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Original Research

Genetics characterization, nutritional and phytochemicals potential of gedi leaves (*Abelmoschus manihot* (L.) Medik) growing in the North Sulawesi of Indonesia as a candidate of poultry feed

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ABSTRACT:

Gedi, local name of *Abelmoschus manihot* (L.) Medik was used by local people in Northern Sulawesi-Indonesia as vegetable, because of its medicinal properties. The potency of gedi leaves in broiler diet has not been reported in literatures. The objective of this research was to investigate a genetic diversity of gedi commonly consumed as a gourmet cuisine in the North Sulawesi of Indonesia, and exploring the potential of this plant as a herb plant for a candidate of poultry feedstuff. Eight morphologically different gedi leaves (GH1, GH2, GH3, GH4, GH5, GH6, GM1 and GM2) that grow in Manado area, North Sulawesi of Indonesia were collected and identified. The leaves were extracted for DNA isolation followed by PCR and DNA sequencing analysis. During DNA isolation, 3 of 6 GH (GH4, GH5, GH6) were discontinued because of difficulty in separating the mucilage properties. Following PCR analysis, GH2 and GH3 did not produce bands and consequently were excluded from further analysis. In addition to that, chemical analysis was also performed to determine the phytochemical and nutritional contents .The results indicated that all gedi leaf samples showed similarity (99%) to species member of *Abelmoschus manihot,* and tribe of Malvaceae. In terms of proximate analysis, gedi leaves showed high crude protein (18.76 - 24.16%) and calcium (2.92-3.70%) content. Also, showed high crude fibre (13.06-17.53%). Together with the presence of alkaloid and steroidal saponin gedi leaves may offer beneficial effects as poultry feedstuff to a special production trait such as cholesterol-less meat.

Keywords:

Abelmoschus manihot, genetic characterization, nutritional analysis, phytochemical constituents.

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INTRODUCTION

Abelmoschus manihot (L.) Medik is a native plant which is 1.2 – 1.8 m height and is widely distributed in the tropical regions. This plant has various local names such as aibika. It was hypothesized that the origin of this plant from the survey of literature the local names of *Abelmoschus manihot* (L.) Medik varies and the data available were largely derived from studies carried out in the polynesian-pacific regions (Preston, 1998). In North Sulawesi of Indonesia this plant is called "gedi" and its leaves provide essential ingredient for cooking porridge as a special gourmet food among the North Sulawesi cuisine. According to Jain and Bari (2010), gedi leaves contain polysaccharides and protein containing mucilage (gum) that enables the porridge to have a special viscosity. Morphologically, gedi plants vary in shape, color and other properties regardless of geographical differences suggesting some genetic variation may occur after a long period of adaptation.

Gedi plants have been reported to posses medicinal properties that may benefit to human health. Puel *et al.,* (2005) reported that the female wistar rat which feeding 15 % of gedi leaves prevent osteopenia that was attributable to the calcium content of gedi leaves. Other authors, Jain *et al.,* (2009) reported that woody stem of gedi plant contain stigmasterol and γ-sitosterol, and also contain isoquercitrin, hyperoside, hibifolin, quercetin and isohamnetin that have anti consulvant and anti depressant-like activity (Guo *et al.,* 2011; Wang *et al.,* 1981; Wang *et al.,* 2004). Gedi leaves have active pharmacological properties against analgesic effect (Jain *et al.,* 2011). Sarwar *et al.,* (2011) stated that *Abelmoschus manihot* has a profound anti-inflammatory and anti-diabetic effect. From these reports it can be concluded that gedi plants posses herbal medicine properties that can be used to manipulate the human and animal health. In spite of its phytopharmaceutical benefits there is paucity in information dealing with genetic diversity of gedi plant in Indonesia. Most information of *Abelmoschus manihot* derived from the studies carried out in the polynesian pacific regions (Preston, 1998).

