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NUTRITIONAL COMPOSITION OF DIOSCOREA HISPIDA FROM DIFFERENT LOCATIONS AROUND LEUSER ECOSYSTEM AREA

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Abstract. *Proximate analysis of Dioscorea hispida tubers, collected from five locations around Leuser ecosystem in Aceh Province, showed variations amongst samples. Standard AOAC method for proximate analysis of the fresh weight showed that the water content varied between 15.8 - 37.8%, crude protein 1.13 - 6.20%, crude lipid 1.99 - 9.36% and ash 0.29 - 1.24%. The total carbohydrate was high, i.e. between 58.3 - 71.9%. The main mineral was phosphorus, with a value of 11.7 - 46.9 mg/100g. These variations could be due to soil, climate and weather factors, as well as postharvest handling. Phytochemical tests showed that all of the samples contained alkaloids and terpenoids. One of the samples (LP) also contained phenol and steroid. The high cyanide content in the tubers (379 - 739 ppm) was easily removed by repeated washing. The cyanide level dropped significantly after the 3rd wash. Information on nutritional content in D. hispida is essential for planning its utilization. Increasing the economic value of D. hispida is expected to attract people around the Leuser ecosystem to cultivate and utilize it, thereby reducing illegal forest encroachment.*

Keywords: *Dioscorea hispida, proximate, Leuser, janeng, gadung, starch*

I INTRODUCTION

Dioscorea spp (generally called yam) is an important source of carbohydrates in many tropical countries. In 2007, world's yam production reached 52 million tons, of which Africa produced 96% and Nigeria alone produced 71% of the total [1]. It is argued that the cultivation of yam has strategic roles and functions in the socioeconomic, cultural and religious aspects of the society in Nigeria [2]. Yam belongs to Dioscoreaceae family, which consists of more than 800 species. Some species have been cultivated (e.g *D. cayenensis, D. rotundata*), while others are still wild (e.g *D. abyssinica and D. praehensilis*). Efforts to domesticate wild yam have been made to utilize their phenotypic or genotypic superiority through crossbreeding with those that have already been cultivated [1]. In Indonesia, yam is rarely used as a major source of carbohydrates. The most frequently cultivated species is *D. alata*. The main component of Dioscorea spp is carbohydrates (in the form of starch). Total carbohydrate content reaches 28%, which is higher than that in potatoes and sweet potatoes but lower than cassava. The caloric value of 118 kcal/100g is interestingly higher than cassava. Potassium and phosphorous content are also higher than that in other tubers. In addition, Dioscorea spp contains several vitamins, of which vitamins A and C are dominant [3]. Yam has also been used as a health food and herbal medicine [4]. It was reported that noodles made with *D. alata* starch showed hypolipidemic and antioxidant effects in mice [5], as well as increased the production of antibodies (IgG and IgA) [6]. The positive effects on health are due to the activity of its secondary metabolites. Four species of yam *D. bulbifera, D. versicolor, D. deltoidea, and D. triphylla* are reported to exhibit high antioxidant activities [7]. *D. pseudojaponica* also contained a bioactive steroid, with anti-cancer activity [8]. This study reports the analysis results of proximate, mineral, phytochemical and cyanide content of *D. hispida* tubers collected from several area in the vicinity of the Leuser ecosystem in Aceh. *D. hispida*, traditionally called Janeng in Aceh, is a tropical plant that grows wild at low and medium altitudes, especially in bush and forest.

This plant is rarely cultivated, thus the results of this study may be used to increase the economic values of the plant. Utilization by communities around the Leuser ecosystem area may reduce forest infringement.

II METHODOLOGY

Tubers of *D. hispida* were collected from several villages directly adjacent to the Leuser ecosystem, i.e in Lokop (East Aceh District), Pengidam (Aceh Tamiang District), Kampung Pisang and Menggamat (South Aceh District). The samples were relatively of the same age. A total of 5 kg of tubers were kept intact until analysis.

