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Quality and Antioxidant Activity of Arrowroot (Maranta arundinaceae L.) Tuber Accessions Collected from Java Island

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ABSTRACT

Arrowroot (*Maranta arundinaceae* L.) is a starch–producing plant that can potentially be used as an alternative substitute for wheat flour. Additionally, arrowroot can serve as a functional food, as it is not only a source of nutrition but also provides health benefits such as improving digestion and regulating blood sugar levels. Arrowroot tubers contain high levels of carbohydrates and bioactive phenolic compounds that function as antioxidants. Currently, no data is available on the nutritional content and antioxidant activity (DPPH assay) of arrowroot tubers from various regions of Java Island. This study aimed to analyze the quality and antioxidant activity of arrowroot tubers obtained from several locations in Java. The results of the proximate analysis (moisture and ash contents) showed that the lowest moisture content was in the Banjar 1 accession (3.44%), while the highest was in the Sragen 3 accession (12.47%). The Banjar 2 accession had the highest ash content (8.94%), while the Sragen1 accession had the lowest (3.98%). The antioxidant activity was indicated by the IC_{50} value, which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals. Antioxidant activity analysis revealed that the Malang 1 accession had the lowest IC_{50} value of 163.16 ppm, indicating the highest antioxidant potential.

Keywords: 1,1–diphenyl–2–picrylhydrazyl, antioxidant, arrowroot, phenol, phytochemicals

INTRODUCTION

Food is a necessity that must be fulfilled daily, but ensuring its availability can be challenging. Besides being a source of energy, food can also function as a health–beneficial functional food (Susiyanti *et al.* 2016). One example is arrowroot (*Maranta arundinaceae* L.), a tuber found in various regions of Indonesia, including Java, Maluku, and Sulawesi (Kementan 2021). Arrowroot is a promising food source that can serve as an alternative to both wheat flour and rice. With its high carbohydrate content, arrowroot tubers offer a viable substitute for traditional carbohydrate sources (Lestari *et al.* 2017; Alifah 2021). Fresh tubers are particularly rich in starch, containing approximately 20% higher than that of sweet potatoes (14.72%) but lower than that cassava (31.09%) (Leonel and Cereda 2002). Additionally, it is rich in fiber, which supports a healthy digestive system (Octavianti and Solikhah 2009).

Arrowroot tubers have anti–cholesterol and anti–ulcer properties due to their low glycemic index (GI) of 32, classifying them as a low–GI food. In addition to their low glycemic index, the tubers contain bioactive compounds that are beneficial for individuals with diabetes mellitus. The flour is rich in functional components, including 2.16 mg/100 g diosgenin, 3.98% water–soluble polysaccharides, 1.49% insoluble dietary fiber, and 1.12% water–soluble fiber (Yogananda and Estiasih 2016). As a result, arrowroot tubers can be used as a functional food, particularly for individuals struggling to regulate glucose and lipid profiles (Deswina and Priadi 2020). The plants, tubers, and arrowroot starch are shown in Figure 1. Arrowroot tubers also contain phenolics, flavonoids, alkaloids, tannins, and saponins, which can inhibit free radicals (Nishaa *et al.* 2012; Putra and Estiasih 2016; Ramadhani *et al.* 2017). Kusbandari and Susanti (2017) reported that arrowroot has a total phenol content of 0.15 g per 100 g of material, indicating its potential as a natural antioxidant.

Arrowroot tubers from various regions of Java offer numerous health benefits. However, their use in the community remains limited, as arrowroots are often left to grow without intensive cultivation. Moreover, no data is currently available on the nutritional content and antioxidant activity of the tubers from different regions of Java. Therefore, this study aimed to analyze the nutritional content and antioxidant activity of various

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arrowroot accessions from Java. The ultimate goal was to identify high–quality arrowroot accessions with strong antioxidant properties for potential cultivation.

METHODS

Research Materials

- Plant materials

Twelve accessions of arrowroot tubers were obtained from various arrowroot–producing areas in Java. These accessions represent several regions of Java, including Yogyakarta, Malang, Banjar, and Sragen. Tubers collected from field exploration (Table 1) were used to prepare the samples.

