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Research Article

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Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from Philippines

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ABSTRACT

The nutritional content and phytochemical composition of Diplazium esculentum (Retz.) Sw., known as "pako" in the Philippines, were determined to ascertain its use as an ingredient in culinary dishes in the country. Pako, also known as fiddlehead fern, is patronized by locals as an ingredient in vegetable dishes and salads. Standard AOAC methods showed that the fresh plant samples contain 91.82 % moisture, 1.42 % ash, 0.28 % crude fat, 0.87 % crude protein, and 0.72 % crude fiber while oven dried plant samples contain 17.39 % ash, 3.40 % crude fat, 10.67 % crude protein, and 9.06 % crude fiber. Qualitative phytochemical screening detected the presence of alkaloids, reducing sugars, anthraquinones, anthranol glycosides, cyanidins, phenols, saponins, and proteins in both the ethanolic and aqueous leaf extracts, while cardiac glycosides, leucoanthocyanins, phytosterols, diterpenes, and triterpenes were only detected in the ethanolic extract. Using the Folin-Ciocalteu method, the total phenolic contents were 125.60 ± 13.44 and 11.65 ± 0.87 mg gallic acid equivalents per 100 g air-dried sample for the ethanolic and aqueous extracts, respectively. The corresponding total flavonoid contents were 110.81 ± 11.16 and 16.21 ± 0.72 mg quercetin equivalents per 100 g air-dried sample for the ethanolic and aqueous extracts, respectively.

Keywords: *Diplazium esculentum*, fern, nutritional content, phytochemical screening, phenolics, flavonoids

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INTRODUCTION

Indigenous plants play a very significant role in the economic and social spectra of a particular locality. In the Philippines, people continue to rely on indigenous plants for food, alternative medicine, and cosmetics. Despite the local population's patronage of many of their diverse indigenous species, many such plants remain understudied.

Pako or fiddlehead fern, (*Diplazium esculentum* (Retz.) Sw.), is an indigenous edible fern in the Philippines that is abundant in the southern parts of the island of Luzon up to the entire central Visayas Region. It belongs to family Athyriaceae. The crisp, palatable taste of the young shoots makes it a desirable ingredient for Filipino salad preparations. Apart from being often served as salad, it is also prepared with coconut milk or stir-fried together with other vegetables, along with meat and seafood. *D. esculentum* is also sometimes grown as a house plant. Aside from the Philippines, it is reported to occur in the southern parts of Asia up to Polynesia. It is known as "linguda" in northern India, "pucuk paku" in Malaysia, and "dhekia" in Assam (Northeast India) [1]. Despite its popularity, there is scant scientific literature about research and development efforts on this plant species.

A few studies on the bioactive properties of *D. esculentum* were reported without emphasis on its phytochemical constituents. Determination of the antioxidant activity of the shoots of the species along with two other local vegetables *Manihot utilissima* (tapioca shoot) and *Sauropous androgynus* (cekur manis) showed significant

differences in properties of boiled and fresh samples of the vegetables. Both the aqueous and organic extracts from fresh and boiled samples of *D. esculentum* gave higher antioxidative activities using the ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) method than α-tocopherol (Vitamin E), which served as positive control [2]. Aqueous and alcoholic extracts of *D. esculentum* showed activity against human and plant pathogenic bacteria like *Escherichia coli, Salmonella arizonae, S. typhi,* and *Staphylococcus aureus*. The reference standard antibiotic used in the study was tetracycline. All extracts that were mixed in equal proportion with the antibiotic were more effective against the bacteria than the antibiotic alone [3].

Phytochemical screening is an important step prior to the determination of the bioactivity of the extract, *e.g.*, antibacterial activity, [4] or the determination of the concentration of specific phytochemicals in the extract, *e.g.*, total phenolic and flavonoid contents [5, 6]. The presence of phytochemicals in the extracts can be qualitatively characterized by screening. The bioactivities of most phytochemical classes are available in the literature and will provide an overall estimate of the bioactivity of the extracts of *D. esculentum.*

The present study deals with the profiling of *D. esculentum:* nutritional content analysis, phytochemical screening, and quantification of phenolics and flavonoids to further assess the health benefits of the plant species and its potential bioactivities.

EXPERIMENTAL SECTION

Sample collection and species verification: *Diplazium esculentum* (Retz.) Sw. samples were obtained from the wet market of the Municipality of Los Baños, Laguna, Philippines. The authenticity of the fern sample was validated and certified to be *Diplazium esculentum* (Retz) Sw. by Prof. Annalee S. Hadsall, Curator of the Botanical Herbarium of the Museum of Natural History, University of the Philippines Los Baños. The upper portion of the plant (1 kg) was subjected to analyses of its nutritional value and another portion (1 kg) was used for solvent extraction for use in phytochemical screening and total phenolic and flavonoid content determination.