Gedi as a culinary herb and medicinal herb may have beneficial effects in animals. The phytochemical and nutritional compounds of leaf material may affect to poultry health and productivity. Cross *et al.,* (2007) reported that culinary herbs in diets affect chick performance, gut health and endogenous secretions. Al-Sultan and Gameel (2004) suggests that feeding *Curcuma longa* (turmeric) to chicken through diet can induce hepatic changes and that these changes are not dose or time dependent. Windisch *et al.,* (2008) cited several research, i.e. that phytogenic product also reduced activities of intestinal and fecal urease enzyme in broilers.

Ashayerizadeh *et al.,* (2009) reported that garlic powder and turmeric powder in diet significantly reduced abdominal fat percent, LDL and VLDL concentration in serum of broiler. Moreover, Yang *et al.,* (2003) reported that green tea by product affect the reduction of body weight gain and meat cholesterol in broilers. Khatun *et al.,* (2010) observed using *in vitro* model that viscous water-soluble portion of the fruit of *Abelmoschus esculentus* (L.) Moench has significant capacity to reduce the glucose diffusion form the dietary fiber-glucose systems.

The study was undertaken to investigate the compositional characterization of gedi. The samples were an alysed for the molecular characterization and identification, the proximate composition of the leaf part, energy content and the phytochemical composition, in order to get some useful information to be used in the preparation of poultry feed. Because there are no major reports in the literature, this would be an information for the detailed utilization of gedi to poultry feed.

MATERIAL AND METHODS

Plant Identification

Eight accessions of gedi (*Abelmoschus manihot*) collected from Manado, the North Sulawesi, Indonesia were used for this study. Herbarium specimens were identified for plant species at the Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia.

DNA extraction, quantification, and sequencing

DNA was extracted from 80-100 mg of fresh leaf tissue from each of the 5 randomly selected samples using a protocol of AxyPrep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences, www.axygenbio.com). Three samples were scored as missing because of unable to isolate the mucilage. The final DNA supernatant were diluted for DNA quantifications with PCR technique. PCR analysis were performed using a Thermocycler machine, and in 50 µl reaction mixture containing 2 µl template of DNA, 2 x master Mix Vivantis 25 µl (Vi Buffer A 1 x; Taq Polimerase 1,25 unit), Primer F1 (10 pmol/µl) 1 µl (0,2 mM), Primer R1318 (10 pmol/µl) 1 µl (0,2 mM), $MgCl_2$ (50 mM) 1,5 µl (3 mM dNTPs 0,4 mM), H_2O 20,5 µl, sample 1 µl.Initial trial was run on 5 samples and Taq quantity was Taq Polimerase 1,25 unit. Two primers were initially screened for amplification in PCR, they are Primer ndhF-F1 with product description 5'-GAA-TAT-GCA-TGG-ATC-ATA-CC-3' (length 20) dan primer ndhF-R1318 with product description 5'-CGA-AAC-ATA-TAA-AAT-GCR-GTT-AAT-CC-3' (length 26). PCR conditions were pre-hot 94°C (5 minutes), denaturation 94°C (45 seconds), annealing 54°C (45 seconds), primerization 72°C (1 minute 30 seconds) in 35 cycles and hold at 72°C (5 minutes). All PCR products were separated by electrophoresis in 1% agarose gel in 1 x TBE ran for 2 hours followed by ethidium bromide staining (5 µg ethidium bromide/ml). The gel was then stained and rinsed in water for about 10 minutes, and after that visualized under UV-light in trans -illuminator.

All PCR products were sequenced. Sequence data were identified at First Base Laboratories Sdn, Bhd (1st base), Taman Serdang Perdana, Selangor, Malaysia. Sequences were aligned using BLAST programme, and the building of a phylogenetic tree was established by Bioedit 7.19 and Mega 5 programme (http:// megasoftware.net).