- Reagents

The chemicals were methanol, $FeCl_3$, HCl 2 N, Mg powder, concentrated HCl, NaOH, H_2SO_4, acetic anhydride, chloroform, Mayer reagent, Wagner reagent, Dragendorff reagent, Liebermann–Burchard reagent, DPPH (1,1–diphenyl–2–picrylhydrazyl), and ascorbic acid.

- Laboratory equipment

Laboratory equipment consists of an ELISA reader, an 99-well microplate, a rotary evaporator, filter paper, micropipette, Eppendorf, volumetric flask, moisture balance, oven, and furnace.

- Preparation of raw plant material

The preparation of raw plant material began with collecting fresh arrowroot tubers from v12 regions of Java Island. The collected material was sorted to separate tubers from other plant parts (roots, stems, and leaves) and thoroughly washed under running water to remove any dirt or soil. The cleaned tubers were chopped and dried at room temperature, away from direct sunlight, until completely dry. The dried material was pulverized using a blender to a fine powder, which was then sieved through an 80–mesh sieve to obtain raw plant material powder. The raw plant material powder was subsequently stored in a dry container, protected from atmospheric moisture. The dry weight of the sample was equivalent to the weight of the raw plant material used (Ermawati *et al.* 2021, with modifications).

Proximate Analysis

- Moisture Content

A 5 g of the dry sample was placed in a moisture balance device to determine the moisture content (%) (Nurhidayanti and Warmiati 2021, with modifications).The measurement was performed in triplicate and calculated using following formula:

(a) (b) (c)

Figure 1 Plant (a), tuber (b), and starch (c) of arrowroot (Personal Documentation).

Table 1 Location of origin and altitude of some arrowroot accessions in Java Island

Accession area	Accession code	Origin of accession	Altitude (m asl)
Yogyakarta	B1	Dusun Brongkol, Desa Argodadi, Sedayu Subdistrict, Bantul Regency, Yogyakarta	87.50
Yogyakarta	B2	Dusun Kadireso, Desa Triwidadi, Pajangan Subdistrict, Bantul Regency, Yogyakarta	100
Yogyakarta	B3	Dusun Pladen, Kel.Sendangrejo, Minggir Subdistrict, Sleman Regency, Yogyakarta	150
Malang	B4	BPP Turen, Bokor, Pagedangan, Turen Subdistrict, Malang Regency, East Java	445
Malang	B5	Dusun Krajan, Desa Sanan Kerto, Turen Subdistrict, Malang Regency, East Java	600
Malang	B6	Dusun Kampung Anyar, Desa Sanan Kerto, Turen Subdistrict, Malang Regency. East Java	600
Banjar	B7	Dusun Pangadegan, Kel. Hegar Sari, Paturuman Subdistrict, Banjar City	97
Banjar	B8	Dusun Siluman Baru, Kel. Purwaharja, Purwaharja Subdistrict, Banjar City	97
Banjar	B9	Dusun Babakan Sari, Kel. Pataruman, Paturuman Subdistrict, Banjar City	85
Sragen	B10	Bonagong, Tanon, Sragen Redgency, Central Java	109
Sragen	B11	Bonagong, Tanon, Sragen Regency, Central Java	109
Sragen	B12	Bonagong, Tanon, Sragen Regency, Central Java	109

$$\text{moisture content (\%)} = \frac{(\text{weight of raw plant material} - \text{dry weight})}{\text{raw plant material}} x\ 100\%$$

- Ash Content

A 2 g dry sample was placed in a crucible and ashed in a furnace at 650°C for approximately three hours. The crucible was then removed and cooled in a desiccator. Once cooled, the ash was weighed multiple times until a constant weight was achieved (Landeng *et al.* 2017). The ash content was determined in triplicate and calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{ash weight}}{\text{weight of raw plant material}} x\ 100\%$$

- Yield Calculation of Tuber Extract

The tuber extract was obtained through maceration (in triplicates) and dried using a rotary evaporator to obtain a concentrated extract. The resulting extract was used to determine the yield using the following formula (Dewatisari *et al.* 2017; Syamsul *et al.* 2020):

$$\text{Yield (\%)} = \frac{\text{weight of extract}}{\text{weight of raw plant material powder}} x\ 100\%$$

Phytochemical Tests

Phytochemical tests were conducted to identify the secondary metabolites in the extract. The metabolites analyzed in this study included flavonoids, saponins, alkaloids, tannins, and terpenoids.