Nutritional content analysis: Fresh *D. esculentum* leaves were analyzed for their moisture, ash, crude fat, crude fiber and crude protein content while oven dried *D. esculentum* were analyzed for their ash, crude fat, crude fiber and crude protein content using standard analytical methods [7].

Solvent extraction: Fresh sample (500 g) was homogenized using a commercial blender (Waring™) and percolated in 95% ethanol for 24 hours, with continuous shaking. The extract was filtered using suction filtration and concentrated by evaporating ethanol under reduced pressure using Büchi Rotavapor R-110 at 50°C. Water extraction was done by boiling fresh sample (500 g) in distilled water for 15 minutes and then cooled. The concentrated extracts were stored in an airtight amber colored bottle and refrigerated prior to analysis.

Phytochemical Screening: The extracts were tested for the presence of different phytochemicals. The following lists the procedure and reagents, along with the resulting positive reaction indicating the presence of specific phytochemicals in the screening process [8, 9].

1. **Alkaloids** (Wagner's test): Two mL of the extracts were treated with few drops of Wagner's reagent (iodine-potassium iodide solution). Formation of reddish brown precipitate indicates presence of alkaloids.
2. **Carbohydrates - Reducing Sugars** (Benedict's test): Two mL of the extracts were treated with few drops of Benedict's reagent ($CuSO_4$, Na_2CO_3, Sodium citrate). Formation of orange red precipitate indicates presence of reducing sugars.
3. **Anthraquinones** (Borntrager's Test): Two mL of the extracts were dried over a water bath and the residue was extracted with 10 mL distilled water then filtered. The filtrate was extracted with 5 mL portions of benzene twice. The benzene extract was divided into two portions, with one portion serving as control. The other portion was treated with 5 mL of NH_3 solution then shaken. Formation of red coloration in the lower alkaline (ammoniacal) layer indicates the presence of anthraquinones.
4. **Anthranol glycosides** (Modified Borntrager's test): Two mL of the extracts were treated with few drops of ferric chloride solution and then immersed in boiling water for 5 mins. The resulting solutions were extracted with equal volume of benzene. The benzene layer was separated using ammonia. Formation of rose-pink colored solution (ammoniacal solution) indicates the presence of anthranol glycosides.
5. **Cardiac glycosides** (Legal's test): Two mL of the extracts were treated with few drops of Sodium nitroprusside in pyridine and NaOH. Formation of pink to blood red colored solution indicates the presence of cardiac glycosides.
6. **Cyanogenic glycosides** (Picrate paper test): Two mL of the extracts were treated with few drops of 10 mL water and 1 mL dilute HCl. Picrate papers (paper strips dipped in saturated aqueous picric acid previously neutralized with

$NaHCO_3$) were suspended above flask containing the solution. The solution was warmed at 45 ^oC for an hour. A picrate paper color change from yellow to red indicates presence of cyanogenic glycosides.

7. **Leucoanthocyanins** (Bate-Smith and Metcalf's test): Two mL of the extracts were treated with two portions of 0.5 mL concentrated HCl and observed for any color changes. The solutions were warmed for 15 minutes using a water bath. The solutions were observed again for at least an hour for further color change. Formation of strong red or violet color indicates the presence of leucoanthocyanins.
8. **Cyanidins** (Willstätter cyanidin test): Two mL of the extracts were treated with two portions of 0.5 mL concentrated HCl. Three to four pieces of magnesium turnings were added in the solution. Color change was observed within 10 minutes. Formation of purple colored solution indicates presence of cyanidin aglycones.
9. **Phenols** (Ferric chloride test): Two mL of the extracts were treated with three drops of ferric chloride solution. Formation of bluish black colored solution indicates presence of phenols.
10. **Tannins** (Gelatin test): Two mL of the extracts were treated with few drops of 1 % gelatin solution containing NaCl. Formation of white precipitate indicates the presence of tannins.
11. **Saponins** (Froth test): Two mL of the extracts were diluted with distilled water up to 20 mL then shaken. Formation of about 1 cm layer of foam indicates the presence of saponins.
12. **Phytosterols** (Liebermann-Burchard's test): Two mL of the extracts were treated with few drops of chloroform and then filtered. The resulting solutions were treated with few drops acetic anhydride, boiled and then cooled. Concentrated H_2SO_4 was added after cooling. Formation of blue green colored solution indicates the presence of phytosterols.
13. **Diterpenes** (Copper acetate test): Two mL of the extracts were treated with three drops of copper acetate solution. Formation of emerald green colored solution indicates the presence of diterpenes.
14. **Triterpenes** (Salkowski's test): Two mL of the extracts were treated with few drops of chloroform and then filtered. The resulting solutions were treated with few drops of concentrated H_2SO_4, shaken, and allowed to stand for 5 minutes. Formation of golden yellow colored solution indicates the presence of triterpenes.
15. **Proteins** (Nitric acid test): Two mL of the extracts were treated with few drops of concentrated nitric acid. Formation of yellow colored solution indicates the presence of proteins.
16. **Free amino acids** (Ninhydrin test): Two mL of the extracts were treated with few drops of 0.25 % w/v ninhydrin and then boiled for 5 minutes. Formation of blue colored solution indicates the presence of free amino acids.