Phytochemical Screening

Chemical tests were carried out to evaluate the presence of the phytochemicals such as alkaloids,

Table 1: Identification/Determination of Gedi Leaves from Manado, North Sulawesi

No	Place of Collection	Species	Tribe
1	(1) (GH4)	*Abelmoschus manihot* (L.) Medik	Malvaceae
2	(2) (GH5)	*Abelmoschus manihot* (L.) Medik	Malvaceae
3	(3) (GH2)	*Abelmoschus manihot* (L.) Medik	Malvaceae
4	(4) (GM2)	*Abelmoschus manihot* (L.) Medik	Malvaceae
5	(6) (GH3)	*Abelmoschus manihot* (L.) Medik	Malvaceae
6	(8) (GM1)	*Abelmoschus manihot* (L.) Medik	Malvaceae
7	(9) (GH1)	*Abelmoschus manihot* (L.) Medik	Malvaceae
8	(11) (GH6)	*Abelmoschus manihot* (L.) Medik	Malvaceae

Notes: GH = green leaf; GM = reddish green leaf; GH1= Bumi Nyiur; GH2 = Wanea; GH3 = Bumi Beringin; GH4 = Teling; GH5 = Bahu; GH6 = Kleak; GM1 = Tingkulu; GM2 = Wanea.

flavonoids, saponins, tannins, triterpenoids/steroids, and hydroquinone in five selected samples; using standard procedures described by Harborne (1987), and one of the five samples was performed for total flavonoid analysis.

Test for alkaloids

One gram of sample was homogenized, added with chloroform and then with 3 ml of ammonia. Chloroform fraction was separated and acidified using H_2SO_4 2M for two minutes. The filtrate was separated and added with few drops of Mayer, Wagner, and Dragendorff's reagent. The sample was contained alkaloid if produced white sediment using Mayer reagent, orange sediment using Dragendorff reagent, and brown sediment using Wagner reagent.

Test for phenolic

Approximately 5 g powder was shaken and then heated to boil and filtered. For testing the presence of flavonoids, filtrate was added with Mg powder, HCl:EtOH (1:1) and amyl alcohol. A yellow solution that turned colorless within few minutes indicated the presence flavonoids. For the evaluation of saponins, filtrate was shaken with distilled water. The presence of saponins was indicated by the appearance of bubbles. For the evaluation of tannins availability, filtrate was added with three drops of $FeCl_3$ 10%. The dark green solution indicated the presence of tannins.

Test for steroids/triterpenoids

Four grams of sample were added with 2 ml hot ethanol. Filtered and heated, and homogenized with 1 ml

Table 2: Nutrients composition and energy values of gedi leaf (dry weight basis)

Nutrients	Types of Gedi				
	GH1	GH2	GH3	GM1	GM2
Dry Matter (%)	81.72	87.33	87.14	86.70	84.76
Ash (%)	11.78	13.22	11.45	12.29	14.27
Crude Protein (%)	20.18	18.76	19.89	22.62	24.16
Crude Fiber (%)	17.53	14.37	15.68	14.37	13.06
Crude Fat (%)	1.06	3.80	2.96	1.63	4.51
N-free extract (%)	31.17	37.18	37.16	35.79	28.76
Ca (%)	3.29	3.70	2.92	3.33	3.36
P (%)	0.39	0.50	0.55	0.48	0.85
GE (Kkal/kg)	3419	3859	3850	3654	3699
Component of Fiber (%):					
NDF	20.78	21.72	25.02	34.09	23.51
ADF	18.44	19.11	16.23	20.10	17.30
Hemicellulose	2.34	2.61	8.79	13.99	6.21
Cellulose	11.39	15.25	11.02	5.50	10.62
Lignin	5.88	3.02	4.54	13.17	6.50
Silica	1.15	0.84	0.66	1.18	0.16

Notes: GH = green leaf; GM = reddish green leaf

Figure 1: Eight accessions of gedi leaf collected from Manado, North Sulawesi. GH1= Bumi Nyiur area, GH2 = Wanea area, GH3 = Bumi Beringin area, GH4 = Teling area, GH5 = Bahu area, GH6 = Kleak area, GM1 = Ting-kulu area, GM2 = Wanea area

diethyl ether. It is added with one drop of H_2SO_4 and one drop of CH_3COOH anhydrate. The presence of steroids was indicated by the alteration of violet to blue or green color. The formation of reddish violet color to the interface was formed that indicating positive sign for triterpenoids.

