Quality Evaluation of Powdered Fern (*Diplazium esculentum*): Physicochemical, Phytochemical, Antioxidant, and Anti-inflammatory Properties in Aqueous Extracts

Erlyn Grace P. Aguilar[3*], Jose S. Valmorida[1], Queenie Ann L. Curayag[1] and Reggie Y. Dela Cruz[2]
[1]College of Human Ecology
[2]Institute of Biological Sciences
Central Mindanao University
Musuan, Bukidnon 8710 Philippines

[3]College of Science and Mathematics
University of Science and Technology of Southern Philippines – Cagayan de Oro
Cagayan de Oro City, 9000 Philippines
[*]*erlyngrace.aguilar@ustp.edu.ph*

Date received: August 08, 2024
Revision accepted: November 20, 2024

Abstract

This study examined the physicochemical, microbiological, nutritional, and mineral properties of Diplazium esculentum powder and the phytochemical, antioxidant, and anti-inflammatory properties of its aqueous products. Fresh D. esculentum leaves were oven dried at 50 °C for 5 h. The powder derived from the dried leaves exhibited a 20% yield and significant nutritional profile, rich in zinc mineral and protein, with low fat content. Additionally, the powder exhibited favorable flow properties (CI: 24.85, HR: 1.33), making it suitable for incorporating into various food products. Aqueous extracts of D. esculentum were prepared using a 2 x 3 factorial design with different extraction conditions, revealing a significant influence of extraction time and temperature on phytochemical content and bioactivity. Treatment 3 (40 °C for 6 h) demonstrated the highest phenolic content of 360.65 mg GAE/g, while the control (ethanolic extraction) yielded the lowest with 115.53 mg GAE/g. Treatment 6 (60 °C for 6 h) showed the highest flavonoid content of 17.92 mg QE/g. Moreover, extracts from longer extraction times showed higher levels of phenols, flavonoids, and antioxidant activity, with an IC_{50} value of 135.55µg/mL. Furthermore, the extracts revealed potent anti-inflammatory properties, particularly inhibiting the COX-2 enzyme. These findings emphasized the potential of D. esculentum as a valuable source of nutrients and bioactive compounds. The powder's nutritional content indicated its potential applications in functional foods such as prebiotic-enriched foods, beverages, food additives, and baked goods. Furthermore, the extracts' antioxidant and anti-inflammatory properties are a potential ingredient in nutraceuticals such as antioxidants and probiotics.

Keywords*:* anti-inflammatory, antioxidant, aqueous extract, *Diplazium esculentum*, phytochemical

1. Introduction

Edible ferns, such as *Diplazium esculentum*, have been a staple in traditional Southeast Asian diets for centuries, offering a rich source of essential nutrients and potential health benefits. As global interest in natural and functional foods grows, these underutilized plants are gaining more attention. *D. esculentum* has shown to possess remarkable nutritional and medicinal properties, including anti-inflammatory and antioxidant activities. Chaudhuri and Roy (2020) reported that *D. esculentum* contains essential nutrients such as iron, calcium, and Vitamins A and C while being low in calories, making it ideal for weight management. It also has a potential to lower blood sugar, reduce inflammation, improve digestion, and a high potential in anti-inflammatory and cardiovascular effects by reducing blood cholesterol and inhibiting LDL-C oxidation (Speer *et al*., 2019).

However, even with the fern's nutritional value and potential health benefits, there is little information on the assessment of its quality and its extraction by aqueous techniques. Previous studies have mainly focused on alcoholic extraction techniques such as maceration, digestion, decoction, infusion, Soxhlet extraction, and microwave-assisted extractions (Abubakar and Haque, 2020), which can introduce contaminants and limit the applicability of the extracts in food products. Aqueous extraction, on the other hand, is a more sustainable and food-friendly method. While it has been successful in extracting bioactive compounds from various plants, its effectiveness has been beneficial for *D. esculentum* is yet to be fully investigated.

A significant gap exists in ensuring the safety and efficacy of *D. esculentum* powder as a food ingredient, particularly its physicochemical, nutritional, and microbiological properties. Moreover, an evaluation of the phytochemical profile, antioxidant, and anti-inflammatory activities of aqueous *D. esculentum* extracts are essential to enhance its potential as a functional food ingredient. By addressing these knowledge gaps, this study aimed to contribute to the valorization of *D. esculentum* as a valuable source for food and pharmaceutical industries. This study provided insights into the quality of *D. esculentum* powder and the potential benefits of its aqueous extracts, providing wider application in innovative food products.

2. Methodology

D. esculentum fern species was used in this study and obtained from the Central Mindanao University-Tuklas Lunas Development Center Fernery (CMU-TLDC), Musuan, Bukidnon. The equipment used were forced air oven dryer (Shel Lab, United States), fabricated industrial miller (Dynamics Development Trade and General Services, Inc., Philippines), sieve (80-mm, China), UV-vis spectrophotometer (MultiSkan Go, ThermoScientific, Finland), vacuum rotary evaporator (RV 10 digital V, IKA, Malaysia), moisture analyzer (A&D MX-50, Japan), chroma meter (CR-400, Konica Minolta, Japan), water activity meter (PAWKIT, Meter Aqualab, USA), pH meter (TS-1, Suntex, China), digital refractometer (MA871, Milwaukee, USA), freeze-dryer (Alpha 2-4 LD plus, Martin Christ, Germany), microplate reader (CLARIOstar®, BMG LABTECH), incubator (Memmert, Germany), colony counter (Model 570, Suntex, China), and microscope (SM201, Svbony, China). The chemicals that were used are Folin-Ciocalteu reagent (Analytical Grade, Sigma-Aldrich, Germany), quercetin (Analytical Grade, Phygene, China), gallic acid (Analytical Grade, Phygene, China), ascorbic acid (Analytical Grade, SCR, China), 2,2-diphenyl-1-picryhydrazyl (DPPH) (Phygene, China), aluminum chloride (Analytical Grade, ChemPUR, Poland), sodium carbonate (Analytical Grade, Sigma-Aldrich, Germany), absolute ethanol (Analytical Grade, Spectrum, CA), COX (Ovine/Human) Inhibitor Screening Assay kit (Cayman Chemicals, Inc., United States), Buffered Peptone Water (HiMedia, United States), Plate Count Agar (HiMedia, United States), Potato Dextrose Agar (HiMedia, United States), and Eosin Methylene Blue Agar (HiMedia, United States). Other materials such as distilled water, glass jars, graduated cylinders, Erlenmeyer flasks, petri plates, test tubes, test tube racks, 96-well microplates, sterilized pipette tips, beakers, filter papers, and stirring rods were also used.

