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Antioxidant Properties of Various Yacon Leaf Water Extracts and Physicochemical Profile of Decoction During Refrigerated Storage

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Abstract

Background: Yacon (Smallanthus sonchifolius (Poepp.) H.Rob.) leaves show promising antioxidant properties, and have traditionally been used for diabetes management in Baturraden, Banyumas, Central Java, Indonesia. This study evaluated the effects of traditional extraction methods and crude drug-to-solvent ratios on the content and activity of antioxidants and physicochemical properties of yacon leaf water extracts during storage. Methods: Crude drugs were extracted by infusion, short-time decoction, and longer-time decoction at ratios of 1:10, 1:20, and 1:100 (w/v). Antioxidant content was analyzed using standard total flavonoid content (TFC) and total phenolic content (TPC). The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric-reducing antioxidant power (FRAP) assays. Yacon leaf water extracts at a ratio of 1:20 (w/v) were stored in tightly closed bottles at $4\pm 2^{\circ}C$ for 26 days. The organoleptic characteristics, color, pH, TFC, TPC, and DPPH scavenging activity were evaluated on days 0,1, 3, 6, 12, 18, and 26. Results: The different methods and crude drug-to-water ratios generated different antioxidant activities and contents of the yacon extracts. Yacon leaf decoction for 15 min in a ratio of 1:20 (w/v) produced extract with the best scavenging activity (450.27±5.48 mM Trolox equivalent (TE)/100 g dry weight (DW)), TFC (6.43±0.18 mg Quercetin equivalent (QE)/g DW), and TPC (3.91±0.04 mg gallic acid equivalent (GAE)/g DW). The yacon leaf decoction started to undergo aroma and PH changes on days 3 and 6, respectively. On day 12, the TFC, TPC, and DPPH SA of yacon leaf decoction remained 93.93±3.70, 96.52±1.81, and 89.99±0.91% of the freshly prepared extract, respectively. Conclusion: Our results suggest that extraction using the decoction method for 15 min at a water-to-crude drug ratio of 1:20 (w/v) generated an extract with the best antioxidant profile, which chemically started to change on day 12 during refrigerated storage.

Keywords: antioxidant, extraction, refrigerated storage, Smallanthus sonchifolius, stability

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INTRODUCTION

Yacon (Smallanthus sonchifolius (Poepp.) H.Rob.) leaves are traditionally used for diabetes treatment by people in Baturraden, Banyumas, Central Java, Indonesia (Utaminingrum et al., 2020). The antidiabetic activities of yacon leaves have been evaluated in in vitro and in vivo models, with promising results (Aligita et al., 2018; Simamora et al., 2020). Yacon leaves may contribute to the antidiabetic activity via indirect antioxidant mechanisms. The ethanolic extracts of yacon leaves showed considerably high 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and ferric-reducing antioxidant power (FRAP), which was strongly correlated with total phenolic content (TPC) (Hartanti et al., (2022).).

The extraction method greatly affects the quantity and quality of extracted antioxidant compounds. Optimal extraction conditions enable high yields of components while maintaining bioactive their antioxidant activity. Infusion and decoction are used to prepare traditional herbal commonly formulations. Infusion is a dilute extract prepared by pouring boiling water into crude drugs and straining it to obtain a lukewarm preparation. On the other hand, a decoction is obtained by boiling crude drugs in water on a decoct apparatus for specific times (Sujarwo et al., 2015). Hence, the temperature and time of extraction are different for both the traditional methods. The extraction temperature and time significantly affected TPC and DPPH scavenging activity of the Clinacanthus nutans (Sulaiman et al., 2017). Furthermore, different extraction methods result in different bioactivity profiles. The extracts obtained from the infusion of a polyherbal formulation showed a better reduction in blood pressure in hypertensive patients than the decoction (Triyono et al., 2018). Similarly, the ratio of plant material to solvent also affected the extraction efficiency. Generally, a small ratio is associated with the saturation of the solvent by the target compounds, which limits the mass transfer during extraction (Abubakar & Haque, 2020). The Indonesian Herbal Pharmacopeia (IHP) suggests a crude drug-to-solvent ratio of 1:10 (w/v). In contrast, *jamu godhog*, a traditional herbal drink, is prepared by decoction at a ratio of 1:20 (w/v) (Hartanti et al., 2023; Indonesian MoH, 2017).

