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Comparative Assessment of Nutritional, Antioxidant and Phytochemical Properties of Wild Yams (*Dioscorea* spp.) Accessions in Peninsular Malaysia

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Abstract

Yams (*Dioscorea* spp.) are one of the important tuber crops for sustainability and food security in the world. However, *Dioscorea* spp. is considered an underutilized crop and is not heavily consumed, especially by local people in Malaysia, due to its limitations in its nutritional composition and beneficial studies. This study was carried out to evaluate various accessions of wild yams in Peninsular Malaysia. Eight different accessions of yams (*D. alata* (DA1-DA5), *D. alata var. purpurea* (DVP), *D. esculenta* (DE) and *D. piscatorum* (DP) were collected from various localities in farmer's farms in Peninsular Malaysia. All the samples were investigated for their proximate composition, polyphenols and flavonoid content and in vitro antioxidant activity. *D. piscatorum* (DP) collected in Kuala Koh, Kelantan, showed the highest amount of carbohydrate content and energy compared to all accessions ($p<0.05$) with 90.8 g/100g and 374.33 kcal/100g, respectively. Meanwhile, a variety of *D. alata* (DA2) collected in Machang, Kelantan, showed a high amount of protein content (10.57 g/100g) ($p<0.05$). The flavonoid/phenolic ratio was highest in the methanolic extract of DVP (0.72), followed by DE (0.64) and DP (0.61), respectively. However, the methanolic extract of DP showed the lowest inhibition concentration value (IC_{50} = 4.73 mg/ml), indicating its stronger scavenging activity towards DPPH free radicals. The comparison and assessment of different accessions of *Dioscorea* spp. on their nutritional composition and phytochemicals highlights the suitability of each sample to accommodate specific dietary or health-related applications.

Keywords: Antioxidant; Dioscoreaceae; polyphenols; ubi; underutilized.

INTRODUCTION

Dioscorea belongs to the Dioscoreaceae family and is also known as yams, greater yam, "Shanyao" in China, "umbi" or “uwi” in Indonesia, “ube’ in the Philippines or “ubi” in Malaysia (Mojiono et al., 2022; Li et al., 2023; Azahana et al., 2023; Ferriol, 2023). The world production share of yams by regions from 2020-2022 in Africa (98%), Americas (1.2%), Oceania (0.5%) and Asia (0.2%), with the top producer countries being Nigeria, Ghana, Côte d'Ivoire and Benin. The increase in yams production worldwide from 81 million tons (2020) to 88 million tons (2022) showed a significant demand for this crop (FAO STAT 2024). Meanwhile, according to the statistics released by the Department of Agriculture of Peninsular Malaysia, the production of *Dioscorea alata* or "ubi badak" in 2023 was estimated at around four metric tons (DOA, 2023). Limited knowledge of its nutritional value compared to other tuberous crops is one of the reasons this crop is not commercially cultivated and consumed in Malaysia. The *Dioscorea* spp. commonly consumed by local people on the East Coast of Peninsular Malaysia and can be found abundantly in local markets, especially during the monsoon season from October to December each year (Azahana et al., 2023).

The tuber flesh of *Dioscorea* spp. is rich in carbohydrates, making it one of the primary staple foods in certain countries, such as those in Africa, and an alternative starchy food in some areas in Malaysia. The carbohydrate content of several species of *Dioscorea* (*D. alata*, *D. esculenta*, *D.rotunda*, *D. cayenensis* and *D. bulbifera*) was 50% - 77% depending on their varieties and location of cultivation (Oko & Famurewa, 2015; Ezeabara & Anona, 2018; Godfrey et al., 2023; Sato et al., 2024). Meanwhile, the protein content in the various