2.1 Experimental Design

A 2 x 3 factorial research design was employed in the study, which involved two independent variables, each with multiple levels. This experimental design was used to investigate the main effect and interaction of the independent variable on the dependent variable in terms of its phytochemical, antioxidant, and anti-inflammatory properties. The control sample in this study was implemented using a standard ethanolic extraction following the established method of CMU-TLDC. This method employed the use of ethanol as a solvent to extract bioactive compounds from the plant. The ethanolic extraction was utilized as a baseline to compare the effectiveness of the aqueous extraction techniques employed in the study.

2.2 Experimental Treatment

The experimental design utilized a 2 x 3 factorial design, incorporating two independent variables which are temperature and time. Temperature had two levels (40 and 60 °C), while time had three levels (1, 3, and 6 h). Table 1 shows the variable set levels, and the six variable combinations generated from the design.

2.3 Powder Preparation

Fresh whole *D. esculentum* fern plant was collected from the CMU-TLDC Fernery. The fern leaves were separated from their stalk and washed with 10 ppm chlorinated water for sanitation, and distilled water for the final washing. The sanitized leave samples were placed in perforated trays and dried using forced air oven dryer at 50 °C for 5 h or until the moisture content was below 10%. The dried leaves were milled using a fabricated industrial miller and sieved through an 80-mesh sieve. The fern powder was packaged in a 50-g aluminum foil stand-up pouch and stored in airtight containers at ambient temperature to ensure its integrity and prevent degradation. The stored powdered samples were utilized for further evaluation

Table 1. 2 x 3 factorial design and variable combinations for fern powder extracts

Treatments	Temperature (°C)	Time (Hours)
Control	30	72
1	40	1
2	40	3
3	40	6
4	60	1
5	60	3
6	60	6

Control is extracted using ethanol; treatments 1-6 were extracted using aqueous water.

2.4 Physicochemical Analysis of Fern Powder

2.4.1 Percentage Yield (% w/w)

The percentage yield of fern powder was calculated using the final product's weight after proper drying and powdering with respect to the fresh fern leaves' initial total weight. The total weight of the fresh fern does not include the stalk. The percentage yield was calculated from Equation 1 as utilized by Majid and Rining (2018):

$$Yield\ (\%) = \frac{Weight\ of\ the\ fern\ powder\ (g)}{Weight\ of\ the\ fresh\ fern\ leaves\ (g)} \times 100 \quad (1)$$

2.4.2 Bulk Density and Tapped Density

The bulk density of the fern powder sample was determined using the Bassey *et al*. (2020) method with modifications. Approximately ten (10) grams of the fern powder sample was weighed and measured into a 100-mL graduated cylinder and was gently tapped until the sample reached the constant volume. The bulk density and tapped density were determined using Equations 2 and 3.

$$Bulk\ Density\ (\frac{g}{mL}) = \frac{Mass\ of\ the\ powdered\ sample\ (g)}{Bulk\ volume\ of\ the\ packing\ (mL)} \quad (2)$$

$$Tapped\ Density\ (\frac{g}{mL}) = \frac{Mass\ of\ the\ powdered\ sample\ (g)}{Final\ volume\ after\ tapping\ (mL)} \quad (3)$$

2.4.3 Flowability

The flowability of the fern powder was determined by identifying the Carr index (CI) and Hausner ratio (HR) (Smita *et al*., 2019). The CI and HR were calculated from the bulk and tapped densities of the fern powder recorded (Shishir *et al*., 2014) as shown in Equations 4 and 5.

$$CI = \frac{Tapped\ density - bulk\ density}{Tapped\ density} \times 100 \quad (4)$$

$$HR = \frac{Tapped\ density}{Bulk\ density} \quad (5)$$

2.4.4. Color

The color of the fern powder was determined using the method of Biswas *et al*. (2023). The color was evaluated using a chroma meter. The chroma meter was calibrated with Minolta standard reference plate at the start of the analysis. About 5 g of the sample were placed on a clean petri dish with white paper underneath and the chroma meter was positioned 8mm above the sample. The L^* (lightness to brightness), a^* (redness to greenness), and b^* (yellowness to blueness) color variables were measured in triplicates from the equator region of each powder sample and the average was determined.

2.4.5. Moisture Content (%)

The moisture content of the fern powder was determined following Putri *et al*. (2015) method using a moisture analyzer. Five (5) grams of fern powder

sample was placed and spread evenly in the sample pan. Then, it was analyzed at 105 °C until it reached the preset termination value, and a buzzer beep noise was created.

2.4.6. Water Activity (*aw*)

In the determination of water activity of samples, a portable water activity meter was used. The procedure followed the PAWKIT operation manual wherein the prepared sample was placed inside the cup-holder instrument and pressed the left button (I) to turn on the instrument. The water activity measurement began and created a beep noise, the results were then displayed in the LCD.

2.4.7. Total Soluble Solids (°Brix)

The determination of total soluble solids of the fern powder sample was measured using a digital refractometer. Instrument was calibrated using distilled water before proceeding the actual analysis. The fern powder samples were diluted in distilled water following the 1:10 ratio and was mixed until powdered sample is completely dissolved. The diluted sample was then placed in the refractometer prism until the reading became constant.

2.4.8. Potential Hydrogen (pH)

The pH of the sample will be determined based on AOAC 981.12 method (Horwitz, 2005) with modification. The pH meter was first calibrated using buffer solutions before starting the analysis. The fern powder samples were diluted with a 1:10 parts ratio of fern powder to distilled water. The mixtures were stirred to homogenize completely. The sample was allowed to stand for 5 min to allow the foam on the surface to subside. The pH was measured by submerging the electrode of the digital pH meter until the pH reading became constant.