- Flavonoid Test

A 0.5–g sample was added to 10 mL of hot distilled water, boiled for 10 min, and filtered while still hot. Then, 5 mL of the filtrate was collected and mixed with 0.1 g of magnesium metal, 1 mL of concentrated HCl, and 2 mL of amyl alcohol. The mixture was shaken thoroughly and allowed to separate. The presence of flavonoids was indicated by the appearance of a red, yellow, or orange color in the amyl alcohol layer (Kalaiselvi *et al.* 2016).

- Saponin Test

A 1–g sample was dissolved in 5 mL of distilled water and shaken. The presence of saponins was confirmed by the formation of stable foam that persisted for at least 10 sec (Kalaiselvi *et al.* 2016).

- Tannin Test

A 0.5–g sample was dissolved in 10 mL of distilled water and filtered using filter paper. Subsequently, 2 mL of the filtrate was collected and combined with 2 drops of 1% $FeCl_3$. The presence of tannins was indicated by the formation of a blue or blackish–green color (Kalaiselvi *et al.* 2016).

- Alkaloid Test

A 3–g extract was dissolved in 3 mL of HCl and 27 mL of distilled water and heated for 6 minutes. The solution was divided into aliquots of 10 mL each for testing with Mayer, Wagner, and Dragendorff reagents. For each test, three drops of the respective reagent were added to 2 mL of the sample extract mixture. A positive result was indicated by the formation of white precipitates (Mayer), orange precipitates (Wagner), and brown precipitates (Dragendorff) (Kalaiselvi *et al.* 2016).

- Triterpenoid Test

A 1–g sample was added to 20 mL of chloroform and then mixed with Liebermann–Burchard reagent (anhydrous acetic acid and concentrated sulfuric acid). A positive reaction for triterpenoids was indicated by the formation of an orange or purple ring, while the presence of steroids was confirmed by a bluish–green color (Kalaiselvi *et al.* 2016).

Antioxidant Activity Assay Using the DPPH (1,1–diphenyl–2–picrylhydrazyl) Method

The antioxidant activity of arrowroot tuber methanol extract was evaluated using DPPH as a source of free radicals (Rafi *et al.* 2018). Arrowroot tuber samples were extracted using methanol.

- Preparation of Methanol Extract

A 500–g of fresh arrowroot tubers was washed under tap water, cut into small pieces, and dried in an oven at 50 °C until a constant dry weight was obtained. The dried sample was ground into a fine powder and sieved using an 80–mesh sieve. A 25 g of arrowroot tuber powder was macerated with 125 mL of methanol (1:5 v/v) for 3 days and shaken once or twice daily. The mixture was filtered through Whatman No. 1 filter paper, and the residue was remacerated with methanol for 3 days. This remaceration process was repeated twice. The collected filtrate was concentrated using a rotary evaporator at 55 °C, followed by evaporation in a water bath until a thick extract was obtained. The final extract was analyzed for yield, moisture content, ash content, phytochemical composition, and antioxidant activity (Kusbandari and Susanti, 2017, with modifications).

- Preparation of Vitamin C Standard Curve

The ascorbic acid standard was prepared by dissolving 1 mg/mL vitamin C in distilled water to obtain a 1000 ppm stock solution. From this, a series of standard concentrations (20, 10, 5, 2.5, 1.25, 0.625, and 0.3125 ppm) were prepared for calibration curve. Arrowroot tuber extract samples were prepared at concentration 2000, 1000, 500, 250, 125, and 62.5 ppm. A 10.000 ppm stock solution was prepared by dissolving 10 mg/mL extract in distilled water and serially diluted to obtain certain concentrations.

