The Antioxidant Potential of Ethanol Extract of Yacon (Smallanthus sonchifolius) Tuber Based on Ferric Reducing Antioxidant Power (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC) Assays

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Abstract. Antioxidants play a vital role in neutralizing free radicals that trigger oxidative stress and contribute to degenerative diseases such as cancer, diabetes, and cardiovascular disorders. Growing public awareness of the risks associated with synthetic antioxidants has increased the demand for natural, safe, and environmentally friendly alternatives. The yacon tuber (Smallanthus sonchifolius), a member of the Asteraceae family, contains bioactive compounds—mainly phenolics and flavonoids—known for their strong antioxidant activity. This study aimed to assess the antioxidant potential of ethanol extracts of yacon tubers using two metal ion–reducing assays: Ferric Reducing Antioxidant Power (FRAP) and Cupric Reducing Antioxidant Capacity (CUPRAC). The extract was obtained through maceration in 95% ethanol and concentrated at 65°C. The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+} under acidic conditions, whereas the CUPRAC assay evaluates the reduction of Cu^{2+} to Cu^{+} under neutral conditions. All methods were measured by spectrophotometer. The antioxidant capacity of ethanol extracts of yacon tubers using the FRAP method was 16.22 ± 0.15 mg AAE/g and CUPRAC method was 4.54 ± 0.20 mg AAE/g. This indicates that the yacon ethanol extract has the iron and copper reduction capacity. The yacon can thus be considered a promising natural source of antioxidants for pharmaceutical and nutraceutical development.

Keywords: *Yacon, Natural antioxidants, FRAP, CUPRAC, Ethanol extract*.

A. Introduction

Reactive oxygen species (ROS) and free radicals are unstable molecules that can damage biomolecules, leading to oxidative stress and the onset of degenerative diseases such as cancer, diabetes, and cardiovascular disease[1,2]. Antioxidants protect biological systems by donating electrons or hydrogen atoms, thereby stabilizing these reactive species [1]. However, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have raised concerns regarding their potential toxicity, especially their hepatotoxicity, nephrotoxicity and neurotoxicity [3].

As a result, research into natural antioxidants derived from plants has expanded significantly [4]. The yacon plant (*Smallanthus sonchifolius*), native to the Andes but now cultivated in Indonesia, is a rich source of phenolic acids and flavonoids compounds recognized for their free radical scavenging potential [4,5]. Four main phenolic compounds such as chlorogenic acid, caffeic acid, coumaric acid and protocatechuic acid, as well as the amino acid tryptophan have been identified in yacon tubers [6].

Previous investigations on plants such as ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) have demonstrated similar antioxidant mechanisms, suggesting that yacon may provide comparable or complementary benefits [7]. Evaluating its antioxidant activity using standardized assays such as FRAP and CUPRAC can help determine its potential as a functional ingredient in health-promoting formulations.

B. Method

Tools

We used instruments such as Pyrex glassware, micropipette, oven, rotary evaporator, spectrophotometer and weighing scale.

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Materials

Ascorbic acid, ferric reducing antioxidant power (FRAP) reagent, cupric reducing antioxidant capacity (CUPRAC), Yacon tuber powder and ethanol 95% were used in this analytical procedures.

Procedure

Sample Preparation and Extraction

Yacon tubers were collected from Manoko, Lembang, West Java, Indonesia. After washing and drying at 35°C to a constant weight, the samples were ground into fine powder. Fifty grams of the powder were macerated with 250 mL of 95% ethanol for 72 hours under dark conditions with occasional stirring. The extract was filtered and concentrated using a rotary evaporator at 65°C until a viscous extract was obtained. The extraction was processed at the Laboratory of Pharmacy Faculty, Universitas Islam Bandung.

Antioxidant Activity Assays

Determination of antioxidant activity with FRAP and CUPRAC methods was carried out at the Pharmacy Laboratory of Universitas Perjuangan Tasikmalaya.

FRAP (Ferric Reducing Antioxidant Power):

The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+}, forming a blue complex with maximum absorbance at 593 nm. Results were expressed as mg ascorbic acid equivalent per gram of extract (mg AAE/g).

The FRAP reagent contained 2.5 ml of a 10 mmol/L TPTZ (2,4,6- tripyridy-s-triazine, Sigma) solution in 40 mmol/L HCl plus 2.5 ml of 20 mmol/L FeCl3 and 25 ml of 0.3 mol/L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C. Aliquots of 40 l sample supernatant were mixed with 0.2 ml distilled water and 1.8 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. The 1 mmol/L FeSO4 was used as the standard solution. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L FeSO4 [8].

