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## Nutrition of local wild edible fern (*Diplazium esculentum*) leaves

Y Koniyo<sup>1\*</sup>, C Lumenta<sup>2</sup>, A H Oli<sup>1</sup>, R O S E Mantiri<sup>2</sup> and N Pasingi<sup>1</sup>

<sup>1</sup> Faculty of Fisheries and Marine Science, Gorontalo State University, Jl. Jendral Sudirman, No. 6, Gorontalo City, 96128, Gorontalo Province, Indonesia

<sup>2</sup> Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Unsrat Bahu, Manado City, 95115, North Sulawesi Province, Indonesia.

Corresponding author: yuniarti.koniyo@ung.ac.id

**Abstract.** Vegetable fern species distributed throughout the world are diverse, but there is a lack of scientific data about the nutritional content of local vegetable ferns. This study aimed to provide preliminary data of *Diplazium esculentum* nutrition obtained from Bone Bolango District, Gorontalo area, Indonesia, in order to support the developing of pharmaceutical and mixed animal feed products. Tests for the proximate characteristics of leaf extracts and the phytochemical analysis were carried out through qualitative screening following Indonesian National Standard (SNI) Method year 2006, while the detail amino acid compound analyzed using Gas Chromatography Mass Spectrometry (GCMS) method. The results showed that *D. esculentum* from Gorontalo Land is rich with proteins and amino acids. The amount of protein and fat is detected as  $21.52 \pm 2.70\%$  and  $2.47 \pm 0.97\%$  respectively. The fern also contains complete secondary metabolites in the form of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides with a high amount.

### 1. Introduction

Ferns are a group of simplicia commonly used as vegetables by Southeast Asia and Islands in the Pacific Ocean residents [1]. Edible ferns are planted that have been used both for dietary and therapeutic purposes [2]. *Diplazium esculentum* (Retz.) Sw. or 'Dheki Shak' is used as an edible leaf for local communities of parts of West Bengal [3]. In Indonesia, ferns are available in almost every region. The high availability of wild ferns on the mainland of Gorontalo indicates the characteristics of the Gorontalo environment to match the plant's growth habitat. However, the utilization of vegetable ferns in Gorontalo is not optimal yet.

Vegetable fern species spreading throughout the world is diverse, reaching 400 species [4]. However, scientific data on the nutritional content of vegetable ferns that live in the tropics, especially in the Gorontalo area, are not provided. In several areas, the results of phytochemical screening of some species of *Taenitis blechnoides*, *Pityrogramma calomelanos*, *Adiantum latifolium*, *Cheilosoria tenuifolia*, *Vittaria ensiformis* contain chemical compounds saponins and steroids. Also, the types of *Vittaria graminifolia* contains alkaloids, flavonoids, and tannins. Moreover, *Pteris vittata* contains terpenoids [5].

While studies on the nutritional and phytochemical aspects of edible ferns are common, but in such elements, the least exploration is to species *D. esculentum*. Preliminary identification of the proximate



content and phytochemical characteristics of this species in the Gorontalo area can underlie the development of pharmaceutical products, food diversification, and mixed animal feed cultivation.

## 2. Materials and methods

The leaves of the *D. esculentum* samples obtained from Tingkohubu Timur Village, Suwawa Sub-district, Bone Bolango District, Gorontalo (Altitude 26 m MSL, N 0°31'41".5452"; E 123°8'57.3756") were washed with running water to remove the inherent contaminants. The leaves are dried to prevent damage, microbial contamination, and are durable to store for a long time. Suitable dry *Simplicia* was selected, weighed, and stored in a closed container.

The vegetable ferns isolation was carried out by maceration. The dry *Simplicia* was extracted using 80% methanol with 3 x 24 hours soaking time to obtain crude extract. The extracted solution was filtered and evaporated by a rotary evaporator to give methanol extract.