species of *Dioscorea* was reported in the range of 1.13% - 6.20% (*D. hispida*), 4.7% - 8.99% (*D. alata*), 7.25% (*D. esculenta*) and 8.83% (*D. bulbifera*) (Saleha et al., 2018; N'dri et al., 2018; Yalindua et al., 2021; Rayamajhi et al., 2024). Recent studies on its biological activities from extracts of *Dioscorea* spp. also revealed interesting activities in antioxidant (Adomėnienė & Venskutonis, 2022), antimicrobial (Induar et al., 2024), antidiabetic (Rayamajhi et al., 2024), antidiarrheal (Islam et al., 2024), antihypertensive (Logan et al., 2024), hypolipidemic (Li et al., 2019; Povydysh et al., 2023), antiproliferative and anticancer (Mainasara et al., 2021; Nurul Makiyah et al., 2023). The astonishing pharmacological activities of *Dioscorea* spp. mainly due to the presence of its secondary metabolites, such as phenolic compounds, including flavonoids and anthocyanins, steroidal saponin, and carotenoids (Ren et al., 2015; Price et al., 2018; Ukom et al., 2020; Safitri et al., 2021; Kuagny et al., 2023; Syahputra et al., 2024; Islam et al., 2024). However, studies on proximate composition and phytochemicals of various *Dioscorea* spp. accessions in Malaysia remain limited. Thus, this study aims to deliver and assess various accessions of *Dioscorea* spp. collected in Peninsular Malaysia for its nutritional values, phenolics and flavonoid content, and antioxidant activity.

MATERIALS AND METHODS

Collection and Preparation of the Sample

Eight different accessions of *Dioscorea* spp. were collected across Peninsular Malaysia (Table 1 and Figure 1). Each species was identified by a botanist at the Agrobiodiversity and Environmental Research Centre, MARDI, Serdang. The flesh color and cross-section reviewed under a stereomicroscope are shown in Figure 2. All the specimens were deposited in the Herbarium, Kompleks MyGenebank, MARDI, Serdang. All the samples were cleaned, and the skin was peeled. The samples were cut into small cubes and dried in the industrial oven at 50°C for three days or until the moisture content remained constant. The samples were ground into a fine powder (18 mesh) using a mechanical grinder (IKA Werke MF 10 Basic, Germany). The sample powder was sealed and kept in the chiller at 4°C until further analysis was needed.

Table 1. Various accessions of *Dioscorea* spp. collected in Peninsular Malaysia.

Sample	Sample Code	Location	Tuber Flesh Color
D. alata	DA1	Bukit Payong, Terengganu	Light white yellowish
D. alata	DA2	Machang, Kelantan	Light white yellowish
D. alata	DA3	Pasar Pasir Putih, Kelantan	Light white yellowish
D. alata	DA4	Marang, Terengganu	Light white yellowish
D. alata	DA5	Wakaf Bharu, Kelantan	Light white yellowish
D. alata var. purpurea	DVP	Wakaf Bharu, Kelantan	Dark purple
D. esculenta	DE	Teluk Pelandok, Negeri Sembilan	Light yellow
D. piscatorum	DP	Kuala Koh, Kelantan	Dark yellow-Orangish

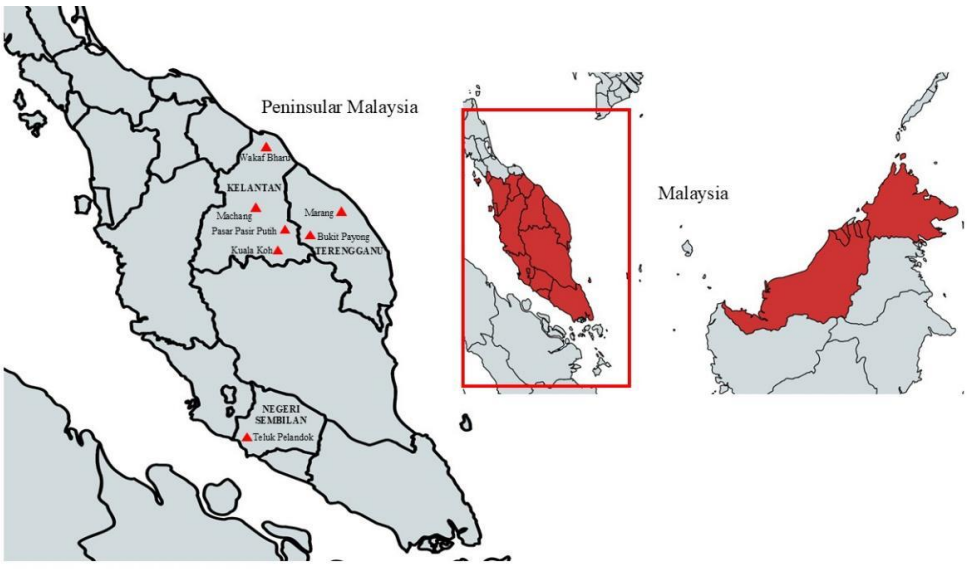

Figure 1. The area of the sample (*Dioscorea* spp.) was collected in Peninsular Malaysia.