Both decoction and infusion are traditionally prepared in small quantities for one-day use only, because they are often susceptible to degradation mechanisms influenced by temperature, light, oxygen exposure, and microbial spoilage if kept longer. Storage water extracts at low temperatures might preserve them longer because such conditions slow down the degradation processes (Jovanović et al., (2022)). . Hence, it is essential to understand the changes in pH, color, TPC, TFC, and DPPH scavenging activity during storage, which are crucial for maintaining their stability and shelf life. This study evaluated the effects of traditional extraction methods and crude drug-to-water ratios on DPPH scavenging activity, FRAP, TPC, and total flavonoid content (TFC) of yacon leaves. The physicochemical profile of the extract with the best antioxidant properties was also evaluated during refrigerated storage for 26 days.

MATERIALS AND METHODS Materials

Reagents such as DPPH, 2,4,6-tris(2-pyridyl)-Striazine (TPTZ), Folin-Ciocalteu reagent, gallic acid, quercetin, Trolox, acetic acid, aluminum chloride, hydrochloric acid, sodium acetate, sodium carbonate, sodium hydroxide, and solvents, that is, chloroform, deionized water, ethanol, and methanol, were of analytical grade (Sigma, United States). Crude drugs were prepared from mature yacon leaves collected from Sumbang, Banyumas, Central Java, Indonesia. The identity of the plants was confirmed to be *Smallanthus sonchifolius* (Poepp.) H.Rob. (Asteraceae) by Tusrianto, the botanist at the Laboratory of Pharmaceutical Biology, Universitas Muhammadiyah Purwokerto, Banyumas, Central Java, Indonesia (Ref. 272-RDS/(2022).

Method Extraction

The yacon leaves were dried using the rack-drying method. The crude drugs were pulverized into a fine powder. Powdered crude drugs were extracted with water by infusion and decoction (Abubakar & Haque, 2020). The crude drugs were poured into freshly boiled water ($95\pm2^{\circ}C$) and incubated for 5 min to obtain an infusion. They were boiled in a water bath (set at 100°C) for 15 minutes (Decoction-15) and 30 minutes (Decoction-30) using the decoction method. Three crude drug-to-solvent ratios were used for each extraction method: 1:10, 1:20, and 1:100 (w/v), respectively. The water extract was filtered and used for further antioxidant content and activity analyses.

Antioxidant content determination

TFC and TPC of the extracts were determined according to the compendial methods of the Indonesian Herbal Pharmacopeia (Indonesian MoH 2017). A 0.5 ml of properly diluted extract sample was homogenously mixed with 1.5 ml of ethanol, 0.1 ml of 10% AlCl₃, 0.1 ml of 1M CH₃COONa, and 2.8 ml of water. After standing at room temperature for 30 min, the absorbance of the reaction mixture was read at 426 nm. The absorbance was plotted on a calibration curve (y=0.0055x-0.1342), and the TFC was presented as mg quercetin equivalent (QE)/g DW crude drugs. For TPC evaluation, appropriately diluted extract samples (1.0 mL) were homogenously mixed with 7.5% Folin-Ciocalteu reagent (5.0 mL). The reaction mixture was allowed to stand for 8 min and was subsequently added to 1% NaOH (4.0 mL). After 40 min, the absorbance was recorded at 741 nm and plotted on a calibration curve (y=0.0401x+0.0437). TPC is presented as mg gallic acid equivalent (GAE)/g DW crude drug.