Total Phenolic and Flavonoid Content: The total phenolic and flavonoid content of the ethanolic and aqueous extract was determined using the Folin-Ciocalteu assay [10].

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis of *D. esculentum* using standard AOAC methods showed that the fresh plant samples contain 91.82 ± 0.43 % moisture, 1.42 ± 0.10 % ash, 0.28 ± 0.004 % crude fat, 0.87 ± 0.004 % crude protein, and 0.72 ± 0.05 % crude fiber while oven dried plant samples contain 17.39 ± 0.82 % ash, 3.40 ± 0.05 % crude fat, 10.67 ± 0.05 % crude protein, and 9.06 ± 0.67 % crude fiber (Figure 1). Fresh *D. esculentum* is very high in water content. Drying removes the water present in the plant tissues, making it easier to quantify the various components of the plant. The analysis shows that *D. esculentum* is high in inorganic minerals (ash content). Additionally, the results show that *D. esculentum* is high in fiber and protein contents. The analysis for total sugars was not directly done so it was not reported. It can be approximated by difference using the values obtained from direct experimental data of other constituents, assuming that the total amount of analyzed material in the samples is 100%.

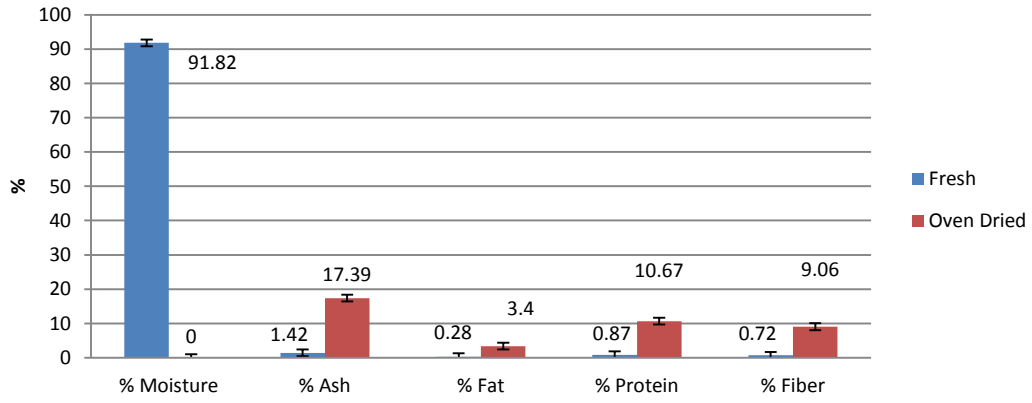

Figure 1. Proximate analysis of fresh and oven dried *D. esculentum* (n = 3) using standard analytical methods [7]

Phytochemical Screening: The amount of phytochemicals which are found in the ethanolic and aqueous pako extracts were quantitatively determined by standard procedures. Slight differences in the quantity of phytochemicals were observed between the ethanolic and aqueous extracts of *D. esculentum* (Table 1). The approximate amount of detected phytochemicals in the samples was based on the intensity of color change observed during screening.