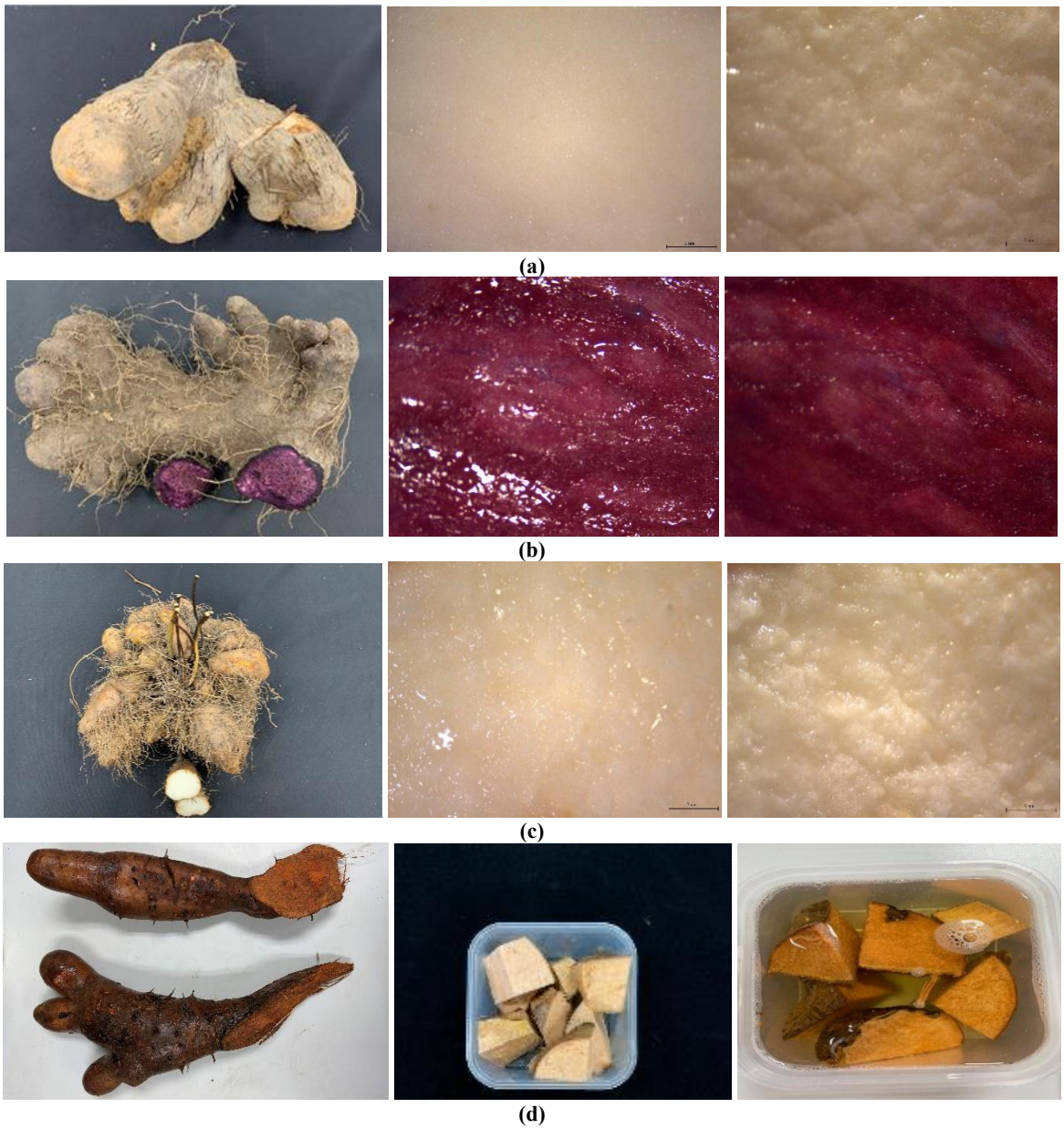

Figure 2. Flesh color and structure were reviewed after cross-section under a stereomicroscope of *Dioscorea* spp. collected. a). *Dioscorea alata* (white) b). *Dioscorea alata var purpurea* c). *Dioscorea esculenta* and d). *Dioscorea piscatorum.*

