalin at a concentration of 250 ppm was 2.37% and 35.16%, respectively. The inhibitory potency of the pure isolated compounds was not substantially greater than that of the crude extract itself. These results led us to conclude that the overall contribution of enhydrin and uvedalin to the extract's DPP-4 inhibitory activity is modest, suggesting the presence of other active compounds within the extract. However, it may provide a new approach to the treatment of type 2 diabetes.

**Keywords**: Dipeptidyl peptidase-4, Yacon, Enhydrin, Uvedalin, antidiabetic

## Introduction

Type 2 diabetes mellitus is one of the metabolic diseases whose prevalence continues to increase globally. According to data from the International Diabetes Federation (IDF), the number of diabetes sufferers worldwide might exceed 700 million people by 2045 if no effective prevention and management efforts are made [1]. One of the strategies in managing type 2 diabetes is to inhibit the activity of the enzyme Dipeptidyl peptidase 4 (DPP-4), which plays a role in the degradation of incretins such as GLP-1 (glucagon-like peptide-1) and glucose-dependent insulinotropic polypeptide (GIP), which are responsible for increasing insulin secretion and reducing glucagon production after meals [2]. DPP-4 inhibitors have been widely used as an effective therapy in increasing incretin levels, thereby helping to control blood glucose levels in patients with type 2 diabetes [3]. Nevertheless, the use of synthetic DPP-4 inhibitors is often associated with undesirable



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side effects, necessitating alternatives derived from natural sources that are safer and more effective.

Yakon (Smallanthus sonchifolius) is a plant known to have potential as a natural source for diabetes treatment [4–6]. Yakon leaves contain various bioactive compounds, including sesquiterpene lactones with significant biological activity [7]. Among these sesquiterpene lactones, the compound enhydrin is known as the main chemotype, while uvedalin is a subtype that also contributes significantly to the biological activity of this plant [8]. Enhydrin and uvedalin have been studied for their various pharmacological activities, including potential as anti-inflammatory, antioxidant, and anticancer agents [9,10]. However, their potential as DPP-4 enzyme inhibitors has not been extensively explored. In-depth studies on the DPP-4 inhibitory activity of these compounds can open new opportunities in the development of safer and more effective natural-based diabetes therapy agents.

Therefore, this research aims to evaluate the DPP-4 enzyme inhibition activity of yacon leaf extract and identify the active compounds responsible for this activity, focusing on sesquiterpene lactones, specifically enhydrin and uvedalin. In addition, the results of this research may provide new insights for further development of yakon leaves as a natural source of DPP-4 inhibitors, offering a safe and effective antidiabetic agent.

## Materials and Methods

### Chemicals and Instruments

DPP-4 Inhibitor Screening Kit (MAK203, Sigma-Aldrich, USA), HPLC-grade methanol and acetonitrile (J.T. Baker, USA), Chloroform and dimethyl sulfoxide (Merck). A Multimode Microplate Reader with fluorescence detection was used in the in vitro bioassay (GloMax® Discover Microplate Reader, Wisconsin, USA). Preparative HPLC from Waters 1525, a 2998 PDA detector, Empower 3 Software, and a Sunfire $C_{18}$ Column (150 × 7.8 mm; 5 µm).

### Plant Materials

Freshly harvested yacon leaves were obtained from a plantation in the Wonosobo region of Central Java, Indonesia. Next, the plant determination was processed at the Department of Biology Pharmacy, Universitas Gadjah Mada, to confirm the plant species. The utilized leaves were mature and exhibited a vibrant green hue. Following collection, the leaf samples underwent washing and subsequent drying in an oven maintained at 50°C for 24 hours.

### Preparation of yacon leaf extract (YLE) and sesquiterpene lactones isolates

Dried yacon leaves were extracted using a 70% ethanol solvent. It was performed as a modified batch extraction process, utilizing kinetic maceration with continuous stirring to transfer the desired components into the solvent effectively. A total of 100 g of dried leaf powder was soaked in 1 L of ethanol, then stirred using a magnetic stirrer at a speed of 200 rpm for 1 hour. The resulting filtrate was then filtered and concentrated using a



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rotary evaporator to produce a crude extract. Then, in accordance with our earlier study protocols, the method of isolating sesquiterpene lactone molecules was conducted [11]. To summarize, a rinse extraction method was used to extract 100 g of dried yacon leaf powder with chloroform at room temperature for one minute. Following that, 10 mL of a 70% methanol solution was added to the crude chloroform extract, which was then kept at -20 °C to promote crystal formation. The resulting crystals were subsequently purified by preparative HPLC until enhydrin and uvedalin, two pure isolates, were effectively obtained. The solvent used for separation by preparative HPLC was a 40% acetonitrile: 60% water gradient on a C18 column (150 × 7.8 mm, 5 µm).