CUPRAC (Cupric Reducing Antioxidant Capacity):

The CUPRAC assay measures the reduction of Cu^{2+}neocuproine complex to Cu^{+}neocuproine at 450 nm. This method operates at near-neutral pH, making it suitable for both hydrophilic and lipophilic antioxidants. Results were expressed as mg AAE/g extract.

Preparation of CUPRAC reagent

Preparation of 0.01 M CuCl2.2H2O solution was prepared by dissolving 0.4262 g of CuCl2.2H2O dissolved in water and diluted to 250 mL. Preparation of 0.0075 M neocuproine ethanolic solution was prepared by dissolving 0.039 g of Nc dissolved in 96% ethanol and diluted to 25 mL. Preparation of 1 M ammonium acetate buffer solution pH 7 was prepared by dissolving 19.27 g of NH4Ac in water and diluting it to 250 mL [9].

Determination of maximum wavelength

The test was carried out by mixing 1 mL of 0.01 M CuCl2.2H2O, adding 1 mL of 0.0075 M neocuproine ethanolic, 1 mL of 1 M ammonium acetate buffer, then adding 1 mL of ethanol p.a. and adding 0.1 mL of distilled water to the vial, then measuring absorption at a wavelength of 400-600 nm

using UV-Vis spectrophotometry. From the running results, the maximum wavelength value was obtained, namely 450 nm[9].

Statistical Analysis

Data were expressed as mean ± standard deviation. After obtaining the absorbance value of the sample and the standard ascorbic acid solution, the antioxidant power of the sample was then calculated by finding the AAE value based on absorbance and concentration data from the linear regression equation y = a + bx obtained from the standard curve

C. Result and Discussion

Initial Findings

Table 1. The antioxidant capacity of yacon tuber extract

METHOD	SAMPLE	Linearity Regression	*R* 2	Antioxidant capacity (mg AAE/g)	SD
FRAP	Yacon	y = 12.188x - 62,5	0.99	16.3242	
	Ethanol			16.0499	
	Extract			16.2743	
			Mean	16.2161	0.15
CUPRAC	Yacon	y = 0.0548x - 0,0016	0.99	4.6460	
	Ethanol Extract			4.6825	
				4.2993	
			Mean	4.5426	0.2

A linear regression equation of yacon tuber extract in FRAP assay are y = 12.188x - 62,5 with a correlation coefficient (R2) = 0,99 (Figure 1).

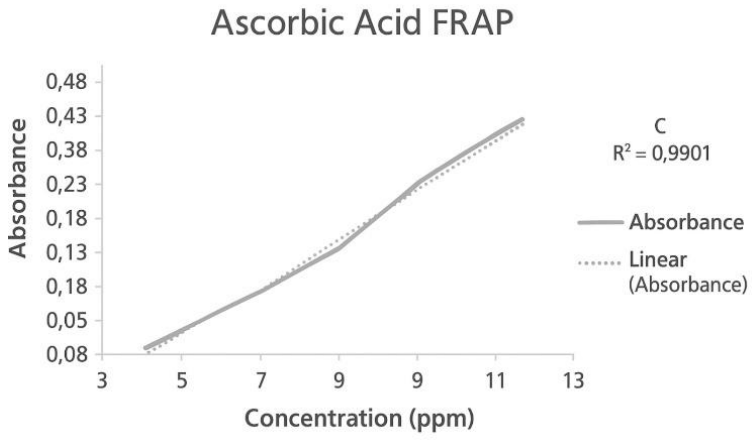

Figure 1. Yacon Tuber Extract FRAP curve

Antioxidant capacity of yacon ethanol extract in CUPRAC assay are plotted in equation y = 0.0548x - 0,0016 with a correlation coefficient (R2) = 0,99 in Figure 2.