2.5 Nutritional and Microbiological Analysis of Fern Powder

2.5.1. Nutritional Composition and Mineral Analysis

The nutritional analyses and mineral analysis on zinc of the fern powder samples were subjected to Department of Science and Technology Region 10 (DOST-10) and FAST Laboratories, respectively. The samples were analyzed based on the standard nutritional labelling requirement such as calories, carbohydrate, total fat (AOAC 932,06), crude protein (AOAC 991.2), ash

(AOAC 930.05), and moisture content (AOAC 930.04). Nutrition facts computation was also calculated by DOST-10 which followed the Food and Nutrition Research Institute (FNRI) values for males aged 19-29.

2.5.2. Microbiological Analysis

The aerobic plate count (Maturin and Peeler, 2001), total yeast and mold count (Tournas *et al*., 2001), and *Escherichia coli* count (Feng *et al*., 2002) were determined following the methods in Bacteriological Analytical Manual (BAM) Chapters 3, 18, and 4, respectively. A total of 10 g of fern powder was homogenized with 90-mL sterile buffered peptone solution and stirred for 10 min. Plate count agar was used for the estimation of total bacterial count after one to two days of incubation at 35 °C. The total yeast and mold count was estimated using Potato Dextrose Agar medium after incubation at 35 °C for three to five days. After the incubation period, cfu/g was calculated. *E. coli* was determined based on its presence or absence using the Eosin methylene blue agar after incubation at 35 °C for 18 h.

2.6 Preparation of Fern Powder Extracts

The powdered fern samples were subjected to extraction. The control sample underwent an ethanol extraction which followed the method of Dadi *et al*. (2019) wherein the dried powder samples were soaked in absolute ethanol in the ratio of 1:5 (m/V) for 72 h with occasional stirring. The extracted samples were filtered using Whatman No. 1 filter paper and dried using a using vacuum rotary evaporator (IKA RV 10 digital) at 40 °C. Aqueous extraction of fern samples was done following the methods of Chai *et al*. (2015) and Hui *et al*. (2018) with slight modifications following the experimental treatment. The sample was then centrifuged at 7,830 rpm at 4 °C for 5 min. The supernatants obtained were freeze-dried to constant weight and extract yield was recorded. The resulting dried extracts were stored in a covered 50-mL beaker at ambient temperature until further analysis.

2.7 Quantitative Phytochemical Screening

Phytochemical screening was performed to confirm the presence of phytochemicals in the extracted *D. esculentum* sample which included the determination of total flavonoid content (TFC) and the determination of total phenolic content (TPC).

The TFC was determined using aluminum chloride colorimetric method (John *et al*., 2015; Vyas *et al*., 2015; Junejo *et al*., 2018). However, in this study, the method was slightly modified wherein a 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was incubated in the dark at room temperature for 30 min. Then, the wavelength was set at λ = 415 nm against the blank sample prepared by substituting aluminum chloride with the same volume of distilled water. A standard calibration curve was prepared with a quercetin of different concentrations (25, 50, 75, 100 and 250 mg/mL) against its absorbance at 415 nm. The total flavonoid content was expressed as μg quercetin equivalents per gram sample (μg QE/g).

The TPC was determined using Folin-Ciocalteu assay method (Dadi *et al*., 2019) with modifications. A 0.5 mL of the fern extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and vortexed. After 8 min, 2 mL of 7.5% sodium carbonate was added, mixed, and kept in the dark at room temperature for 2 h. The same procedure was used for the blank and gallic acid standards at different concentrations (25, 50, 75, 100 and 250 mg/mL) to develop a standard curve. The absorbance was measured at 750 nm using a UV-vis spectrophotometer. The total phenolic content was expressed as mg of gallic acid equivalent per gram of microencapsulates (mg GAE/g dw).

2.8 Determination of Antioxidant Activity

The antioxidant activity of the samples was determined through a modified 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity method (Dela Cruz *et al*., 2017; Junejo *et al*., 2018). A 0.1 mm DPPH solution in ethanol was prepared and 1 mL of this solution was added to 3 mL solution in ethanol at varying concentrations (25, 50, 75, 100 and 250 ppm). The mixtures were shaken vigorously and incubated in a dark room for 30 min at ambient temperature. The absorbance was measured using a UV-vis spectrophotometer at a wavelength of 517 nm under dim light. Deionized water was used for blank reading, and ascorbic acid was used as a standard solution. Equation 6 was used to calculate the percentage of scavenging activity.

$$\%\, scavenging\ activity = \left[\frac{A_c - A_s}{A_c}\right] \div \left[\frac{A_c - A_{AA}}{A_c}\right] \times 100 \quad (6)$$

where % scavenging activity is the DPPH radical scavenging activity percentage; A_c = absorbance of control; A_s = sample absorbance; A_{AA} = ascorbic acid absorbance.

The plot of scavenging activity against the concentration of the extract and the standard solution was drawn, and the regression equation for the linear curve was found. Using statistically programmed software, the antioxidant activity (IC_{50}) value was calculated for the sample and the standard (Mistriyani *et al*., 2018).

2.9 Determination of Anti-inflammatory Activity

The anti-inflammatory activity of the samples was determined using COX-Inhibition Assay method (Ang *et al*., 2022) with slight modifications. This determined the ability of the extracts to inhibit the enzyme cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1) in a red-lighted dark room using a spectrophotometric method.