- DPPH (1,1–diphenyl–2–picrylhydrazyl) Radical Scavenging Assay

The DPPH free radical scavenging activity was measured by mixing 100 µL of sample or standard with 100 µL of a DPPH solution (125 µM in methanol). The mixture was mixed thoroughly and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a microplate reader, with methanol as the control. Each sample and standard were measured in duplicate (Rafi *et al.* 2018, with modifications).

Data Analysis

Proximate analysis data, such as moisture content, ash content, yield, and antioxidant activity, were analyzed using Microsoft Excel. Phytochemical screening data were obtained through direct observation of color changes, precipitate formation, or other reactions during laboratory testing.

RESULTS AND DISCUSSION

The moisture content of arrowroot tubers from various accessions ranged from 3.44% to 12.47%. In this study, the lowest moisture content (3.44%) was found in accession B7 (Banjar), whereas the highest (12.47%) was observed in accession B12 (Sragen). The ash content of the arrowroot tubers ranged from 3.98% to 8.94%. The highest (8.94%) was recorded in accession B8 (Banjar), which originated from Banjar city at an altitude of 97 m above sea level (masl). In contrast, the lowest (3.98%) was found in accession B10 (Sragen) (Table 2). The result indicates that arrowroot tuber sample B12 (Sragen) has a high moisture content, potentially making it less suitable for long–term storage. According to Sutomo *et al.* (2021), variations in growing locations influence both specific and non–specific extract parameters. Specific parameters include organoleptic properties and chemical composition, while non–specific parameters cover moisture, ash, drying shrinkage, and juice content. These variations in moisture and ash content are influenced by factors such as altitude, rainfall, air humidity, light intensity, nutrient availability, and soil mineral composition in the growing area. According to the Indonesian Herbal Pharmacopoeia (Depkes RI 2017), the recommended moisture content for high–quality raw plant material should be less than 10%, while the ash content should not exceed 10.6%. A moisture content exceeding 10%, as observed in sample B12, potentially increases the risk of microbial contamination during storage. The moisture and ash content found in this study were lower than those reported by Rajeshekara *et al.* (2020), with 12% and 13%, respectively. This contrasts with findings by Hossain *et al.* (2021) on tea leaves, where moisture and ash content were not significantly affected by altitude. The variation in moisture content is likely due to environmental factors such as weather conditions, rainfall, and humidity, which influence moisture retention in plant materials. The yield of methanol extracted arrowroot tubers from different accessions in this study varied from 0.91% to 2.77% (Table 3).

The highest extract yield was observed in accession B6 (Malang) at 2.77%, followed by B12 (Sragen) at 2.72% and B4 (Malang) at 2.41%. The yield of raw plant material is influenced by several factors, including particle size, solvent type, solubility properties, and maceration duration (Hidayanti *et al.* 2017). In this study, we used a ratio of solvent–to–sample 1:5. Remaceration was performed three times to maximize the yield. The final yield was obtained by combining the filtrates from both the initial maceration and remacerations. The extract yield was influenced by the environmental conditions in which these accessions grew. According to Subaryanti *et al.* (2023), the productivity and quality of tubers are affected by genetic and environmental. Additionally, the bioactive components produced through plant metabolic processes are affected by intrinsic and extrinsic factors. Intrinsic factors include genetic traits, while extrinsic factors include environmental factors, such as soil quality and climate. These factors play a crucial role in the photosynthetic process, as photosynthates produced in the leaves (source) are distributed to the tubers (sink) (Buntoro *et al.* 2014). Photosynthates, primarily in the form of starch, are not solely used for energy and growth (primary metabolites) but are also

Table 2 Average moisture and ash contents of arrowroot tubers (*M. arundinaceae* L.)