Antioxidant activity evaluation

The DPPH scavenging activity and FRAP of the extracts were analyzed using a previously reported method (Hartanti et al., (2022)). . A 0.5 ml properly diluted extract was homogenously mixed with 25 µg/ml DPPH solution (5.0 mL) in ethanol. After incubating at room temperature, the reaction mixture was read at 517 nm and protected from light for 30 min. The absorbance of each sample was calculated as the inhibitory percentage of the blank. The percentage inhibition was plotted on a calibration curve (y=0.0654x+9.1889), and the DPPH scavenging activity was expressed as mM Trolox equivalent (TE)/100 g DW crude drugs. For FRAP analysis, 0.21 ml of properly diluted extract sample was homogenously mixed with freshly prepared FRAP reagent (4.0 mL). The reaction mixture was allowed to stand for 40 min and the absorbance was recorded at 596 nm. The absorbance of the samples was plotted on a calibration curve (y=0.0401x+0.0437). TPC is presented as mM TE/g DW of crude drugs.

Storage condition

The yacon leaf water extracts in a crude drug-tosolvent ratio of 1:20 (w/v) were stored for evaluation of physicochemical properties following a previously reported method (Vongsak et al., 2013). A total of 15 ml of the extract was stored in tightly closed containers at 4±2°C for 26 days.

Physicochemical properties evaluation

Physicochemical properties were evaluated on days 0,1, 3, 6, 12, 18, and 26. Three untrained panelists organoleptically evaluated the taste, aroma, and color of the extracts. The color of the extract was read using a Chroma Meter (Konica Minolta, Japan) and reported as the color distance (Prommachart et al., 2020). The TFC, TPC, and DPPH scavenging activities of the extracts were determined using the same methods used to determine antioxidant content and activity.

Data analysis

The effect of the extraction method and crude drug-to-solvent ratio on the TFC, TPC, DPPH scavenging activity, and FRAP of the extracts was evaluated by two-way ANOVA. The effect of storage time on pH, TFC, TPC, and DPPH SA was analyzed using one-way ANOVA. The mean separation of the variants was evaluated using Duncan's post-hoc test. The correlation between TFC-TPC and DPPH scavenging activity (FRAP) was analyzed using Pearson's correlation test. Significant effects, differences, and correlations were considered at p < 0.05. All analyses were conducted utilizing SPSS ver. 26 (IBM, US).

RESULTS AND DISCUSSION

Infusion and decoction differed according to the heating intensity of the plant materials and target compounds. In this study, contact with the high temperature at the highest intensity occurred in the decoction for 30 min, followed by the decoction for 15 min and infusion. The temperature of the decoction was assumed to be approximately 90°C. Temperature plays a significant role in the extraction process. Heat enhances the extraction efficiency as it improves the solubility of the target compounds in the solvent and modifies the transfer of the compound outside the plant materials. However, heat may cause reactions that eventually lead to the degradation of thermolabile compounds (Abubakar & Haque, 2020). The crude drugs used in this study were characterized, and the TFC and TPC were chosen as the chemical contents of the crude drugs for the standardization process (Hartanti et al., (2022).).

Both the extraction method and crude drug-tosolvent ratio affected the extracted flavonoids in yacon leaf crude drugs, with decoction for 30 min of 100 parts of crude drugs in a part of water generated the extract with the highest TFC (9.08 \pm 0.08 mg QE/g DW). Similarly, the extraction method and ratio significantly affected the TPC of crude drugs. Infusion and short-time decoction in a ratio of 1:20 (*w*/*v*) generated the highest extracted phenolic compounds from yacon leaf crude drugs, with values of 6.06 \pm 0.37 and 3.91 \pm 0.04 mg GAE/g DW, respectively (Figure 1).