A 5,184 uL of 100 mM pH 8 Tris buffer was added to a clean vial. A 96 uL of 250 U/mL of COX-2 and COX-1 enzymes and 480 uL of 20 uM Hematin was mixed separately and added into the vial with buffer. The mixture constituted the enzyme-cofactor solution. A 120 uL of the enzyme-cofactor mixture was then placed in each well that was dispensed with 50 uL of the same buffer. Then, 10 uL of 200 ug/mL of plant extracts in dimethyl sulfoxide (DMSO) was added to make a final well concentration of 10 ug/mL. An 8-mM indomethacin in 100% DMSO and 30:70 Water:DMSO ratio (final well concentrations) were used as the positive control. After the incubation of the mixture at 25 °C for 15 min, a 10 uL of 200 uM Amplex Red (10-acetyl-3,7-dihydroxyphenoxazine) and 10 uL of 2,000 uM arachidonic acid were added to each well. The reaction mixture was mixed and purged with N2. The reaction was then monitored for 2 min using a microplate reader at an excitation wavelength of 535 nm and emission wavelength of 590 nm. The fluorescence intensity was measured at 12 seconds intervals. The positive control and the % inhibition of the samples were determined based on the average slope of each replicate by using equation 7 below:

$$\%\ inhibition = \left[\frac{Slope_{unihibited} - Slope_{inhibited}}{Slope_{unihibited}}\right] \times 100 \quad (7)$$

where *% inhibition* is the inhibition percentage of COX enzyme; $slope_{unhibited}$ = slope of the line from the fluorescence vs time plot of the negative control group; $slope_{inhibited}$ is the slope of the line from the fluorescence vs time plot of the positive control group.

2.10 Statistical Analysis

Experiments were carried out in triplicates. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 through One-way Analysis of Variance (ANOVA) and Tukey's honestly significant difference test for the comparison between extraction treatments of *D. esculentum* powder. Linear regressions were carried out using Microsoft Office Excel 2016. Data values were presented as mean ± standard deviation.

3. Results and Discussion

3.1 Physicochemical Evaluation of D. esculentum Powder

The dried fern powder's physicochemical characteristics were evaluated, and the results are summarized in Table 2. The powdered sample showed an impressive 20% recovery rate, surpassing other vegetable leaf powders like *Amaranthys gangetics* (12.2%), *Chenopodium album* (7.8%), *Centella asaitia* (14.3%), *Amaranthus tricolor* (11.6%), and *Trigonella foenumgrecum* (12.4%) as stated by Joshi *et al.* (2019). This effective preservation of fern weight during the drying and milling process not only enhances economic viability but also influences the sustainability of production. It ensures a greater quantity of *D. esculentum* powder available for various applications in the food and medicine industries.

Table 2. Results for physicochemical properties of *D. esculentum* powder

Properties	*D. esculentum* powder
Percentage yield (%)	20
Bulk density (g/mL)	0.35 ± 0.01
Tapped density (g/mL)	0.47 ± 0.01
Carr's compressibility index	24.85 ± 0.66
Hausner's ratio	1.33 ± 0.01
Color	
*L**	71.24 ± 2.41
*a**	-15.69 ± 0.42
*b**	38.18 ± 0.72
Moisture content	8.80 ± 0.02
Water activity	0.56 ± 0.00
Total soluble solids (°Brix)	3.27 ± 0.06
pH	6.22 ± 0.01

Values are represented as Mean ± SD of triplicate determination.

The micrometric properties of the fern powder were evaluated to assess its flowability. The bulk density was measured at 0.35 ± 0.01 g/mL, which is relatively higher than the bulk density range of 0.18-0.34 g/mL for *Nephrolepis* species (Bassey *et al*., 2020), 0.26 ± 0.01 g/mL for mamaku pith powder (Bish *et al*., 2023), and 0.62g/mL for *Diplazium maximum* young fronds (Sareen *et al*., 2021). A higher bulk density is desirable for powders as it allows more efficient packaging and enhances particle flow during processing (Huang *et al*., 2020). The tapped density, calculated to be 0.470.01 g/mL, indicates that the powder was more compressed. This indicates its cohesiveness and can contribute to its flowability and the texture of baked goods. Carr's Index (CI) and Hausner Ratio (HR) values were calculated as 24.850.66 and 1.330.01, respectively, indicating passable flowability and intermediate cohesiveness for the powder. These values indicate that the powder particles maintain some mobility without excessive compaction or agglomeration, maintaining their structure and integrity (Hamalainen, 2021). The flow properties of the powder obtained in this study were significantly higher than those of *B. balcooa leaf* (CI: 20.83, HR: 1.26) based on the study of Brahma *et al*. (2022), and it was lower than the CI range of 31.00-36.67 and HR range of 1.43-1.58 reported for *Nephrolepis* species (Bassey *et al*., 2020).

The color of *D. esculentum* powder plays a vital role in consumer acceptance as it is the first feature assessed during purchase (Aranibar *et al*., 2018). The color results showed L^* value of 71.24 ± 2.41 which suggested a white shade, a green undertone represented by the a^* parameter of -15.69 ± 0.42, and a positive b^* value of 38.18 ± 0.72 indicated a preference for a yellow hue. The color can vary from light to dark green, influenced by fern species and the enzymatic and non-enzymatic browning reactions (Koniyo *et al*., 2019; Tamuno, 2020). Furthermore, the average moisture content of the fern powder in the study was 8.80±0.02 which is lower than the published studies such as *D. esculentum* leaves (10.80%) and *Nephrolepis exaltata* leaves (21.78%) (Dash *et al*., 2017; Sharma *et al*., 2020). The fern powder water activity was 0.56 ± 0.00, which resulted in a decrease in water activity compared to fresh fern leaves. This reduction is significant because it lowers the chances of bacterial and fungal growth (Tapia *et al*., 2020).

The TSS content was 3.27±0.06, which was relatively low due to ferns' lower sugar content (Chaudhuri & Roy, 2020). A study by Junejo *et al*. (2018) reported that *D. esculentum* significantly decreased the blood glucose level in streptozotocin-induced diabetic rats, indicating the antidiabetic activity of the fern. The pH value was found to be slightly acidic at pH 6.22 ± 0.01 which

enhances solubility in water and emulsifying capacity for food applications (Ma *et al*., 2020). A mildly acidic pH is ideal for gastrointestinal tolerance and food processing to reduce sour taste (Li and Liu, 2015).

The physicochemical properties of *D. esculentum* powder have significant implications for its processing and storage applications. The relatively high bulk density and intermediate flow properties indicate that the powder can be easily packaged and processed. However, its cohesiveness may require careful handling to prevent caking and ensure a smooth flow during production. The low moisture content and water activity are particularly beneficial for long-term storage, as they reduce the risk of microbial growth and deterioration. This characteristic also enhances the powder's stability during processing, reducing the risk of spoilage. The slightly acidic pH can contribute to the powder's compatibility with other ingredients and its potential for various applications. It may require modifications in formulation to achieve desired pH levels in food products. Additionally, the low sugar content could contribute to the sensory properties of food products, particularly sweetness.