Analysis of the proximate composition of samples was carried out at the Fisheries Product Quality Testing and Development Centre, Gorontalo. The characteristic of water, ash content, alcohol soluble extract, protein, and fat follow Indonesian National Standard (SNI) 2006, while the qualitative phytochemical screening for the extraction was conducted at the Laboratory of Spice and Medicinal Crops Research Institute, Agency for Agricultural Research and Development, Bogor, Indonesia. The phytochemical screening using different reagents as follows: **Alkaloids test**: Two grams of extract were dropped from Dragendorff's reagent. An orange precipitate was formed by the addition of the Dragendorff's reagent indicated the presence of Alkaloids. **Saponins test**: Two grams of the extract was treated with 2 ml of hot water, then shaken and treated with a few drops of HCl. The permanent foam indicated a positive result. **Tannin test**: Two grams of the extract were added FeCl [3]. The formation of a blackish green color indicated the presence of tannins. **Phenolic test**: Two grams of the extract were added FeCl [3]. The formation of blackish-blue indicated the presence of polyphenols. **Flavonoids test**: The filtrate was added concentrated HCl solution and then heated. The color changes to yellowish-green indicated the presence of Flavonoids. **Triterpenoid test**: Two drops of anhydrous acetic acid were added in 1 gram of extract, which had been dissolved in 2 ml of chloroform. This mixture was pressed with H<sub>2</sub>SO<sub>4</sub>. The formation of brownish or violet rings indicates the presence of a triterpenoid. **Steroids test**: 2 ml of chloroform was added to 5 grams of sample. The red color is formed when dripping concentrated H<sub>2</sub>SO<sub>4</sub> indicated the presence of Steroids. **Glycoside test**: The examination of glycosides was carried out with the Lieberman-Buchard reaction. The extract was dissolved in 5 ml of acetic anhydride then H<sub>2</sub>SO<sub>4</sub> was dropped. The formation of blue or green indicated the presence of glycosides.

Further testing to find out the constituent compounds for each type of amino acid was carried out using the Gas Chromatography-Mass Spectrometry (GCMS) method. A liquid extract of fern leaf was injected into the GCMS injector (inlet) of 1 µL. The analysis took place according to the temperature program specified in the Standard Instrument Control Parameters, which was 60°C for 10 min, then 7°C / min to 300°C for 36 min in 80.286 minutes run time.

## 3. Results and discussion

The results of the identification of the proximate contents of *D. esculentum* leaves extract presented in Table 1 to show that this vegetable species has a variety of minerals, proteins, and fats. Further testing through phytochemical tests (Table 2) and the detail compounds (Table 3) indicate that *D. esculentum* leaves contain a complete amino acid composition.

**Table 1.** Proximate extract of vegetable fern, *D. esculentum*, leaves

| No | Proximate Composition   | Amount (%)   |
|----|-------------------------|--------------|
| 1  | Water                   | 16.16 ± 0.69 |
| 2  | Total Ash               | 2.67 ± 0.79  |
| 3  | Acid insoluble ash      | 2.83 ± 0.05  |
| 4  | Alcohol soluble extract | 27.46 ± 4.82 |
| 5  | Protein                 | 21.52 ± 2.70 |
| 6  | Fat                     | 2.47 ± 0.97  |

In general, all the samples were analysed for higher proximate content. Variations in the results of testing the proximate content of the species *D. esculentum* obtained from different locations indicate that habitat factors determine the proximate content. Plants require multiple nutrients and generally acquire them from the soil solution [6].

**Table 2.** Phytochemical test of vegetable fern, *D. esculentum*, leaves

| No | Amino acids   | The screening test from replication |   |   |   |
|----|---------------|-------------------------------------|---|---|---|
|    |               | 1                                   | 2 | 3 | 4 |
| 1  | Alkaloids     | +                                   | + | + | + |
| 2  | Saponins      | +                                   | + | + | + |
| 3  | Tannins       | +                                   | + | + | + |
| 4  | Phenolic      | -                                   | + | + | + |
| 5  | Flavonoids    | +                                   | + | + | + |
| 6  | Triterpenoids | +                                   | + | + | + |
| 7  | Steroids      | +                                   | + | + | + |
| 8  | Glycosides    | +                                   | + | + | + |

(+: present) (-: absent/undetected)