Proximate and Nutritional Composition

Approximately 250g of dried samples were weighed and sent to the accredited laboratory for proximate composition analysis. Each reading was done in triplicate.

Total Phenolic Content (TPC)

The total phenolic contents of the sample were determined using the Folin–Ciocalteu method with some modifications (Mirfat et al., 2013). A 2g dried powder sample was extracted in 20 ml of 70% methanol. Crude extract (50µL) was mixed with 100µL of Folin Ciocalteau's phenol reagent (Merck, Germany). After 3 minutes, 100µL of 10% sodium carbonate (Na_2CO_3) (Sigma Aldrich, USA) was added to the reaction mixture and allowed to stand in the dark for 60 minutes. The absorbance was measured at 725 nm, and the total phenolic contents were calculated from a calibration curve using gallic acid (0-10 µg/mL) as a standard reference. The estimation of phenolic contents was conducted in triplicate. The results were presented as mean values ± standard deviations and expressed as mg gallic acid equivalent per 100 g of samples (mg GAE/100g) in dry weight (DW).

Total Flavonoid Content (TFC)

The total flavonoid content was measured using the previously published method with minor modifications (Mirfat et al., 2013). Approximately 100µl of extract was diluted with 400 µl of distilled water. Subsequently, a 5% sodium nitrite ($NaNO_2$) solution (30µl) was added and allowed to react for 5 min. Afterwards, 10%

aluminium chloride ($AlCl_3.6H_20$) (20µl) was added and left for 5 min. Finally, 200µl of sodium hydroxide (NaOH) was added, and the mixture was mixed using a vortex. All samples were analyzed in triplicate, and the absorbance was measured immediately at 510nm. Quercetin was used to calculate the standard curve, and the results were expressed as mg quercetin per 100 g sample (mg QE/100g) in dry weight (DW).

Free Radical Scavenging Assay

All the powder samples underwent extraction and were assessed for their free radical scavenging ability, following the previously described procedure with minor modification (Salahuddin et al., 2020; Zulkhairi et al., 2021). The assay was conducted using a 96-well plate, with 2g of dried powder sample extracted in 20 ml of 70% methanol. The stock solution was diluted to the desired concentration for the working solution. The final volume obtained (7µL) was mixed with 280µL of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, USA). The plate was covered with aluminium foil to prevent exposure to sunlight and was kept in a dark place for 30 minutes. Analysis was performed using a UV spectrophotometer (Eon Biotek Instrument) at 517 nm. The results were expressed as the inhibition concentration (IC_{50}) value in mg/mL, representing the concentration at which DPPH radicals were scavenged by 50%.

Statistical Analysis

All triplicate samples were analyzed using Duncan's Multiple Range Test (DMRT) (Mean ± SEM, n = 3) with SAS Version 9.4.

RESULTS AND DISCUSSION

Proximate and Nutritional Composition

Proximate and nutritional composition of *Dioscorea* spp. shown in Table 2. Accessions DA1-DA5, DVP and DE showed a range of carbohydrate content <85 g/100g, while DP had significantly the highest carbohydrate content with 90.8 g/100g ($p<0.05$). Additionally, DP also provides significantly ($p<0.05$) higher energy in 100g compared to other *Dioscorea* accessions with 374.33 kcal/100g, while DA1-DA5 with 352.67 – 371.0 kcal/100g, DVP (368.67 kcal/100g) and DE (366.0 kcal/100g), respectively.

Table 2 Proximate Composition of *Dioscorea* spp. collected in Peninsular Malaysia.