3.2 Nutritional Composition of D. esculentum Powder

The nutritional value of *D. esculentum* was determined to assess its effectiveness as a food ingredient and results are presented in Table 3. The fern powder exhibited a total energy content of 325.90 kcal/100 g, falling within the range of other Diplazium species like *D. maximum* fronds at 319.42 kcal/100 g (Gupta *et al*., 2020), indicating a good calorific value. Notably, the energy content of *D. esculentum* powder surpassed that of other leafy vegetables such as spinach powder (292 kcal/100 g), moringa leaf powder (324.4 kcal/100 g), and fiddlehead ferns (González-Burgos *et al*., 2021).

Table 3. Proximate composition of *D. esculentum* powder

Properties	*D. esculentum* powder
Ash (g/100g)	12.42
Moisture content (g/100g)	8.73
Crude protein (g/100g)	16.11
Fat, total (g/100g)	2.10
Total carbohydrates (g/100g)	60.64
Zinc (mg/kg)	0.95
Energy (kcal/100g)	325.90

Samples were analyzed by DOST 10 and FAST Laboratories.

The ash content was relatively high at 12.42 g/100 g, indicating a source of trace minerals and inorganic matter (Harris and Marshall, 2017). This exceeds the ash content of 9-11% reported in other studies, such as *D. maximum* young fronds has 10.4 g/100 g and red fern ash is in the range of 0.93 to 0.95 g/100 g (Saragih *et al*., 2017). The moisture content at 8.73 g/100 g contributes to food preservation and enzymatic stability (Ahn *et al*., 2014; Kiaya, 2014). The powder also reported a high crude protein content of 16.11 g/100g, making it a valuable protein source with essential nutrients (Hermann, 2019; Riaz *et al*., 2022). Total fat content was low at 2.10 g/100g, promoting longer shelf life (Awuch, 2019), while the carbohydrate content of 60.64 g/100g fell within the reported range, making it a good carbohydrate source (Pradhan *et al*., 2015; Zihad *et al*., 2019). The powder also contained 0.95 mg/kg zinc, an essential mineral for various bodily functions. The presence of zinc and the richness in bioactive compounds, vitamins, minerals, protein, and energy further highlight the nutritional potential of fern powder for product processing (Pietrak *et al*., 2022).

Furthermore, the computed Recommended Energy Nutrient Intake (RENI) for energy, protein, and zinc per 100 g of fern powder was tested, indicating promising results, particularly for infants aged 0 to 5 months as shown in Table 4.

Table 4. PDRI for energy, protein, and zinc of *D. esculentum* powder (per 100 g)

Age	Energy (kcal)		Protein (g)		Zinc (mg)	
	M	F	M	F	M	F
Infants, mo.						
0 – 5	2.42	2.68	11.11*	12.50*	1.81	1.81
6 – 11	2.08	2.38	5.88	6.67	0.90	1.03
Children, yr.						
1 – 2	1.50	1.63	5.56	5.88	0.93	0.95
3 – 5	1.11	1.19	4.55	4.76	0.76	0.79
6 – 9	0.94	1.02	3.33	3.45	0.75	0.76
10 – 12	0.73	0.76	2.33	2.17	0.58	0.62
13 – 15	0.56	0.69	1.61	1.75	0.41	0.51
16 – 18	0.50	0.66	1.37	1.64	0.42	0.53
Adults, yr.						
19 – 29	0.59	0.78	1.41	1.61	0.58	0.83
30 – 49	0.62	0.80	1.41	1.61	0.58	0.83
50 – 59	0.62	0.80	1.41	1.61	0.58	0.83
60 – 69	0.70	0.93	1.41	1.61	0.58	0.83
≥ 70	0.77	0.97	1.41	1.61	0.58	0.83
Pregnant		0.69		1.15		0.39
Lactating		0.63		1.12		0.34

*Good source

However, for other age groups, a 4-g serving had a relatively minor impact on nutrient intake. Infants aged 0 to 5 months have an acceptable macronutrient distribution range (AMDR) range of 5% for protein intake as their energy source, which the computed Philippine Dietary Recommended Intake (PDRI) for protein exceeds, along with the recommended daily nutrient intake (FNRI, 2018). The table shows that fern powder has a lower percentage of RENI for energy, protein, and zinc. Although it can enhance nutritional value, it should not replace a balanced diet but rather serve as a supplement. Increasing the serving size is recommended for individuals in different age groups. Overall, *D. esculentum* powder is a valuable ingredient to increase the nutritional value of food products, providing health benefits when incorporated into recipes and food products.

3.3 Microbiological Evaluation of D. esculentum Powder

A microbiological analysis was conducted to determine the microbial loads in the oven-dried *D. esculentum* powder. The microbiological properties of fern powder were examined and are presented in Table 5. It was observed that the aerobic plate count, yeast and mold count, and *Escherichia coli* analysis of the sample were within the acceptable level of dried fruits and vegetables based on the FDA Circular No. 2022-012. The low microbial load of the oven-dried *D. esculentum* powder is a crucial element in ensuring food safety. This indicates that the powder is free from harmful bacteria, yeast, and mold, which can lead to foodborne illnesses. The absence of *E. coli*, a common indicator of fecal contamination, further confirms the product's safety (World Health Organization, 2018).

Table 5. Microbiological analysis of *D. esculentum* powder

Parameters	Count (CFU/g)	Acceptable level (Dried fruits and vegetables)*
Aerobic plate count	< 5	10^4
Yeast and mold count	< 5	10^3
E. coli	Negative	Negative

*FDA Circular 2022-012

The low moisture content and water activity of the powder contribute to its bacterial stability. These factors create an unfavorable environment for microbial growth, reducing the likelihood of spoilage and contamination. This ensures that the powder is suitable for long-term storage without compromising its safety. Overall, the low microbial load and favorable moisture content of the *D. esculentum* powder demonstrated its potential as a

safe and stable food ingredient. This can lead to its wider application in various food products, promoting food safety and consumer health.