Proximate analysis
Water content, total minerals, crude fat, crude protein and carbohydrates were determined in accordance with AOAC methods [9]. Water content was determined by thermogravimetry method, based on the difference in sample weight before and after heating. Mineral content was determined by dry ashing method. The difference of samples weight before and after heating at 550°C for several hours indicates total mineral content. Crude fat was determined by soxhlet extraction using diethyl ether as the solvent. Meanwhile, the determination of crude protein was performed using the Kjeldhal method. The total carbohydrate determination was done by calculation by difference. The residual weight after subtracting water, protein, lipids and minerals content indicated the total carbohydrate.

Analysis of minerals
The mineral content of calcium, iron and phosphorus in the ash sample was determined by a combination of gravimetric and titration methods according to AOAC [9]. Determination of calcium was done by titration method using potassium permanganate. Previously, ash samples in solution were treated to produce calcium oxide. The solution was then titrated using potassium permanganate. The amount of 1 ml of 0.1N potassium permanganate used for titration is equivalent to 0.0028 g of calcium oxide. The determination of iron was also done by titration using potassium permanganate. After undergone various reactions, precipitation and washing, the solution was titrated with potassium permanganate. The amount of 1 ml $KMnO_4$ 0.1N is equivalent to 0.005585 g Fe. The phosphorus was determined by firstly converting it to dimagnesium pyrophosphate ($Mg_2P_2O_7$). The weight of ortho phosphate (P_2O_5) was calculated from the weight of $Mg_2P_2O_7$ using the formula of P_2O_5 (g/100ml solution) = 0.6377 x $Mg_2P_2O_7$ (g).

Phytochemical test
Alkaloids, steroids, terpenoids, saponins and flavonoids in each sample was determined using standard method of Harborne [10].

Alkaloids. The presence of alkaloids was qualitatively determined by adding Meyer, Dragendorf, and picric acid reagents to the treated samples. The formation of white, reddish and yellow deposits for the consecutive reagents indicates the presence of alkaloids.

Steroids, terpenoids and saponins. The finely crushed sample was extracted with hot methanol and filtered. The filtrate was further extracted with diethyl ether and the mixture was shaken firmly. The existence of stable foam indicated the presence of saponins. The filtrate was subsequently hydrolysed with 2N hydrochloric acid and filtered. The purple colour in the solution indicates the presence of triterpenes while green/blue colour indicates the presence of saponins. Diethyl ether extracts were further tested with the Liberman-Bourchard reagent. The blue or green color indicates the presence of steroids, while red color indicates triterpenoid.

Flavonoids. Sample were extracted with methanol and concentrated, followed by extraction using n-hexane. The residue was then further extracted with 10 mL of 80% ethanol. Then, 0.5 mg magnesium and 0.5 m HCl were added to the extract. The pink or purple color indicates the presence of flavonoids.

Cyanide. Finely crused sample was mixed with aquades (1:5 w/v), shaken for 2 minutes and filtered. A few drops of potassium chromate were then added to a certain volume of filtrate. The solution was titrated with silver nitrate until the amount of precipitate became constant. The cyanide content is equivalent to the volume of 0.1N silver nitrate used for titration. The remaining precipitate at the first filtration was then mixed with aquadest (1:5 w/v) for 2 minutes and treated as described above.

III RESULTS AND DISCUSSION

Table 1 presents data on proximate analysis of water, crude protein, lipids, total carbohydrates and ash. Water content in each of *D. hispida* samples was in the range of 15.8 - 37.8%. The lowest was observed in the MG sample, while the highest was in the LP and P samples. Water content of two varieties of *D. hispida* collected from the same site differed significantly.

Tabel 1 Result of proximate analysis of *D. hispida* tubers

Parameter	LP	LK	P	MG	KP
Water (%)	37.8 ± 0.08	16.5 ± 0.07	37.8 ± 0.23	15.8 ± 0.06	27.6 ± 0.04
Lipid (%)	1.99 ± 0.03	9.36 ± 0.41	2.22 ± 0.07	5.42 ± 0.08	3.39 ± 0.03
Crude protein (%)	1.13 ± 0.35	2.03 ± 0.23	1.33 ± 0.20	6.20 ± 0.02	6.09 ± 0.75
Total carbohydrate (%)	58.7 ± 0.43	71.9 ± 0.57	58.3 ± 0.53	71.4 ± 0.05	62.3 ± 0.77
Ash (%)	0.38 ± 0.02	0.29 ± 0.01	0.34 ± 0.03	1.24 ± 0.01	0.59 ± 0.02