Sample	Ash	Fat	Protein	Carbohydrate	Energy
			g / 100 g		kcal / 100g
DA1	3.07^{b}	0.10^{cd}	7.43^{c}	82.23^{d}	358.67^{f}
DA2	2.23^{d}	0.13^{c}	10.57^{a}	80.17^{e}	364.0^{e}
DA3	2.73^{c}	0.00^{d}	8.0^{b}	84.67^{b}	371.0^{b}
DA4	2.73^{c}	0.40^{b}	7.23^{c}	80.03^{e}	352.67^{g}
DA5	2.87^{c}	0.53^{a}	6.0^{e}	83.60^{c}	363.33^{e}
DVP	1.90^{e}	0.17^{c}	8.03^{b}	83.77^{c}	368.67^{c}
DE	3.83^{a}	0.57^{a}	6.50^{d}	83.67^{c}	366.0^{d}
DP	1.60^{f}	0.00^{d}	2.77^{f}	90.80^{a}	374.33^{a}

Means followed by the same letter within a column are not significantly different at ($P\leq0.05$) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at $P<0.05$, 0.01 and 0.001 respectively and NS= not significant.

The carbohydrate content in all accessions of *Dioscorea* spp. does not vary from the previously published data. Five different varieties of *D. alata* also showed a range of carbohydrate content ranging from 70.88 to 73.90 g/100g (Oko & Famurewa 2015), while research conducted by Ezeabara & Anona et al. (2018) showed that the carbohydrate content of *D. alata* in Nigeria was 76.26 g/100g. Meanwhile, the carbohydrate content in different varieties of *Dioscorea* spp. showed carbohydrate content ranging from 20.46 g/100g to 92.50 g/100g DW (Kulasinghe & Ranaweera, 2019). Additionally, recent research by Godfrey et al. (2023) on comparative assessment of the proximate composition in *D. alata, D. rotunda*, and *D.bulbifera* revealed the carbohydrate composition in the range of 57.38 to 77. 51g/100g.

Meanwhile, DA2 showed the highest protein content (10.57 g/100g) ($p<0.05$), while DP had the lowest protein content (2.77 g/ 100g). These findings and values agreed with the previous data on nutritional compositions of various landraces of *Dioscorea* spp. Twenty-five landraces of *D.alata*, *D.opposita, D. fordii* and *D.persimilis* in China were studied with a protein content range from 6.3% to 12.2%, while 36 yams landraces were studied in Southwest Ethiopia with protein content from 6.25% to 8.28% (Wu et al., 2016; Mulualem et al., 2018). A study by Padhan et al. (2020) on eight wild yams and one cultivated *Dioscorea* revealed a high protein content in the wild yam of *D. pubera* with 10.3%. Our findings were also aligned with the theory that different accessions of yams contribute to different nutritional compositions. For example, studies on five cultivars of *D.alata* flour fractions (yellow yam, orange

yam, light purple yam, purple yam and dark purple yam) were compared, and the protein content in various flour fractions revealed light purple of *D.alata* had the highest of protein content (9.85%)(Nadia et al. 2015).

Phytochemical Analysis

Meanwhile, the total phenolic content from the methanolic extract of various accessions of *Dioscorea* spp. is shown in Table 3. *D. piscatorum* (DP) had the highest content of phenolics (300.55 mg GAE/100g) ($p<0.05$) compared to other accessions. Meanwhile, the phenolics content in all accessions of *D. alata* (light yellow variation, DA1-DA5) exhibited the amount of phenolics ranging from 38.9 mg GAE/100g - 74.1 mg GAE/100g, with DA3 showing the highest amount of phenolics content (74.1 mg GAE/100g). Meanwhile, the methanolic extracts of DVP and DE displayed phenolic content of 60.92 mg GAE/100g and 84.70 mg GAE/100g, respectively.

Table 3. Total Phenolic, Total Flavonoid, Flavonoid/Phenolic Ratio and Inhibition Concentration of *Dioscorea* spp.