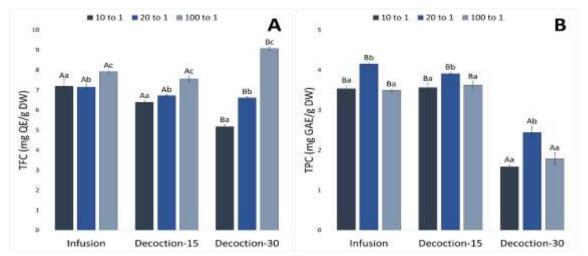


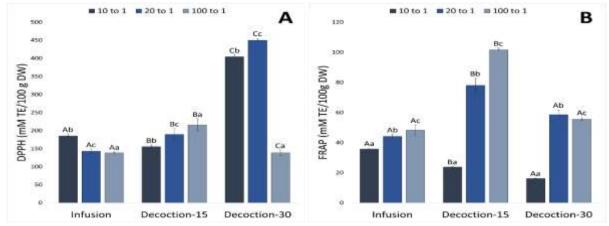
Figure 1. TFC (A) and TPC (B) of crude yacon leaf drugs. Ten to 1, 20 to 1, and 100 to 1 represent the crude drug-tosolvent ratios of 1:10, 1:20, and 1:100 (w/v), respectively. The different uppercase and lowercase alphabets on each bar represented significantly different values by extraction method and crude drug-to-solvent ratio, respectively (n = 3)

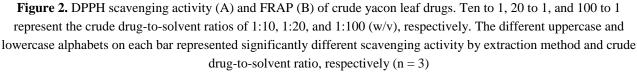
Considerably large quantities of apigenin, luteolin, myricetin, and rutin, both in free and bound forms, have been identified in yacon leaves (Khajehei et al., 2017; Padilla-González et al., 2020; Russo et al., 2015 Russo et al., 2015). The higher TFC of extracts from the decoction for 30 min resulted from the extended contact between the plant materials and heat. This result was similar to that of a study that reported that a longer extraction time resulted in mangosteen leaf extract with a higher flavonoid content (Rusli et al., 2024). Our results also indicate that the extracted flavonoids are likely to be stable under these conditions. Hence, our results are similar to those of Actinidia arguta and Actinidia deliciosa fruits, in which extracts obtained from infusion contained lower TFC than the decoction (Silva et al., 2019). However, longer contact with heat during decoction resulted in more flavonoid degradation. After heating at 90°C for 15 and 30 min in a food heat-treatment model, rutin was degraded by approximately 15% and 25%, respectively (Ioannou et al., 2020). Compared to ethanol extracts from an ultrasonic-assisted process of the same crude drugs in the same crude drug-to-solvent ratio, yacon water extract contained a lower TFC (Hartanti et al., (2022)). . Our results suggested that extraction using a crude drug-to-solvent ratio of 1:100 (w/v) resulted in the highest flavonoid-containing extracts, regardless of the method used. Higher flavonoid content in extracts obtained from a higher ratio of crude drug-to-solvent has also been reported in maceration-processed Moringa oleifera seeds, Rosa canina, Hippophae rhamnoides, and Crataegus monogyna fruit extracts (Ghafar et al., 2017; Predescu et al., 2016).

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Several phenolic compounds such as caffeic acid, caffeoylquinic acid, chlorogenic acid, p-coumaric acid, and ferulic acid have been identified in considerable quantities in yacon leaves (Khajehei et al., 2017; Padilla-González et al., 2020; Russo et al., 2015; Russo et al., 2015). Similar to flavonoids, the extraction of phenolic compounds from plant matrices is also a temperature-sensitive process. The optimum temperature for the extraction of these compounds from the aerial parts of O. basilicum and Robinia pseudoacacia flowers was 90.7 and 60°C, respectively (Do et al., 2020; Gajic et al., 2019). However, our yacon leaf extract results showed that contact with heat at approximately 90°C for 30 min resulted in the highest TPC-containing extracts, comparable to its ethanol counterpart (Hartanti et al., (2022)). . A higher TPC in extracts from decoctions over infusions has also been reported in Actinidia arguta and Actinidia deliciosa fruits and Centella asiatica aerial parts (Silva et al., 2019; Zainal et al., 2019). Extraction using a crude drug-to-solvent ratio of 1:20 (w/v) is commonly used to prepare jamu gendong, which showed the highest TPC. Hence, our TFC results support the traditional preparation methods.