3.4 Extraction Yield

The highest extraction yield was achieved with aqueous extract, where Treatment 1 had the highest yield (6.05%), followed by Treatment 3 (5.33%), Treatment 2 (5.05%), Treatment 5 (2.47%), Treatment 4 (2.24%), and Treatment 6 (1.30%). The control ethanol extract had the lowest yield (0.82%) as shown in Figure 1. This suggests that lower extraction temperatures and shorter extraction times resulted in higher yields (Tambunan *et al*., 2017).

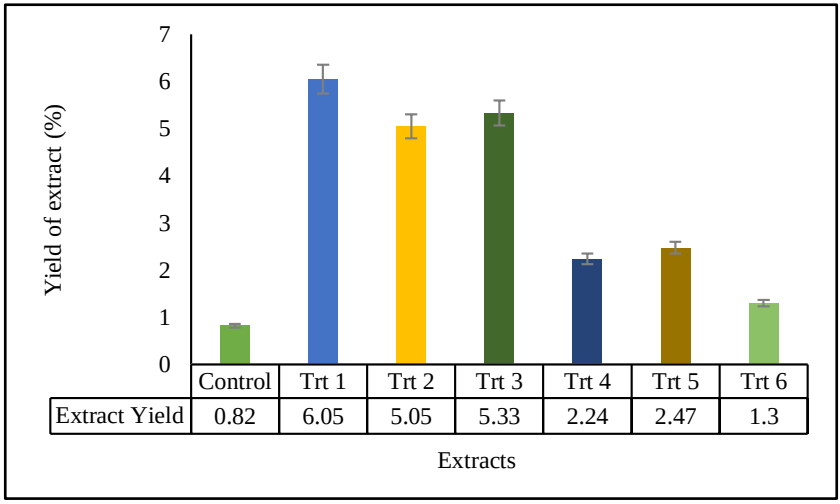


Figure 1. Effect of different treatments on extract yield; error bars are SEM

Aqueous extraction methods were found to be more effective than ethanol in extracting compounds from *D. esculentum* powder. Lower extraction temperatures and shorter extraction times resulted in higher yields, suggesting that excessive heat and prolonged exposure can reduce bioactive compounds. This benefit is attributed to the superior extraction kinetics and higher yields obtained with pure water. Additionally, the polarity of the solvent is crucial. Water, being more polar, can effectively extract polar compounds, while ethanol, being less polar, may be more suitable for non-polar compounds (Alonso-Riaño *et al*., 2020). The dark brown color observed in aqueous extracts obtained through lyophilization may indicate oxidation and degradation, likely due to water addition and heat treatment (Singh *et al*., 2018; Dias *et al*., 2020). In contrast, the ethanol extract had a dark green color,

possibly indicating the presence of chlorophyll or other green pigments from the fern powder. The stability of chlorophyll may be linked to the presence of zinc in the powder (Ozkan and Bilek, 2015), and the green color was preserved through proper storage and protection from light (Ferreira *et al.*, 2023). Parameters such as pH, solid-liquid ratio, particle size, temperature, solvent choice, solubility, and extraction time (Pasrija and Anandharamkrishnan, 2015) should be assessed to determine optimal conditions for maximizing the recovery of bioactive compounds. The obtained extracts were further investigated for phytochemicals, antioxidants, and anti-inflammatory properties.

3.5 Phytochemical Screening

The total phenolic and flavonoid content of the fern powder extracts was assessed quantitatively. The TPC varied from 115.53 to 360.65 mg GAE/g sample as shown in Figure 2, with Treatment 3 displaying the highest TPC.

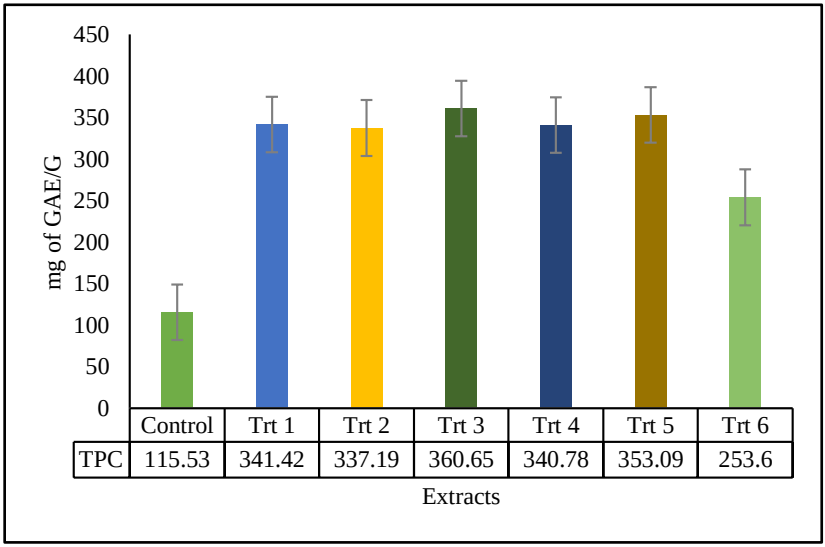


Figure 2. Total phenolic content (mg GAE/g sample) of different extracts; error bars are SEM

The figure also demonstrates a positive correlation between time and TPC at 40 °C (Treatments 1, 2 and 3) but an inverse relationship at 60°C (Treatments 4, 5, and 6), signifying polyphenol degradation at higher temperatures and longer extraction times, potentially enhancing antioxidant properties (Antony

and Farid, 2022). In the study, freezing-drying increased free phenolics compared with the ethanol-extracted control, resulting in reduced phenolic content. Aqueous extracts exceeded the typical range of total phenolic content for non-violet-colored vegetables (Tongco *et al*., 2014).

The TFC ranged from 6.96 to 17.92 mg QE/g sample as presented in Figure 3, with Treatment 6 having the highest TFC which signifies that the higher the temperature and the longer the time of extraction the fern is subjected, the more flavonoid content it contains. As compared to the study of Tongco *et al.* (2014), the *D. maximum* has a TFC of 3.13 (aqueous extract) and 2.89 (alcoholic extract) which is still lower than the obtained results in the study.