LP = Lokop (Putih), LK = Lokop (Kuning), P = Pengidam, MG = Menggamat, KP = Kampung Pisang

Tabel 2 Phosphorus, calcium and iron minerals in *D. hispida* tuber (mg/100g fresh weight)

Parameter	LP	LK	P	MG	KP
Phosphorus	46.9 ± 0.46	40.8 ± 0.95	24.2 ± 0.90	12.4 ± 0.03	11.7 ± 0.003
Calcium	4.40 ± 0.16	5.26 ± 0.08	5.78 ± 0.06	4.31 ± 0.01	1.18 ± 0.01
Iron	0.06 ± 0.01	0.03 ± 0.002	0.08 ± 0.001	0.02± 0.00001	0.04± 0.00001

LP = Lokop (Putih), LK = Lokop (Kuning), P = Pengidam, MG = Menggamat, KP =Kampung Pisang

The water content of LP was 37.8%, while the LK was only 17.5%. The value was much lower than that in other types of yam, such as *D. bulbifera* (69.5%), *D. deltoidea* (80.2%), *D. versicolor* (80.2%) and *D. triphylla* (76.9%) [11]. Protein content in *D. hispida* also varied, ranging from 1.13% (LP) to 6.20% (MG), which was much lower than that of *D. alata* (6.4 - 9.5%), *D. opposite* (6.7 - 10.6%), *D. persimilis* (7.7 - 8.3%) and *D. fordii* (9.8 -10.2%) [13]. Low protein content was also reported for *D. bulbifera, D. deltoidea, D. versicolor* and *D. triphylla*, ranging from 1.6 to 3.1% [11]. Low protein content of 2.62% was also observed in *D. cayenensis* [12]. Like water and protein, lipis in *D. hispida* samples also varied, ranging from 1.99% (LP) to 9.36% (LK). The lipid levels were however much greater than that reported previously [10]. *D. bulbifera, D. deltoidea, D. versicolor and D. triphylla* contained only 0.2 to 0.3% of lipid. Total carbohydrates were calculated after the other major biomolecules were determined. The content of carbohydrate in *D. hispida* samples was 58.3 - 71.9%. Total carbohydrates in *D. bulbifera, D. deltoidea, D. versicolor* and *D. triphylla* were only in the range of 17.4 - 25.9% of the fresh weight [11]. *D. cayenensis* also contained a low carbohydrate of about 29.5% [12]. Meanwhile, total carbohydrates in some cultivars of *D. alata* were 81.6-87.6% of the dry weight. Carbohydrates in *D. opposite D. persimilis and D. fordii* were 65.7, 69.5 and 76.5%, consecutively [13].

Mineral content of *D. hispida*

Ash indicated the total mineral content in *D. hispida* tubers (Table 1). The highest ash content was observed in the MG sample (1.24%), while the lowest was in the LK sample (0.29%). This study only analyzed calcium, iron and phosphorus (Table 2). Phosphorus and calcium were the major minerals found in *D. hispida*, varying from 11.7 (KP) - 46.9 (LP) mg/100 fresh weight. The highest proportion of phosphorus was present in the LP sample, followed by the KP sample. Meanwhile, the highest proportion of calcium was in the MG sample. The presence of iron was relatively low in all samples. Phosphorus content in *D. hispida* was equivalent to that in *D. bulbifera, D. deltoidea, D. versicolor and D. triphylla* with values of 61, 47, 14, and 40 mg/100g fresh weight, respectively [11].