Both the extraction methods and ratios affected the scavenging activity and FRAP of yacon leaf crude drugs. 30-min decoctions in crude drug-to-solvent ratio of 1:20 (w/v) produced extract with the best DPPH scavenging activity, with the value of 450.27 ± 5.48 mM TE/100g DW. On the other hand, decoction of the crude drugs for 15 min produced FRAP of 101.73 ± 1.04 mM TE/100g DW (Figure 2).





Longer extraction times and higher extraction temperatures generally increased the extraction of antioxidant compounds. However, antioxidant activity might decrease with increasing temperature. For example, the optimum temperature for preserving the antioxidant flavonoids in Dryopteris erythrosora leaves was 75°C (Zhang et al., 2019). The antioxidant activity of water extracts obtained from infusions and decoctions and the solvent-to-solid ratio can vary depending on several factors. Some plant infusions showed higher activity, whereas others showed higher free scavenging activity. For example, the Thymus sipyleus aerial part infusion was much higher than that of its decoction counterpart (Ustuner et al., 2019). The same trend was observed in Ayapana triplinervis, Dodonaea viscosa, Hubertia ambavilla, and Pelargonium graveolens. However, comparable DPPH scavenging activity was shown by infusion and decoction of Aphloia theiformis, Hypericum

lanceolatum, Psiloxylon mauritianum, and *Syzygium cumini* (Checkouri et al., (2022).). FRAP of decoction of *Lavandula angustifolia* and *Lavandula x intermedia* was also significantly higher than that of their infusion (Dobros et al., 2022).). Similar results were also demonstrated in Morrocan-originated *Haloxylon scoparium* aerial parts (Lachkar et al. 2021).

Correlations between antioxidant content and activity varied from none to strong, both positive and negative. Strong positive correlations were observed between TPC and DPPH scavenging activity in extracts obtained from decoctions of all evaluated crude drug-to-solvent ratios. In contrast, TPC and FRAP in yacon leaves were observed in the short-term decoction at a ratio of 1:100 (w/v). On the other hand, TFC and DPPH scavenging activities were strongly correlated in the 15-min decoction, while those of TFC and FRAP were observed in extracts from the 15-min decoction at ratios of 1:100 (w/v) (Table 1).

Table 1. Correlation between antioxidant content an	d antioxidant activity of yacon leaf extracts
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Method or ratio	Content	Pearson's correlation coefficient	
		DPPH	FRAP
Infusion	TPC	-0.437	0.214
	TFC	-0.516	0.623
Decoction-15	TPC	0.807*	0.809*
	TFC	0.890*	0.878*
Decoction-30	TPC	0.957*	-0.034
	TFC	-0.872*	0.736*
1:10 (w/v)	TPC	0.950*	-0.901*
	TFC	-0.856*	0.945*
1:20 (w/v)	TPC	0.973*	-0.165
	TFC	-0.721*	-0.630
1:100 (w/v)	TPC	0.966*	0.997*
. ,	TFC	-0.622	-0.578

The asterisk indicates a significant correlation between antioxidant content and activity

Aspect`	Day						
	0	1	3	6	12	18	26
Aroma	Grassy,						
	unpleasant,						
	aromatic						
Taste	Bitter +,	Bitter +,	Bitter ++,	Bitter ++,	Bitter +++,	Bitter +++,	Bitter +++,
	astringent						
	+	+	++	++	++	++	++
Color	Dark green						

Table 2. Organoleptic characters of yacon leaf decoctions during storage

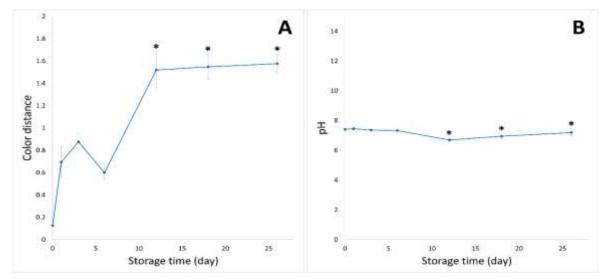


Figure 3. Color distance (A) and pH (B) of the yacon leaf decoction during refrigerated storage. The asterisk indicated a significantly different value from that of the freshly prepared extract (n = 3)