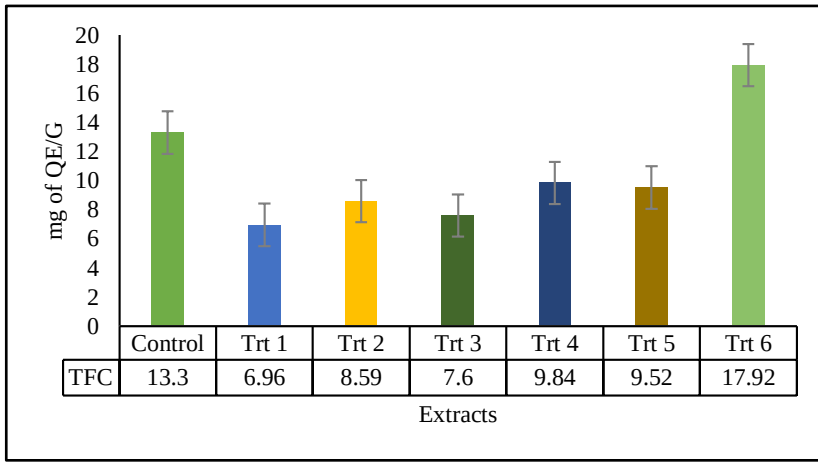


Figure 3. Total flavonoid content (mg QE/g sample) of different extracts; error bars are SEM

As observed in both Figures 2 and 3, there was a significant difference between all treatments, suggesting that the phytochemical content of the extracts is influenced by both time and temperature (Khoza *et al*., 2014). The data presented in both figures clearly indicate that TPC is considerably higher than the TFC value. The phenolic compounds and flavonoid content are both classes of metabolites found in plants and are present in the aqueous extracts of fern powder. Also, the diversity of phenolic compounds is present in plant and may possibly lose or lead to degradation during the extraction process due to heat treatment, and oxidation (Albuquerque *et al*., 2021). However, the phytochemicals present in the *D. esculentum* extracts indicate a greater potential as a source of antioxidant and biological activities.

The increased levels of total phenolic and flavonoid content in the *D. esculentum* extracts signify their potential as a rich source of antioxidants. Phytochemical compounds, particularly phenols and flavonoids, possess their potent antioxidant properties, which can neutralize harmful free radicals and reduce oxidative stress. This can contribute to the prevention of various chronic diseases, such as cardiovascular disease, cancer, and neurodegenerative diseases.

3.6 Antioxidant Activity by DPPH Radical Scavenging Activity Assay

The antioxidant activities of aqueous and ethanol extracts are outlined in Figures 4 and 5. All extracts demonstrated DPPH scavenging activities related to ascorbic acid, ranging from 7.82% (Treatment 1) to 80.47% (Treatment 6) at various concentrations (25, 50, 75, 100, and 250 ppm). The highest inhibition occurred with Treatment 2 at 25 ppm (18.61 ± 1.78), while Treatment 6 at 250 ppm demonstrated the highest inhibition (80.47 ± 0.26). These results align with Gupta *et al*. (2004), showing significant DPPH scavenging effects with increasing concentration. The *D. esculentum* extracts exhibited strong antioxidant activity, comparable to or even surpassing some well-known antioxidant-rich plants.

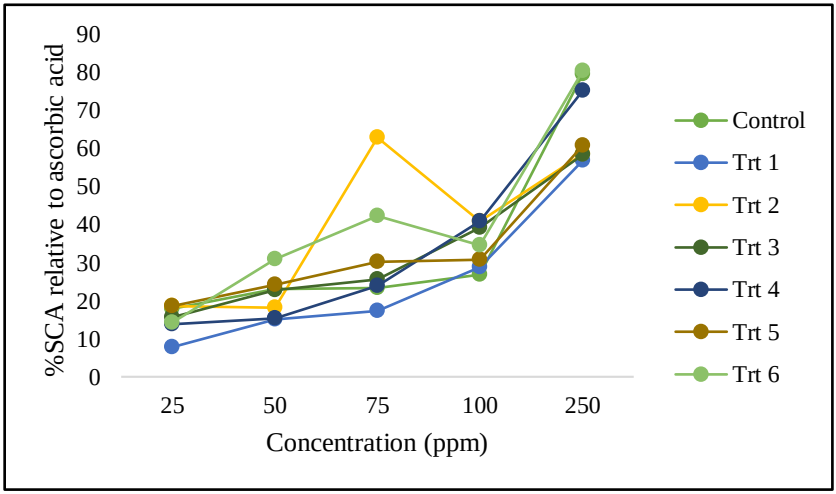


Figure 4. %DPPH radical scavenging activity of treatment extracts

Figure 5 illustrates IC_{50} values, highlighting strong antioxidant properties for all treatments. The order of IC_{50} values for DPPH scavenging activity is Treatment 6 > Control (Ethanol extract) > Treatment 4 > Treatment 2 >

Treatment 5 > Treatment 3 > Treatment 1. Treatment 6 had the highest DPPH activity with an IC_{50} value of 135.55 µg/mL, indicating strong antioxidant activity. The Control also had a relatively high activity with an IC_{50} of 156.03 µg/mL, in the moderate range. Treatment 4 had an IC_{50} of 156.91 µg/mL, which was also in the moderate range.