Accession area	Accession code	Ash content (%)	Moisture content (%)
Yogyakarta	B1	6.22±0.21	6.85±1.22
Yogyakarta	B2	5.47±0.72	7.11±1.15
Yogyakarta	B3	5.71±0.30	5.51±1.95
Malang	B4	5.95±2.60	5.74±2.16
Malang	B5	5.32±3.52	5.63±2.78
Malang	B6	4.65±1.13	5.31±1.12
Banjar	B7	4.80±2.08	3.44±0.30
Banjar	B8	8.94±1.08	8.92±0.37
Banjar	B9	6.97±1.71	5.37±1.58
Sragen	B10	3.98±2.61	4.80±1.80
Sragen	B11	5.92±0.83	8.56±3.88
Sragen	B12	5.73±0.78	12.47±0.18

Table 3 Yield of methanol extract from some accessions of arrowroot tubers (*M. arundinacea* L.)

Accession area	Accession code	Extract weight (g)	Raw material weight (g)	Extract yield
Yogyakarta	B1	0.23	25.28	0.91
Yogyakarta	B2	0.35	25.14	1.41
Yogyakarta	B3	0.46	25.11	1.83
Malang	B4	0.60	25.02	2.41
Malang	B5	0.31	25.12	1.24
Malang	B6	0.69	25.12	2.77
Banjar	B7	0.44	25.06	1.75
Banjar	B8	0.45	25.02	1.80
Banjar	B9	0.50	25.13	2.00
Sragen	B10	0.55	25.08	2.20
Sragen	B11	0.59	25.13	2.33
Sragen	B12	0.68	25.09	2.72

Table 4 Phytochemical screening results of some accessions of arrowroot tubers (M. *arundinacea* L.)

Accession area	Accession code	Component					
		Starch	Alkaloids	Tannins	Flavonoids	Saponins	Terpenoids
Yogyakarta	B1	+	+	+	+	+	–
Yogyakarta	B2	+	+	+	+	+	–
Yogyakarta	B3	+	+	+	+	+	–
Malang	B4	+	+	+	+	+	–
Malang	B5	+	+	+	+	+	–
Malang	B6	+	+	+	+	+	–
Banjar	B7	+	+	+	+	+	–
Banjar	B8	+	+	+	+	+	–
Banjar	B9	+	+	+	+	+	–
Sragen	B10	+	+	+	+	+	–
Sragen	B11	+	+	+	+	+	–
Sragen	B12	+	+	+	+	+	–

converted into other secondary metabolites (Widiyanto and Siarudin 2013).

Phytochemical tests on tuber yielded positive results for alkaloids, flavonoids, saponins, and tannins, while the terpenoid test gave negative response (Table 4). These findings contrast with those of Rajeshekhara *et al.* (2020), who identified terpenoids in arrowroot tuber extract. This variation may be attributed to secondary metabolite production that was affected by internal factors, such as gene expression of constitutive phytochemical compounds, rather than external factors like altitude. The production of secondary metabolites, such as antifeedants, phytoanticipins, and phytoalexins, plays a crucial role in the plant's defense system (Verpoorte and Memelink 2002). These metabolites play various roles in plant growth and development, including defense mechanisms and adaptation to environmental stress. Structure, function, and concentration of these metabolites vary among plant species (Stephanie 2014). In this study, accession B4 (Malang) exhibited the highest antioxidant activity, as indicated by its IC_{50} of 163.16 ppm (Table 5). This suggests that the antioxidant activity of arrowroot starch from accession B4 (Malang) was higher than that of the other accessions. Bahriul *et al.* (2014) stated that lower IC_{50} corresponds to higher antioxidant activity. Antioxidant activity is classified into five categories: very strong (IC_{50} < 50 ppm), strong (50 < IC_{50} < 100 ppm), moderate (100 < IC_{50} < 150 ppm), weak (150 < IC_{50} < 200 ppm), and very weak (IC_{50} > 200 ppm). Ascorbic acid (vitamin C) is known as an antioxidant because it can donate hydrogen atoms and form relatively more stable ascorbyl free radicals. Therefore, ascorbic acid is commonly used as a standard in determining antioxidant capacity (Rozi *et al.* 2023). The antioxidant activity observed in this study falls within the weak category, yet it is higher than that reported by Ruba and Mohan (2013), who found an IC_{50} of 293.4 ppm in the ethanol extract of arrowroot tuber. Conversely, Kusbandari and Susanti (2017) reported an IC_{50} of 1.78 ppm for fresh arrowroot tuber extract, indicating significantly higher antioxidant activity. This substantial difference is likely attributed to the use of fresh material, which may contain a higher concentration of bioactive compounds. In contrast, Hanum (2016) found an IC_{50} of 1660 ppm in arrowroot tuber, indicating very weak antioxidant activity. The antioxidant activity of the arrowroot tuber is affected by the presence of secondary metabolites, which act as free radical scavengers and contribute to oxidative stress reduction. Flavonoids, a major class of phenolic compounds, exhibit antioxidant properties due to their complex phenolic structure and high degree of hydroxylation, which enables them to donate hydrogen atoms and neutralize free radicals.