**The DPP-4 enzyme inhibition activity assay**

The Dipeptidyl peptidase-4 (DPP-4) enzyme inhibition activity was assessed using the DPP4 Inhibitor Screening Kit (Sigma-Aldrich, MAK203), which relies on the fluorometric detection of the enzymatic reaction products. A fluorescence microplate reader with an excitation wavelength of 365 nm and an emission wavelength of 445 nm (λex = 365 nm/λem = 445 nm) was used to conduct this test in a 96-well format. For sample preparation, our test samples—whether the yacon leaf extract or its isolated compounds—were first dissolved in DMSO to a stock concentration four times (4x) greater than the final desired concentration. For controls, we prepared an Enzyme Control containing only the Assay Buffer (no inhibitor) and an Inhibitor Control using sitagliptin, the standard inhibitor provided with the kit.

The assay began by pipetting 25 µL of each sample solution into the wells of the 96-well plate in triplicate. These samples included the yacon extract at various concentrations (1000, 500, 250, 125, and 62.5 ppm), enhydrin (250 ppm), uvedalin (250 ppm), and our control solutions. Next, we prepared a reaction mixture containing 49 µL of DPP4 Assay Buffer and 1 µL of the DPP4 Enzyme, and then added 50 µL of this mixture to each well. The microplate was then incubated at 37 °C for 10 minutes and protected from light. Following this initial incubation, we added 25 µL of the substrate mixture (consisting of 23 µL DPP4 Assay Buffer and 2 µL DPP4 Substrate) to each well. After ensuring thorough mixing, the plate was incubated again at 37 °C.

Fluorescence readings were taken in kinetic mode, with measurements recorded every minute over 30 minutes. The resulting fluorescence data were plotted against time to determine the slope of the curve (ΔFLU/minute), which reflects the rate of enzymatic activity. We then used this slope value to calculate the percentage of inhibition relative to the enzyme control using the following formula:

$$\text{Percentage of relative inhibition (\%)} = \frac{SlopeEC - SlopeSM}{SlopeEC} \times 100\%$$

SlopeEC = The Slope of the Enzyme Control
SlopeSM = The Slope of the Sample Inhibitor



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## Results and Discussions

### Extraction and Isolation of Sesquiterpene Lactones from Yacon Leaves

The antidiabetic properties of yacon leaf extract are well-documented in various studies [7]. Although researchers have extensively investigated the active compounds responsible for this effect, no single component has been conclusively identified as the primary contributor to its hypoglycemic activity. Sesquiterpene lactones are one of the major constituents found in yacon leaf extract and can be utilized as characteristic marker compounds [12].

In this work, we successfully isolated two sesquiterpenoid lactone compounds from yacon leaves. During the isolation process, we initially used recrystallization to obtain the target compounds. Interestingly, the crystals that formed were not a single substance but a mixture of two distinct sesquiterpenoids. Further analysis by HPLC revealed that compound 1 was dominant, making up about 65% of the mix, while the second compound was present in roughly half that amount (Figure 1). To separate them, we employed preparative HPLC, which allowed us to obtain both compound 1 and compound 2 in their pure forms. We had previously identified these two compounds in our earlier research using detailed $^{1}H$ and $^{13}C$ NMR spectroscopy along with LC-MS analysis [11]. Through that work, they were confirmed to be **enhydrin** (compound 1) and **uvedalin** (compound 2).

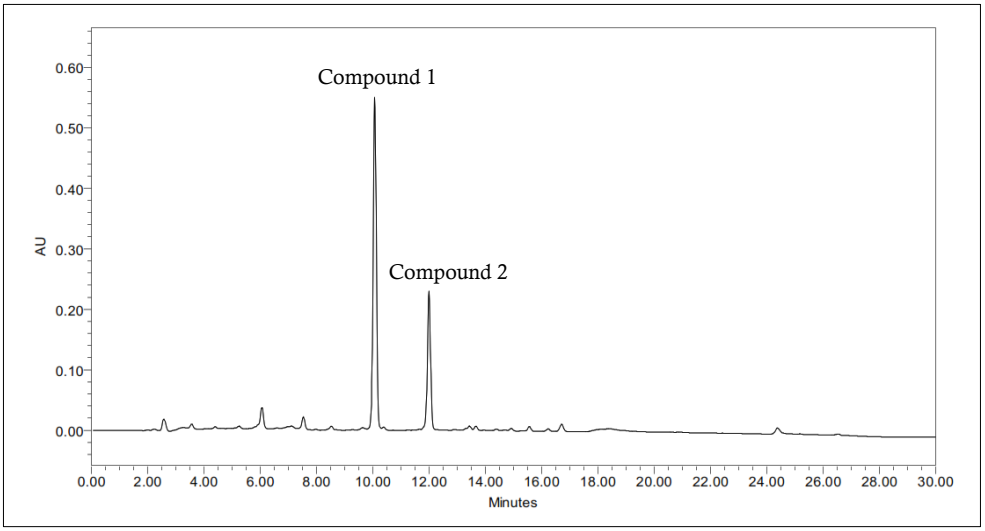


**Figure 1.** Chromatogram of the recrystallized yacon leaf extract, showing two major compounds: **compound 1** (61.33% peak area) and **compound 2** (25.65% peak area)