Phosphorus in some *D. alata* varieties was reported about 180-340 mg/100g fresh weight [14]. High phosphorus content was also reported in *D. opposite, D. persimilis and D. fordii*, with values of 472, 470 and 294 mg/kg dry weight, respectively. The magnesium content in these three yam species was higher than phosphorous, which was in the range of 443-474 mg/kg of the dry weight [13]. Calcium in *D. hispida* (1.18 - 5.78 mg/100g) was much lower than that in four Dioscorea species, which was in the range of 14.3 to 46.9 mg/100g [11]. Calcium in some *D. alata* cultivars was reported about 60-80 mg/100g of the fresh weight [14]. Iron content in *D. hispida* (0.02-0.08 mg/kg fresh weight) was lower than that in some other Dioscorea tubers. Iron content was between 0.39 - 2.92 mg/kg fresh weight was reported for *D. bulbifera, D. deltoidea, D. versicolor* and *D. triphylla* [11]. *D. opposite, D. persimilis* and *D. fordii* contained 18.4 - 19.2 mg iron/kg of the dry weight [13].

The data in Table 1 shows no correlation amongst proximate parameters in each sample. The nutritional differences amongst species and sampling location are most likely influenced by the location where the plant grows. Several influencing factors are soil conditions (pH,

available nutrients, organic matter and soil moisture), climate (temperature, precipitation and light intensity) and postharvest handling and storage [15]. Water content is one of the important parameters in food processing, as it affects stability and quality [16]. The water in starch of *D. alata* greatly influences rheology and physicochemical properties, thus affecting its processing [17].

D. hispida contains a large portion of carbohydrates, although not identified in this study. The type of carbohydrate from *D. alata* has been previously reported. The main carbohydrate was in the form of starch. Total carbohydrates from fresh weight in D. *bulbifera, D. versicolor, D. deltoidea* and *D. triphylla* were 25.9, 17.5, 17.4 and 20.0% [11], respectively. The carbohydrate content in *D. alata* was about 81.6 - 87.6% of the dry weight [14]. The content ratio between amylose and amylopectin in Dioscorea spp was reported higher than that in other tubers. For example, the amylose content of *D. alata* was reported to vary between 29.2% [18] and 36.2% [19]. This value is equivalent to amylose in wheat starch and higher than potato starch. Short chain amylopectin (DP 6-12) in *D. alata* was also greater (19%) than that of potato starch [18]. The amount and types of carbohydrates, proteins and lipids greatly affect the way food processing is performed. The functional properties of carbohydrates are determined from the water absorption capacity and gel formation. The structure of starch (crystalline and amorphous ratios) also greatly affects its functionality [18]. However, the viscosity and crystallinity of starch of *D. hispida* can be adjusted by hydrothermal treatment, without affecting the integrity of the structure and its chemical properties [21].

Secondary metabolites in *D. hispida*

The phytochemical test showed that all *D. hispida* samples contained secondary metabolites of alkaloids and terpenoids and only LP samples contained steroids and phenols (Table 3). The difference in secondary metabolite products of samples is likely affected by growth location, soil conditions and climate.

Table 3 Results of phytochemical test of *D. hispida*

Sample	Steroid	Fenol	Alkaloid	Terpenoid
LP	+	++	+	+
LK	-	-	+	+
P	-	-	+	+
MG	-	-	+	+
KP	-	-	+	+

LP = Lokop (Putih), LK = Lokop (Kuning), P = Pengidam, MG = Menggamat, KP = Kampung Pisang

One of the steroid compounds normally found in the Dioscorea genus is diosgenin [22]. Diosgenin is a hydrolysis product of saponins. Diosgenin is commercially used for the synthesis of cortisone, pregnenolone, progesterone, and other steroidal products. In addition to steroids, the LP sample also contained phenolic compounds, which are also common secondary metabolites in plants. Their aromatic structures give raise to antioxidant activity. D. *bulbifera* was reported to have a total polyphenols of 166 mg/100g, while *D. versicolor* and *D. triphylla* contained total polyphenols of 41 and 13 mg/100g [7], respectively. *D. alata* and *D. cayenensis* were also known to contain total polyphenols of 16.03 and 3.43 mg/100g, respectively [22].