According to Hanin and Pratiwi (2017), phenolics are secondary metabolites that act as antioxidants by donating hydrogen atoms or electrons to neutralize free

Table 5 Antioxidant activity of arrowroot tuber (M. *arundinaceae* L.)

Accession area	Accession code	IC_{50} (ppm)
Yogyakarta	B1	288.30±20.14
Yogyakarta	B2	462.60±66.43
Yogyakarta	B3	406.66±16.78
Malang	B4	163.16±22.75
Malang	B5	204.58±19.61
Malang	B6	377.22±4.82
Banjar	B7	282.43±23.06
Banjar	B8	457.14±39.66
Banjar	B9	263,81±87,68
Sragen	B10	270.61±9.02
Sragen	B11	263.30±37.48
Sragen	B12	303.59±16.00

radicals, thereby preventing oxidative damage. Since the altitudes in this study were nearly identical, the influence of altitude on metabolite variation is likely minimal. However, other environmental factors such as soil quality, temperature, and humidity may still contribute to differences in metabolite content. Juwita *et al.* (2017) stated that a higher ash in food products may indicate lower suitability for consumption due to increased inorganic residue. Furthermore, a higher ash content reflects an increased concentration of inorganic minerals that remain after combustion.

Phenolics are produced by certain plants in response to environmental stress and play a protective role by preventing oxidative damage and inhibiting DNA dimerization, which can lead to mutations. The low IC_{50} observed in accession B4 (Malang), which grows at an altitude of 445 m asl, may be attributed to the plant's adaptive response to altitude–induced stress, which stimulates the production of antioxidant secondary metabolites. Arrowroot typically thrives at an altitude of 60–90 m asl. However, at altitudes above 250 m asl, the plant may adapt to altitude–induced stress by increasing the production of secondary metabolites, such as flavonoids and phenolics, which function as antioxidants and defense compounds. This is supported by the findings of Puspitasari *et al.* (2019), that arrowroot can grow at altitudes ranging from 0 m to 1000 m asl, with the highest tuber production (43.07%) occurring at 250 m asl, compared to lower yields at 1100 m asl. The increased tuber production at 250 m asl is likely due to optimal environmental conditions at this altitude, which enhance photosynthetic efficiency and promote a higher allocation of photosynthates to the tubers. These findings suggest that cultivation of arrowroots in lowland areas may lead to high tuber yields, as environmental conditions at lower elevations are generally more favorable for plant growth and development.

CONCLUSION

The exploration of arrowroot tubers in various regions of Java led to the identification of 12 accessions, from Yogyakarta, Malang, Banjar, and Sragen. Accession B4 (Malang) had a moisture content of 5.95% and an ash content of 5.74%, placing it within the medium range. The methanol extract yield from this accession was 2.41%. The high antioxidant activity observed in accession B4 (Malang), with an IC_{50} of 163.16 ppm, highlights its potential for cultivation and further utilization.

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