**Table 3.** Compounds of the identified amino acids of vegetable fern, *D. esculentum*, leaves

| No | Compounds  | Amino Acids | PCT Area |
|----|--|-------------|----------|
| 1  | Tetrahydrocyclo Hexane Carboxylic Acid                                 | Tannins     | 8.6658   |
| 2  | gamma,-Sitosterol,807596,000083-47-6,99                                | Steroids    | 7.8705   |
| 3  | Neophytadiene,460917,000504-96-1,99                                    | Steroids    | 6.7438   |
| 4  | decahydrobenzo   | Saponins    | 4.3542   |
| 5  | Decahydiberzo-   | Saponins    | 4.3541   |
| 6  | Hexadecanoic acid  | Alkaloids   | 4.2542   |
| 7  | 1,4-Benzenediol  | Flavonoids  | 3.5192   |
| 8  | 5-Hydroxymethylfurfural  | Glycosides  | 3.4544   |
| 9  | n- Hexadecanoic acid   | Alkaloids   | 2.9183   |
| 10 | Phytol   | Tripennoids | 2.9183   |
| 11 | 1,2-Benzenediol  | Flavonoids  | 1.9802   |
| 12 | Phenol   | Phenolic    | 1.9071   |
| 13 | 2- Hexadecen-1-ol, 3,7,11,15- tetramethyl-, [R-[R <sup>*</sup> -(E)]]- | Alkaloids   | 1.7814   |
| 14 | Benzeneethanol, 4-hydroxy-   | Tannins     | 1.7387   |
| 15 | 2-Pentadecanone, 6,10,14-trimethyl                                     | Tannins     | 1.6919   |
| 16 | BETA.-D4-HEXAMETHYLENEOXIDE,   | Tripennoids | 1.6027   |
| 17 | Trisilane  | Phenolic    | 1.5893   |
| 18 | 2,5-Dimethylfuran-3,4(2H,5H)-dione                                     | Phenolic    | 1.3539   |
| 19 | (3methyl,24R)-ergost-5-en-3-ol   | Glycosides  | 1.3041   |
| 20 | Tetrahydrocyclo Hexane Carboxylic Acid                                 | Alkaloids   | 1.264    |
| 21 | 3-Ethylthio-1-propene  | Tannins     | 1.2591   |

|    |   |             |        |
|----|---|-------------|--------|
| 22 | “11,13-Dimethyl-12-tetradecen-1-ol acetate”                                   | Tripernoids | 1.2568 |
| 23 | Protoanemonine  | Phenolic    | 1.1799 |
| 24 | 6-trimethyloctanal  | Saponins    | 1.1789 |
| 25 | -4-2,(but-2enylidene)-3   | Saponins    | 1.1711 |
| 26 | Benzo[b]cyclopropa[1m]fluorenone  | Glycosides  | 1.1676 |
| 27 | 1,2-O-(1-METHYLETHYLIDENE) HEXOFURANOSE                                       | Glycosides  | 1.1411 |
| 28 | 3-Penten-2-one,4-methyl-  | Phenolic    | 1.0613 |
| 29 | Carbonic acid-2   | Saponins    | 1.0359 |
| 30 | Cycloheptanone, 19599,000502-42-1,42  | Steroids    | 1.0053 |
| 31 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol  | Tannins     | 0.9669 |
| 32 | Catechol  | Glycosides  | 0.9536 |
| 33 | 2-Furanmethanol   | Phenolic    | 0.9047 |
| 34 | 2,2-Dimethyl-6-methoxynaphtho [1,2-b]pyran                                    | Flavonoids  | 0.8978 |
| 35 | Quinoline,1,2,3,4-tetrahydro-1-((2-phenylcyclopropyl)sulfonyl-,trans-         | Tannins     | 0.8935 |
| 36 | Tetrahydrocyclo Hexane Carboxylic Acid  | Alkaloids   | 0.8698 |
| 37 | Tetrahydrocyclo Hexane Carboxylic Acid  | Alkaloids   | 0.822  |
| 38 | Stigmatan-3, 5-Diene  | Flavonoids  | 0.8036 |
| 39 | 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-                            | Flavonoids  | 0.7662 |
| 40 | n- Hexadecanoic acid  | Alkaloids   | 0.7095 |
| 41 | 1,6-diethylamin0-2-(3,4-dimethoxyphenyl)-7-methoxynaphthalene                 | Glycosides  | 0.6325 |
| 42 | “Butane,1-(ethenylthio)”  | Tripernoids | 0.5937 |
| 43 | “S-[2-[N,N-Dimethylamino]ethyl]N,N-dimethylcarbamoyl thiocarboximate”         | Tripernoids | 0.5796 |
| 44 | Cyclopropanecarboxylic acid, 2-methylene-, methyl ester", 18861,088787-23-9,1 | Steroids    | 0.5697 |
| 45 | Stigmast-4-En-3-One   | Flavonoids  | 0.5317 |
| 46 | Cycloheptanone, 19597,000502-42-1,25  | Steroids    | 0.5131 |
| 47 | Diglycerol, 109127,000627-82-7,32   | Steroids    | 0.5094 |
| 48 | Vitamin E   | Vitamin E   | 0.4473 |
| 49 | 14.alpha.-Cheilanth-12-enic Methyl Ester,                                     | Tripernoids | 0.3608 |
| 50 | blithyaldehyde  | Saponins    | 0.0525 |

*D. esculentum* extract is high in water content seen in this study ( $16.16 \pm 0.69\%$ ). Drying removes the water present in the plant tissues, making it easier to quantify the various components of the plant [7].