Test for hydroquinons

One gram sample was boiled with methanol for few minutes. The filtrate was allowed to cool and then added with 3 drops of NaOH 10%. The appearance of red color indicated the presence of hydroquinone.

Nutritional Analysis

The proximate analysis were carried out in duplicates and the results obtained were the average values. The proximate analysis (protein, crude fiber, crude fat, carbohydrate and ash) of five types of gedi leaf were determined by using the Association of Official of Analytical Chemists (AOAC) methods (1980). Nutrient contents were valued in percentage. The energy value was determined by bomb calorie meter.

RESULTS AND DISCUSSION

Plant Identification

Two typical colors of gedi leaves (green and reddish green leaves) growing at eight locations in Manado area were presented in Figure 1. All leaves of this plant do not have the same size or even appearance. They vary in size, color, and even shape. The results of plant identification of eight accessions of gedi leaf were summarized in Table 1. Those have been recognized that all of eight accessions of gedi leaf in this research were species of *Abelmoschus manihot* (L.) Medik, tribe Malvaceae. Breen (2012) reported that leaves are often the basis for identifying plants since they are so easily observed.

The boundaries of the eight accessions of gedi from the different locations of Manado area were based on morphological features of the species. The phylogenetic hypotheses were tested using chloroplast DNA sequence of ndhF. Total genomic DNA were extracted from eight accessions of fresh leaf material, and the ndhF gene was amplified in PCR using primer.

In this research, DNA fragments of the expected size were amplified from five samples to obtain the isolation product of electrophoresis, as shown at Figure 2. Based on DNA fragments, according to their molecular weights those products indicated that there were no different chloroplast type of gedi leaf color characteristics between green leaf (GH) and reddish green leaf (GM) with bands of 1.3 kb (Figure 2). Moreover, profile (external shape) of gedi leaf from the two color types were analysed as shown in Figure 2. Two samples of reddish green leaf (GM) and one sample of green leaf (GH) were used in the analysis of gedi leaf profile (Figure 3).

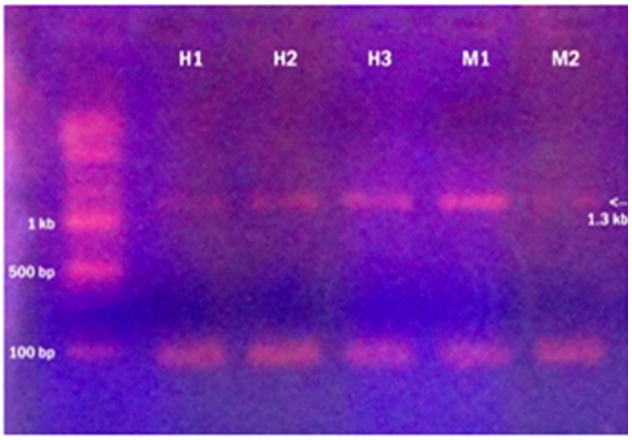

Figure 2: Electrophoresis of 5 samples of gedi leaf isolation product

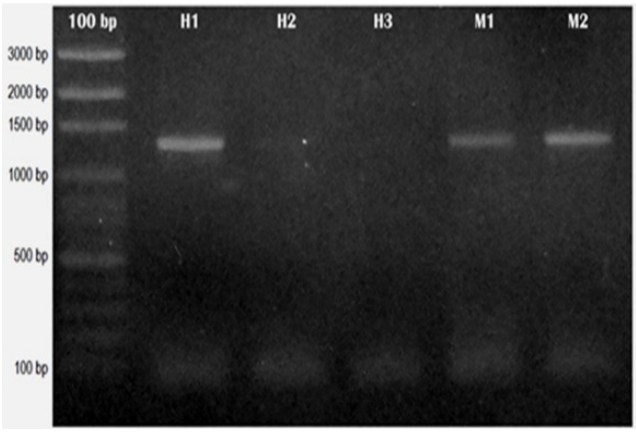

Figure 3: PCR amplification and electrophoresis product for profiles of gedi leaf obtained from 3 samples

> gb|AF384639.1| Abelmoschus manihot NADH dehydrogenase component NdhF (ndhF)gene, partial cds; chloroplast gene for chloroplast product