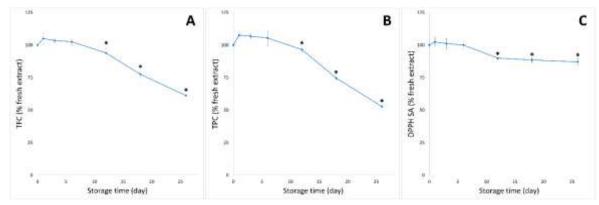


Figure 4. TFC (A), TPC (B), and DPPH scavenging activity (C) of the yacon leaf decoction during refrigerated storage. The asterisk indicated a significantly different value from that of the freshly prepared extract (n=3)

Flavonoids and phenolic compounds in the yacon leaf and Malayan cherry fruit contributed significantly to their antioxidant activities. The phenolic groups of these compounds enable the transfer of hydrogen atoms, while their single electrons scavenge free radicals. Both mechanisms were evaluated using the DPPH scavenging activity assay. The double bonds of these compounds facilitated single-electron transfer, which was exclusively determined in FRAP assays (Santos-Sánchez et al., 2019). The same correlation between these parameters has been previously reported in germany-grown yacon tubers (Khajehei et al., 2018).

The yacon leaf decoction occurred as a dark green liquid with a grassy, unpleasant, aromatic odor, and bitter and astringent taste. The bitterness and astringency of the extracts increased with the storage time (Table 2). The yacon leaf decoction undergoes a slight color change during storage. In contrast, the pH began to decrease on day 12 (Figure 3). The profiles of TFC, TPC, and DPPH scavenging activity of the yacon leaf decoction during storage were similar. The TFC of the yacon leaf decoction started to decrease on day 12, remaining at $93.93\pm3.70\%$ of the fresh extracts. Similarly, the TPC and DPPH scavenging activity also began to decrease at day 12, with 96.52 ± 1.81 and $89.99\pm0.91\%$ of the new counterpart, respectively.

Changes in the color of the decoction represented changes due to chemical reactions or degradation processes of the compounds. For instance, phenolic compounds in the extract may be oxidized or condensed, leading to changes in color. In addition, exposure to light and oxygen might degrade certain phenolic compounds, resulting in pigments altering the color of the solutions. However, a decrease in pH might occur due to the degradation of compounds. This phenomenon has been observed in apple and chokeberry liqueurs (Petrović et al. 2021). The degradation of phenolic compounds and flavonoids may be responsible for the changes in the color and pH of the decoction. During storage, these compounds may undergo various reactions under exposure to light, oxygen, enzymatic activity, and interactions with other components present in the water extract. Degradation of flavonoids and phenolic compounds and decreasing antioxidant activities during refrigerated storage were observed in watermelon and carrot juices (Hwang et al., 2023; Salin et al., (2022).

CONCLUSION

The traditional extraction method and crude drugto-water ratio significantly affected the TFC, TPC, DPPH scavenging activity, and FRAP of the yacon leaf water extracts. Extraction by decoction for 15 min at a crude drug-to-solvent ratio of 1:20 (w/v), as in the traditional preparation of *jamu*, generated yacon leaf extracts with the best antioxidant properties. The storage time affected the physicochemical properties of the yacon leaf decoction, in which changes in physicochemical parameters started to be noticeable on day 12. It is recommended to store the yacon leaf water extract under refrigerated storage and consume it within a week.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.H., A.H.; Methodology, D.H., A.H.; Validation, D.H., A.H.; Formal Analysis, A.H., D.H..; Investigation, F.W.; Resources, D.H., A.H.; Data Curration; D.H., A.H., F.W.; Writing -Original Draft, D.H.; Writing - Review & Editing, DH, A.H., F.W.; Visualization, D.H.; Supervision, D.H., A.H.; Project Administration, D.H.; Funding Acquisition, D.H., A.H.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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