Oven-dried plant samples from Los Angeles, Laguna, Philippines contain  $17.39 \pm 0.82\%$  ash [7]. Moreover, samples from Sikkim include  $16.2 \pm 0.7\%$  ash [8]. These values are higher than the total ash content from this research ( $2.67 \pm 0.79\%$ ).

The acid-insoluble ash content in the current study ( $2.83 \pm 0.05\%$ ) was high compared to the samples of *D. esculentum* from Sikkim, the Himalayas containing  $1.33\%$  ash [9]. Additionally, fresh plant samples from Wet Market of the Municipality of Los Baños, Laguna, Philippines, contain  $1.42 \pm 0.10\%$  ash [7].

The content of the soluble alcoholic extract of *D. esculentum* in this study was higher ( $27.46 \pm 4.82$ ) than this of soluble ethanol extract of the Girimukti Mountains Singajaya District, Garut Regency, West Java Province which amounted to only  $13.82\%$  [10]. There are contained amounts of protein in ferns in the current study.

The content was estimated at  $21.52 \pm 2.70\%$ . These values were lower than the protein contents from several previous research. An earlier study on *D. esculentum* from Sikkim, the Himalayas, has protein to be  $31.2 \pm 1.0\%$  [8]. Besides, another report from the district of Assam, India, said contained 34.28% protein for sonicated extraction [11]. Moreover, the oven-dried *D. esculentum* from the wet market of Los Baños contained  $10.67 \pm 0.05\%$  crude protein [7].

*D. esculentum* in this study had a lower amount of fat ( $2.47 \pm 0.97\%$ ) compared to previous studies reported that oven-dried *D. esculentum* from the Municipality of Los Baños wet market contained  $3.40 \pm 0.05\%$  crude fat [7].

Amino acid composition of *D. esculentum* leaf extract obtained from Gorontalo land turned out to show results in harmony with the result of the leaves from Central Kalimantan, Indonesia [12]. Unlike the leaves obtained from Blangkejeren, Aceh, Indonesia, where secondary metabolites of Alkaloids and Flavonoids are not found in the methanol extract of leaves of *D. esculentum* [13]. Alkaloids, terpenoids, steroids, and proteins, and amino acids were absent in the methanol extract from leaves originating from the mainland Ulu Kuang, Perak, Malaysia [14]. The results of the qualitative screening of leaf ethanol extract from the Municipality of Los Baños, Laguna, Philippines samples showed absent Tannin compound [7]. Alkaloids of the fern leaves from Chandraprabha Vanrai in Dapoli, Ratnagiri District of Maharashtra, were absent [14].

The distribution of *D. esculentum* is throughout Asia and Oceania. It is widely used by the village and tribal communities and is known as a vegetable fern [15]. *D. esculentum* is one of the most popular ferns, which is also used for medicinal purposes [16]. Its leaf is traditionally used in headache, pain, fever, wounds, dysentery, glandular swellings, diarrhea, and various skin infections. It can be used as a natural antioxidant, antimicrobial, and cytotoxic agent. However, further investigation is required to isolate and characterize the active chemical constituents responsible for the given activities [17]. benefits of *D. esculentum* vegetable ferns due to the presence of several active compounds they contain.

The nutritional content of *D. esculentum* vegetable foliage extract in the Gorontalo plains has the potential to be developed into fishery products such as natural animal feed formulations. Another fern species, *Cycas revoluta*, has been reported that containing Steroid compounds and began to be developed into a stimulant molting of tiger shrimp [18]. A similar leafy vegetable study reported that artificial diets enriched by spinach extract effectively stimulated soft shell crab production [19]. Nevertheless, more detailed and comprehensive research on the concentration of each nutrient is needed to support the development of this kind of fishery product.

#### 4. Conclusion

From the description of the present study, it can be concluded that the *D. esculentum* from Gorontalo Land contains high proteins and complete amino acid composition, which is the potential for the development of pharmaceutical products and mixed animal feed cultivation.

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