Length=1257

Score = 2242 bits (1214), Expect = 0.0

Identities = 1223/1229 (99%), Gaps = 2/1229 (0%)

Strand=Plus/Plus

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Sbjct  1    CTACTTTTTCCGACGGCAACAAAAAATCTTCGTCGTAGGTGGGCTTTTCCCAATATTTTA  60

Query  89   TTGTTAAGTATAGTTATGATTTTTTCGGTCGATCTGTCTATTCAACAAATAAATGGAAGT  148
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Query  149  TCTATCTATCAATATGTATGGTCTTGGACCATCAATAATGATTTTTCTTTCGAGTTTGGC  208
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Query  269  GTTCTTATTTATAGTGACAATTATATGTCTCATGATCAAGGCTATTTGAGATTTTTTGCT  328
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Sbjct 1201 TATTCACCGATTTTTGCAATAATAGCTTG 1229
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Figure 4: DNA Sequence Alignment with BLAST Method

Based on DNA bands, the gedi leaf color type of GH and GM had the same positions of bands of 1.3 bp indicating the similar profiles. By sequencing the PCR product, additional useful taxonomic and genome information were successfully obtained from three samples. The ndhF data sets have aligned lengths of 1257 bases, and the sequence data were shown in Figure 4.

Comparisons were done with a few selected DNA sequences, using closest relationship in a BLAST search. Analysis showed that this sequence was very similar to *Abelmoschus manihot* (L.) Medik (99%), as shown in Figure 4. The phylogenetic analysis was done based on ndhF sequences from each of the available three sample accessions of gedi (Figure 5). The three samples were clearly obtained asa member of the species *Abelmoschus manihot* (L.) Medik, tribe Malvaceae, and the sample GH1 was 96% similar to *Abelmoschus manihot.*(L.) Medik.

Nutritional Analysis

The proximate concentration of five samples of gedi were expressed on dry basis listed in Table 2. The proximate analysis showed that the gedi leaves contained ash (11.45-14.27%), crude protein (18.76-24.16%), crude fibre (13.06-17.53%), crude fat (1.06-4.51), N-free extract (28.76-37.18%) and gross energy (3419-3859 Kkal/kg), and minerals were calcium (2.92-3.70%) and phosphorous (0.39-0.85%). In terms of proximate analysis, gedi leaves showed high crude protein (18.76 - 24.16 %) and calcium (2.92-3.70%) content. Also, it showed high crude fiber (13.06-17.53%). In addition, the component of fiber were NDF (20.78-34.09), ADF

Table 3: Phytochemical screening of gedi leaf

Phytochemicals		Qualitative					Quantitative (%) (w/w) (n=3)
		Green			Reddish green		
		GH1	GH2	GH3	GM1	GM2	GH1
Alkaloid	Wagner	+	+	+	-	-	
	Meyer	+	-	+	-	-	
	Dragendorf	-	+	-	-	++	
Hidroquinon		-	-	-	-	-	
Tannin		-	-	-	-	-	
Flavonoid		++	++	-	+	+	0.48
Saponin		+	++	+	-	+	
Steroid		+++	+++	+++	+++	+++	
Triterpenoid		-	-	-	-	-	

Notes: - = nothing; + = weak positive; ++ = positive; +++ = strong positive

(16.23-20.10%), hemicellulose (2.34-13.99%), cellulose (5.50-15.25%), lignin (3.02-13.17%), and silica (0.16-1.18%). Prasad, *et al*., (2010) reported that the biological effects of estimated proximate components (moisture, protein, fiber, fat, ash, and energy) in living system strongly depend on their concentration. Therefore, it should be carefully controlled when herbs are used as food component. Energy and nutrient values of herb plant samples are mainly used to translate herb samples intakes as intakes of food components.The result of this study indicated that *Abelmoschus manihot* (L.) Medik from The North Sulawesi might be the best alternative source of nutrient. High protein and fiber obtained in this study confirms that *Abelmoschus manihot* can be used as good alternative source of protein and crude fiber.