Plants containing alkaloids have been widely used as traditional medicine and today are better utilized as raw materials of modern medicine. Alkaloids have various pharmacological activities, e.g. anti-antibacterial [23]. Terpenoids are organic compounds consisting of various groups of terpen, diterpenes, and sesquiterpenes. Terpen compounds have been studied and known to have many medicinal activities. Plant-based terpenoids have been used in food, pharmaceutical, and chemical industries, and studied for biofuel production [24]. Some types of yam, such as *D. alata, D. opposite, D. persimilis* and *D. fordii* were also known to contain allantoin and dioscin [13]. Allantoin is a purine degradation product and is used as an active ingredient in cosmetics and moisturizers. Meanwhile, the dioscin has anti-inflammatory properties, lipid-lowering activity and anti-tumor levels.

Cyanide content in *D. hispida*

Apart from having good nutrition and medicinal compounds, *D. hispida* also contains toxic compounds, such as dioscorine and histamine. The suppressive effect of dioscorine on acetylcholine in the nervous system was thought to contribute to its poisonous properties [25]. This may be the reason why *D. hispida* tubers have been rarely used as a staple food. Other toxic compound found in the genus Dioscorea is cyanogen (in the form of HCN). Although not exceeding the lethal dose to humans (0.5 - 3.5 mg/kg body weight), the cyanogen content (as HCN equivalent) in *D. bulbifera, D. versicolor, D. deltoidea*, and *D. triphylla* were reported 3.3, 6.0, 3.2, and 3.3 ppm, respectively [26]. Cyanogen exists due to the damage to the tuber's tissue, most often by peeling or slicing. When the tissue is damaged, two cyanide precursors (linamarin and lotaustralin) will contact with air and react to produce cyanohydrin and glucose, catalyzed by linamarase. Cyanohydrin, at room temperature and pH > 5.0 will break down to HCN and acetone [27]. Linamarin and lotaustralin are however very

soluble in water and not heat resistant, thus they could be easily removed. In addition to removing linamarin and lotaustralin, washing with water also dissolves dioscorin.

Traditionally, the removal of toxins in *D. hispida* tubers is done by washing, soaking, and heating or drying the cut or sliced tubers. Another way to remove the toxins is to pile up the tubers and sun-dry them for a certain period. Soaking tubers in salt solution for several days or boiling them for 30 minutes could also remove the toxins. The method seemingly inhibits the activity of linamarase and glucosidase enzymes, thus stopping the pathway of cyanide formation. Repeated washing significantly reduced cyanide levels after the third wash (Table 4). The lowest remaining cyanide was observed in sample P (13%). The cyanide level after the second washing was still about 76 - 96% of the first. Cyanide in LP, LK and P samples seemed to be easily removed. In contrast, the cyanide in KP and MG samples was difficult to remove. Cyanide after the third washing in both samples was 68% and 46%, respectively, compared to cyanide in first washing. If washing continued, cyanide levels are predicted to further decrease. One of the new techniques to remove dioscorine was by microwave-assisted solvents extraction [28]. This separated the alkaloids from the starch by using less volume of solvents. This will shorten the extraction time and reduce environmental pollution due to solvent waste.

Table 4 Cyanide content in *D. hispida* tubers after several washing

Sample	Washing frequency	Cyanide in filtrate (mg/Kg)	Residual (%)
LP	1	378	-
	2	351	93
	3	108	29
LK	1	729	-
	2	594	81
	3	108	15
P	1	621	-
	2	594	96
	3	81	13
MG	1	439	-
	2	392	89
	3	201	46
KP	1	544	-
	2	413	76
	3	372	68

LP = Lokop Putih, LK = Lokop Kuning, P = Pengidam, MG = Menggamat, KP = Kampung Pisang

CONCLUSIONS

The information on the nutritional content and the potential drug compounds in *D. hispida* is essential to determine its utilization strategy. The results showed that the nutritional content of *D. hispida*, mainly the high carbohydrate (58 - 72%), was comparable with other Dioscorea families. High carbohydrate content makes *D. hispida* may be used as staple food. The starch functionality may also be increased by modification, for example as substrates for probiotic food, glucose production and bioethanol fermentation, as well as basic materials for biofilm. Further research is however needed to study the structure of the active compounds, as well as to test their various activities. The presence of dioscorine in the tuber can be simply overcome with good postharvest handling. In addition, the toxins can be easily removed by repeated washing.

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