### Activity and Potency of the Crude Yacon Leaf Extract in DPP-4 Inhibition

Our investigation revealed a strong and highly linear correlation between the concentration of the yacon leaf extract and its ability to inhibit the DPP-4 enzyme. This relationship is described by the linear regression equation y=0.0407x+15.123, with a near-



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perfect coefficient of determination ($R^2$) of 0.9965, indicating the excellent validity of the model.

To quantitatively measure the extract's potency, we calculated its $IC_{50}$ value (the concentration required to inhibit 50% of the enzyme's activity), which was determined to be 856.9 ppm. It is important to note that this value comes from a crude extract, a complex mixture of various compounds. Nevertheless, this level of potency is quite significant for an unrefined extract [13]. Our findings also align with previous research by Riyanti *et al.* [14], who reported that a yacon leaf extract at 1000 ppm (1 mg/mL) inhibited DPP-4 by 52.2%, a figure that is comparable to our results.

**Table 1.** Relative inhibition of yacon leaf extract and its isolated compounds (enhydrin and uvedalin) against the DPP-4 enzyme activity

| Samples | Relative inhibition (%) ± (SD) |
|---|---|
| Sitagliptin (inhibition control) | 99.79 ± 0.22 |
| Yacon leaf extract 62.5 ppm | 16.76 ± 0.97 |
| Yacon leaf extract 125 ppm | 19.88 ± 2.61 |
| Yacon leaf extract 250 ppm | 26.84 ± 4.10 |
| Yacon leaf extract 500 ppm | 35.49 ± 4.87 |
| Yacon leaf extract 1000 ppm | 55.58 ± 3.89 |
| Compound 1 (enhydrin) 250 ppm | 2.37 ± 1.28 |
| Compound 2 (uvedalin) 250 ppm | 35.16 ± 1.40 |

## Effect of Sesquiterpene Lactones in DPP-4 Inhibition: Enhydrin and Uvedalin

The promising activity of the crude extract prompted us to identify the compounds responsible. We chose to isolate enhydrin and uvedalin, two sesquiterpenoid lactones known to be the characteristic *chemotype*, or chemical markers, of yacon leaves. The structures of both compounds were identified using $^{1}H$ and $^{13}C$ NMR, as well as LC–MS. Comparison with reference standards confirmed that both compounds were identical to their respective references. The details of these identifications have been described in our previous report [11]. Upon testing, these two pure compounds displayed different inhibitory activities (Table 1). Uvedalin, which has one epoxide group, showed more potent inhibition than enhydrin, which has two. This difference is likely due to their structural variations, specifically the number of epoxide groups. We suspect that the



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presence of a second epoxide group on enhydrin creates greater steric hindrance, impeding its ability to fit optimally into the enzyme's active site and thus reducing its effectiveness [15].

The inhibitory potency of the pure isolated compounds was not substantially greater than that of the crude extract itself. These findings led us to conclude that the overall contribution of enhydrin and uvedalin to the extract's DPP-4 inhibitory activity is actually modest. This conclusion is supported by an *in silico* study from Adianingsih *et al.* [16], which found that sesquiterpenoids generally have a weaker binding affinity for the DPP-4 enzyme. That same study highlighted that compounds from the phenolic class, such as phenolic acids and flavonoids, possess a much stronger affinity for specific receptors.

This finding is particularly relevant because yacon leaves are known to be rich in phenolic compounds in addition to their sesquiterpenoid lactone content. Therefore, we hypothesize that the antidiabetic efficacy of yacon leaf extract as a DPP-4 inhibitor is likely not driven solely by sesquiterpenoid lactones, but is significantly contributed to by the phenolic compounds it contains.

## Conclusion

According to this study, yacon leaf extract may be helpful as an inhibitor of the DPP-4 enzyme. Nevertheless, enhydrin and uvedalin, the two identified sesquiterpene lactone molecules, did not play a significant role in this activity. It might be inferred that other bioactive components in the extract are more responsible for the observed inhibitory effects due to their limited individual potential. However, it could provide a new approach for treating type 2 diabetes.

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