These results recommended high rank for the leaves of *Abelmoschus manihot* as the best in terms of essential nutrients composition if compared with those of other edible leaves in the literature.

The results of phytochemical screening of five types of gedi leaf were summarized in Table 3. Result depicted that all samples had rich steroid but had no tannin. Four samples contained saponin and flavonoid, while three samples contained alkaloid. The result of this study indicated that *Abelmoschus manihot* (L.) Medik from Manado is a good alternative source of phychemical steroid, flavonoid and saponin.

The phytochemical steroid was detected in all types of gedi leaf, and this phytochemical was found in maximum content. Alkaloids were detected with Wagner reagent only in green leaves GH1, GH2, and GH3. Flavonoids were found at the adequate amount in green leaf GH1 and GH2 while flavonoids in reddish green leaf were at the minimum amount. Quantification of total phenolic content from sample GH1 showed its phenolic content as 0.48% (w/w). The results suggested that all samples of gedi had the potential in steroid, flavonoid and saponin, and free of anti nutritional tannin. Flavonoids had been reported in rat brain, and might represent the potential bioactive component of *A. manihot* and contributed to its anticonsulvant and anti depressant-like activity *in vivo* (Guo *et al.*, 2011). Jain *et al.,* (2011) reported that the phytochemical analysis

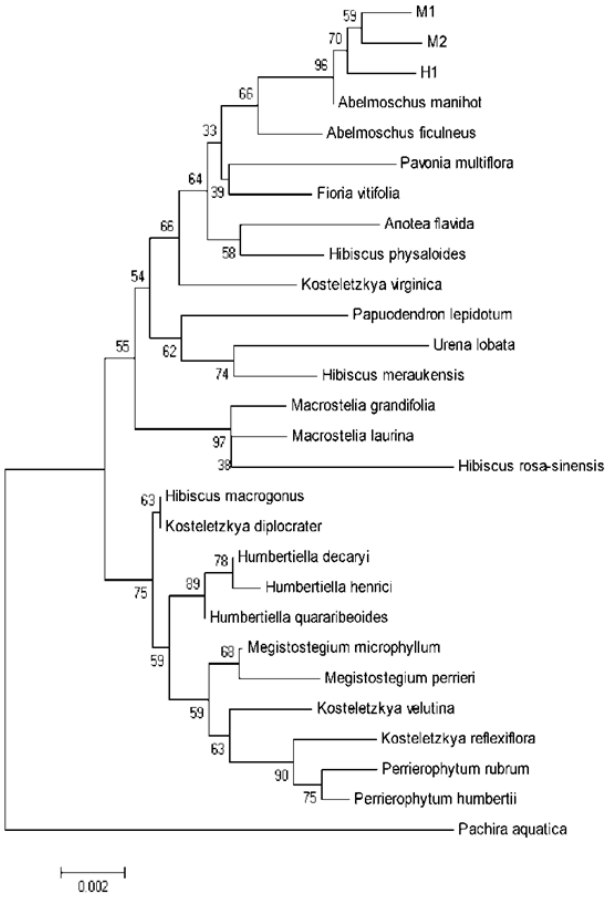

Figure 5: The phylogenetic tree of gedi leaves based on ndhF-gen with Kimura-2 model parameter. Data on the branch are bootstrap maximum likelihood values

showed the presence of steroids, triterpenoids and flavonoids in petroleum ether and methanol extract, respectively which possesses analgesic, antioxidant and anti-inflammatory activity. Saponins that were steroid or triterpenoid glycosides are important in animal nutrition. Some saponins increase the permeability of intestinal mucosal cells in vitro, inhibit active mucosal transport and facilitate uptake of substances that are normally not absorbed (Francis *et al.,* 2002).

CONCLUSION

The characterization, nutritional analysis and phytochemical analysis of *Abelmoschus manihot* leaf by genetical and chemical analysis recommended the potential value of these feedstuff to those populations who rely upon them as poultry feed or supplements to poultry diet. The next step is to assess the bioavailability of the essential nutrients and phytochemicals in these plants. Further study have to focus on the digestibility of protein, fibre, and lipid, and phytochemicals.

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