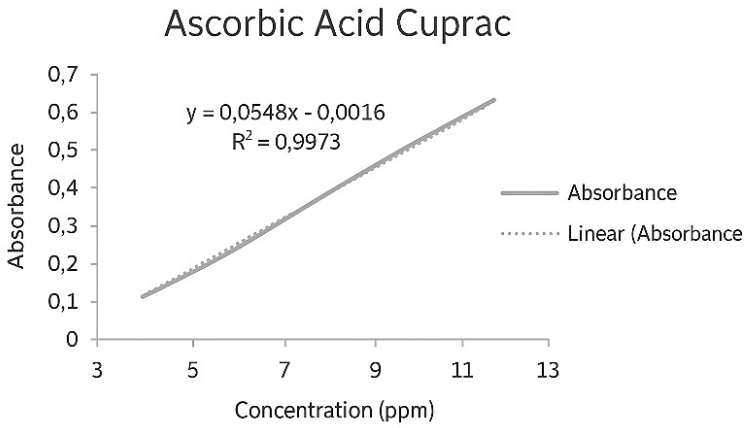

Figure 2. Yacon Tuber Extract CUPRAC curve

Analysis and Discussion

Oxidative stress in the body can damage directly cellular structure leading to many diseases. Free radicals such as reactive oxygen species, reactive nitrogen species and reactive sulfur species are produced naturally and can be restricted by antioxidant system. Free radicals are formed due to molecular oxygen that are catalyzed by oxidative stress in the cell and by trace metals such as iron, cobalt, and manganese in the connective tissue. The balance between free radicals and antioxidant system is needed to maintain the healthy body [10].

The endogenous, natural and synthetic antioxidant have been used as a defence against free radical. The composition of fruit, vegetable, leaves, tubers and roots can reduce oxidative stress and increase quality of life. Natural antioxidant consist of ascorbic acid, alpha tocopherol, beta carotene, lycopene and flavonoids[11,12].

There are several assay that can measure antioxidant capacity of bioactive compounds. The chemical antioxidant assay that can analyze electron transport include ferric ion reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC). Ascorbic acid has been established and therefore be used as a model for co-delivery bioactive component of natural antioxidant. Ascorbic acid is a hydrophylic antioxidant within aqueous environments such as plasma or cytosol. Ascorbic acid can scavenge free radicals like hydroxyl radicals [2,11].

The higher FRAP value of yacon extract suggests that the yacon tubers possesses a strong capacity to donate electrons and reduce Fe^{3+} to Fe^{2+}, indicative of potent redox potential. This result aligns with the high phenolic content previously reported for *Smallanthus sonchifolius* [5]. In contrast, the lower CUPRAC value reflects differences in redox potential between Fe^{3+}/Fe^{2+} and Cu^{2+}/Cu^{+} systems, as well as the sensitivity of each method to specific antioxidant classes [9].

The FRAP method is rapid, sensitive, and reproducible for phenolic compounds but operates in acidic conditions, which may limit its ability to measure antioxidants unstable at low pH. CUPRAC, by comparison, functions at physiological pH and can detect both hydrophilic and lipophilic antioxidants, though it may underestimate strongly reducing agents that react more efficiently under acidic conditions [13]. The CUPRAC method is simple, selective, stable and sensitive for thiol antioxidants, and can measure the ability of phenol compounds [14]. CUPRAC was chosen as the antioxidant activity test method because this method is more suitable for determining the antioxidant activity of a plant extract [9].

When compared to other plants, yacon exhibits antioxidant activity comparable to ginger (9–12 mg AAE/g, FRAP) and turmeric (3.8–5.5 mg AAE/g, CUPRAC) [7]. This finding highlights yacon's potential as a viable alternative natural antioxidant source. Additionally, yacon's phenolic acids chlorogenic, ferulic, and caffeic acids are known contributors to its antioxidant profile, while its fructooligosaccharides and inulin add prebiotic and metabolic benefits [5].

Phenolic acid antioxidant properties result from the presence of an aromatic ring, a carboxyl group and hydroxyl or methoxyl groups [4]. The ferulic acid antioxidant capacity based on the restriction of radical nitrogen species and radical oxygen species formation and neutralization of free radicals.

This compound also acts as a hydrogen donor. The ferulic acid can bind with copper and iron and avoid the generation of hydroxyl radicals[2].

The flavonoids in yacon tubers were found to have a relationship with lipid peroxidation, acetylcholinesterase, and butyrylcholinesterase inhibition. Tryptophan, known as a precursor of serotonin and melatonin, is the most abundant amino acid in yacon tuber. Tryptophan has antioxidant properties[5]. The yacon extract had antioxidant activity in protecting DNA from oxidative degradation, which was contributed by its phenolic compound composition. The scavenging of H2O2 by phenolic compounds is an important antioxidant defense mechanism [15]. This study was limited to in vitro antioxidant assays without further identification of bioactive compounds. Future studies should incorporate LC-MS/MS analysis and in vivo models to better understand the bioavailability and mechanisms of yacon's antioxidant constituents [5].

D. Conclusion

The ethanol extract of yacon tubers exhibits substantial antioxidant potential, with stronger reducing activity observed in the FRAP assay and CUPRAC assay. These findings support the use of yacon as a natural antioxidant source with potential applications in pharmaceutical, nutraceutical, and cosmeceutical formulations. Its strong phenolic based activity and environmentally friendly origin make it a valuable candidate for the development of sustainable health products.

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