Sample	Total Phenolic (mg GAE/100 g)	Total Flavonoid (mg QE/100 g)	Flavonoid/Phenolic Ratio	Inhibition Concentration (IC_{50} mg/ml)
DA1	38.90^{d}	11.97^{d}	0.31	7.31^{b}
DA2	39.52^{d}	19.51^{d}	0.49	15.61^{h}
DA3	74.10^{b}	38.53^{c}	0.52	9.55^{d}
DA4	49.85^{cd}	20.05^{d}	0.40	8.13^{c}
DA5	43.44^{d}	12.98^{d}	0.30	15.07^{g}
DVP	60.92^{c}	$43.76b^{c}$	0.72	9.69^{e}
DE	84.70^{b}	54.52^{b}	0.64	14.59^{f}
DP	300.55^{a}	183.03^{a}	0.61	4.73^{a}

Means followed by the same letter within a column are not significantly different at ($P \leq 0.05$) by Duncan's Multiple Range Test (DMRT) (Mean SEM, n=3). *, ** and *** significantly difference at $P<0.05$, 0.01 and 0.001 respectively and NS= not significant. IC_{50} = Inhibition concentration at which DPPH radicals were scavenged by 50%.

The methanolic extracts of DP, DE and DVP demonstrated the highest flavonoid content with 183.03 mg QE/100g, 54.52 mg QE/100g and 43.76 mg QE/100g, respectively. The flavonoid/phenolic ratio in the DVP was high compared to other accessions, with 0.72, followed by DE (0.64) and DP (0.61), while DA1-DA5 had values ranging from 0.30 to 0.52. The variations of phytochemicals in different accessions of *Dioscorea* spp. due to the many contributing factors. The dark purple hue of the flesh from DVP is due to the high amount of anthocyanin pigment, which is then proven to have the highest flavonoid/phenolic ratio across all the accessions. Alatanin, a class of cyanidin in anthocyanins, was identified as a significant chemical compound present in *D. alata* (purple variety) in Thailand and the Philippines (Moriya et al., 2015; Srivichai & Hongsprabhas 2020). Meanwhile, the study of phenolic content from different landraces of *Dioscorea* spp. including *D. esculenta* (highland and lowland) in the Philippines was conducted, whereby the phenolics content in the highland variety showed an impeccable higher phenolics content as compared to the lowland with 156.5 mg GAE/100g and 112. 4 mg GAE/100g, respectively (Cornago et al., 2011). The development of the HP-TLC method to quantify the secondary metabolites in the *D. esculenta* in Vanuatu showed the presence of epicatechin, a flavonoid class of compound, along with saponin (Lebot et al., 2019). Additionally, the effects of pigment colours from the different landraces of *D.alata* in India and their correlation with the phenolics content showed that the yellow and purple flesh tuber share higher phenolics content and their antioxidant activity (Jose et al., 2019).

Meanwhile, the in vitro antioxidant activity was measured using a free radical scavenging assay on eight extracts of *Dioscorea* spp, and a significant difference in the inhibition concentration was revealed. The methanolic extracts of DA1, DA3, DA4, DVP and DP showed IC_{50} values < 10 mg/ml, with DP having the lowest IC_{50} value of 4.73 mg/ml ($p<0.05$), indicating a more potent antioxidant activity compared to the others accessions. However, the flavonoid/phenolic ratio in DP is lower compared to DVP. Still, it exhibits the most potent antioxidant activity (IC_{50} = 4.73 mg/ml), probably due to the presence of carotenoids, which is attributed to its dark yellow-orangish hue of the flesh tuber that is present in various accessions of *Dioscorea* spp. (Nadia et al., 2015; Jose et al., 2019; Ukom et al., 2020).

CONCLUSIONS

The studies on different accessions of *Dioscorea* spp. showed different proximate compositions, phenolics and flavonoid content. The free radical scavenging assay towards methanolic extracts also showed diverse

antioxidant activities depending on their varieties and localities. *D. piscatorum* (DP) showed the highest carbohydrate content and provided more energy than other accessions. This accession exhibits exceptional antioxidant activity that could benefit health applications emphasizing oxidative stress reduction. Meanwhile, despite the high protein content in the variety of *D. alata* (DA2), its antioxidant activity was weak compared to other accessions. In conclusion, the samples vary significantly in both nutritional and functional properties. These differences highlight the suitability of each sample for specific dietary or health-related applications.

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