Table 1. Qualitative Phytochemical Screening of Ethanolic and Aqueous Extracts of Pako (*Diplazium esculentum*) Leaves

Phytochemical Test	Ethanolic Extract	Water Extract
Alkaloids (Wagner's test)	++	+
Carbohydrates - Reducing Sugars (Benedict's test)	+	++
Anthraquinones (Borntrager's Test)	+	+
Anthranol Glycosides (Modified Borntrager's test)	+	+
Cardiac Glycosides (Legal's test)	+	-
Cyanogenic Glycosides (Picrate paper test)	-	-
Leucoanthocyanins (Bate-Smith and Metcalf's test)	+	-
Cyanidin (Willstätter cyanidin test)	++	+
Phenols (Ferric chloride test)	++	+
Tannins (Gelatin test)	-	-
Saponins (Froth test)	+	++
Phytosterols (Liebermann-Burchard's test)	+	-
Diterpenes (Copper acetate test)	++	-
Triterpenes (Salkowski's test)	++	-
Proteins (Nitric acid test)	++	+
Free amino acids (Ninhydrin test)	-	-

(-) not detected/present; (+) present in low amounts; (++) present in high amounts

In the study, ethanol was used as solvent in an attempt to determine if the use of ethanol could lead to an extract that will respond to specific bioassays over that of the more toxic methanol; although the latter can extract a broader range of phytochemicals [9]. On the other hand, the aqueous extract provides information that can correlate directly with human consumption of the plant. Summarized in Table 2 are the bioactivities of some the phytochemical classes or secondary metabolites whose presence was indicated in the phytochemical screening tests.

Table 2. Biological activity of phytochemicals present in *D. esculentum* as reported in literature [9]

Phytochemical	Biological Activity
Alkaloids	Antimicrobial, Anthelmintic, Antidiarrheal
Anthraquinones	Antimicrobial
Glycosides	Antidiarrheal
Leucoanthocyanins	Antimicrobial, Antidiarrheal
Cyanidin	Antimicrobial, Antidiarrheal
Phenols	Antimicrobial, Anthelmintic, Antidiarrheal
Saponins	Antidiarrheal
Terpenoids (Phytosterols, Diterpenes, Triterpenes)	Antimicrobial, Antidiarrheal

Pako appears to contain antimicrobial, anthelmintic, and antidiarrheal activities. Still, further and more specific bioassays should be conducted to validate the preliminary findings and so as to adequately assess the potential functions of the plant in human systems.

Cardiac glycosides are known to be toxic because they affect the heart and atrial fibrillation [11]. It is important to note that the presence of cardiac glycosides were observed in *D. esculentum* but undetected when water was used. The exact levels of these compounds need to be ascertained because of the danger posed to humans by over consumption of the plant as food. The screening also showed the absence of cyanogenic glycosides that are much more toxic than the cardiac counterpart [12]. Their absence in *D. esculentum* indicates that adverse effects associated with cyanide poisoning will not be experienced when the plant is ingested and metabolized by humans.

Total Phenolic and Flavonoid Content: A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers. In the study, total phenolic content is presented in gallic acid equivalents while total flavonoid content is in quercetin equivalents (Table 3). The ethanolic extract gave a total phenolic content of 125.60 ± 13.44 mg GAE/100 g dried sample and a total flavonoid content of 110.80 ± 11.16 mg QE/100 g dried sample while the aqueous extract gave total phenolic content of 11.65 ± 0.87 mg GAE/100 g dried sample and total flavonoid content of 16.21 ± 0.72 mg QE/100 g dried sample. The average range of total phenolic content of non-violet colored vegetables is 80 to 150 mg, most of which are categorized as flavonoids.

Table 3. Total Phenolic and Flavonoid Content of *Diplazium esculentum* (Retz.) Sw. extracts

	Ethanolic Extract	Water Extract
Total Phenolic Content (mg GAE/100 g air-dried sample)	125.6 ± 13.4	11.7 ± 0.9
Total Flavonoid Content (mg QE/100 g air-dried sample)	110.8 ± 11.2	16.2 ± 0.7

The difference in phenolic and flavonoid content of the extracts may be attributed to the solvent used on extraction. Phenolic compounds, in general, inhibit various types of oxidizing enzymes. The diverse biological roles of phenolic compounds in *D. esculentum* leaves create opportunities for searching specific phytochemicals with their corresponding health benefits.

These results suggest the conduct of additional studies using other solvents that can extract other phytochemicals, and the potential of *D. esculentum* as food and as source of phytochemicals that possess free radical scavenging activity and other promising health benefits.

CONCLUSION

Moisture, ash, crude protein, crude fat, crude fiber contents of pako (*D. esculentum*) were determined using standard AOAC methods. Phytochemical screening shows that *D. esculentum* contains compounds that possess possible bioactivities. Ethanol is able to extract more groups of phytochemicals than water alone. *D. esculentum* may hold potential in providing benefits for human nutrition and health. Furthermore, the ethanolic extract contains more phenolics and flavonoids than the aqueous extract. Generally, the phenolic content of *D. esculentum*, which is largely flavonoid in nature, is relatively higher than average range of values shown to be present in most plants.

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