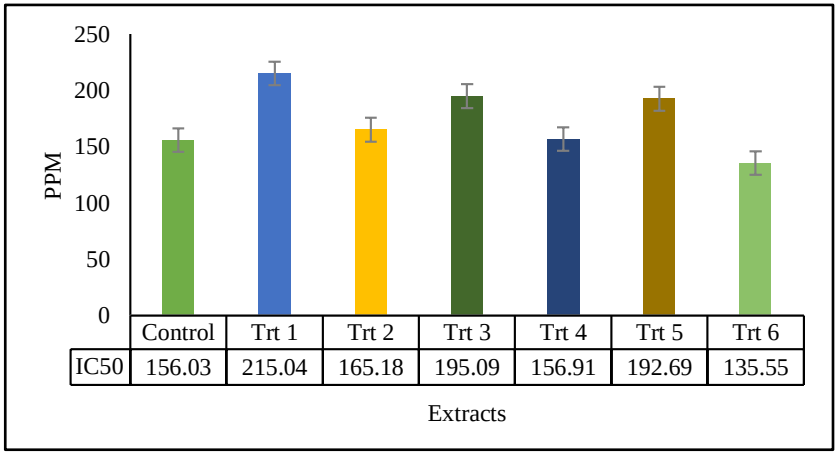


Figure 5. IC_{50} values of treatment extracts

Comparatively, the IC_{50} values obtained in this study were lower than those reported for *Dioscorea villosa* leaves with an IC_{50} value of 21.36 (aqueous extract), indicating a higher antioxidant potency. Additionally, the IC_{50} values reported by Bohara *et al*. (2020) which had 184.60 (aqueous extract) and 135.20 (ethanol extract) for *D. esculentum* were comparable to the obtained results in the study, highlighting the consistent antioxidant potential of *D. esculentum.* Extraction temperature and time significantly contributed to antioxidant activity, with higher values obtained at elevated temperatures and longer durations (Vergara-Salinas, 2012). Furthermore, the denaturation of phenols can reduce antioxidant activity (Arina and Harisun, 2019; Onyebuchi and Kavaz, 2020). The study found a significant correlation between phytochemical content and antioxidant activity, attributed to different radical antioxidant mechanisms and the contribution of phytochemicals in free radical scavenging. *D. esculentum* extracts have significant potential as a natural source of antioxidants, which could contribute to the prevention of oxidative stress-related diseases.

3.7 Anti-inflammatory Activity

The cyclooxygenase (COX) inhibitor activity of ethanol and aqueous extracts was observed (Table 6 and Figure 6). The results indicated that crude extracts generally exhibit higher anti-inflammatory activity against COX-2 than COX-1, except for Treatment 1. Treatments 4 to 7 demonstrated ≥ 50% COX-2 inhibition and a selectivity index (COX-2/COX-1 ratio) ≥ 1.00, indicating their active and selective nature towards COX-2. This is consistent with the study by Ang *et al*. (2022) indicating a lower COX-2 inhibition and selectivity ratio for *D. esculentum* frond (ethanolic extract). Control (Ethanol) extract had the highest percent COX-1 inhibition, while Treatment 2 (40 °C for 3 h) exhibited the lowest. Percent COX-2 inhibition followed the order of Treatment 1 < Treatment 2 < Treatment 3 < Treatment 4 < Treatment 5 < Treatment 6 < Control. However, only Treatments 4 to 7 were considered to be effective against both COX-2 and COX-2.

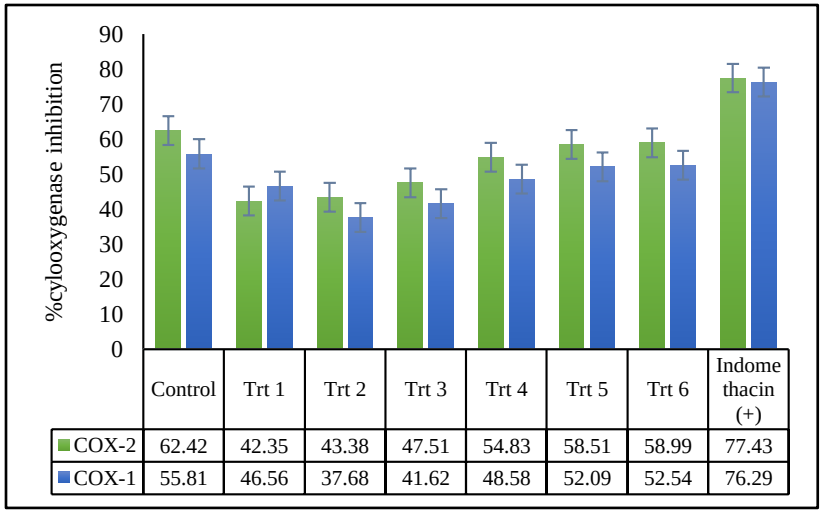
Table 6. Cyclooxygenase Inhibition Activity (%) of *D. esculentum* extracts

Extracts	% Cyclooxygenase inhibition (10 ppm) Selectivity index (COX-2 / COX-1)
Control (30 °C, 72 h)	1.12
Trt 1 (40 °C, 1 h)	0.91
Trt 2 (40 °C, 3 h)	1.15
Trt 3 (40 °C, 6 h)	1.14
Trt 4 (60 °C, 1 h)	1.13
Trt 5 (60 °C, 3 h)	1.12
Trt 6 (60 °C, 6 h)	1.12
Indomethacin (+ Control)	1.01

The means of the same letter superscript within the column are not significantly different at $p \leq 0.05$.

Further, Treatments 2 to 6 and control displayed a selectivity ratio of ≥ 1.00, indicating their relative selectivity towards COX-2. This suggested potential sources of COX-2 selective anti-inflammatory compounds in the ethanolic and aqueous extracts of *D. esculentum* powder, which could be employed in food products and pharmaceuticals. Extraction temperature and time can lead to COX enzyme activity, with excessively high temperatures leading to enzyme denaturation. The observed anti-inflammatory properties in Treatments 4 to 6 and control may be attributed to the presence of which likely inhibited inflammation through multiple mechanisms, including enzyme inhibition (Ang *et al*., 2022). Specifically, the observed selectivity towards COX-2 suggested that the active compounds may target this enzyme more effectively than COX-1. COX-2 is known to play a crucial role in the

inflammatory response by catalyzing the production of pro-inflammatory prostaglandins. By inhibiting COX-2, the extracts can reduce inflammation and alleviate symptoms associated with inflammatory diseases. Potential therapeutic applications of these extracts include the treatment of conditions such as arthritis, inflammatory bowel disease, and other inflammatory disorders.



	Control	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Indomethacin (+)
COX-2	62.42	42.35	43.38	47.51	54.83	58.51	58.99	77.43
COX-1	55.81	46.56	37.68	41.62	48.58	52.09	52.54	76.29

Figure 6. Inhibitory activity of *D. esculentum* extracts against COX-1 and COX-2; error bars are SEM

4. Conclusion and Recommendation

The study on the quality evaluation of *D. esculentum* powder highlighted its potential as a valuable functional food ingredient due to its favorable physicochemical properties, rich nutritional composition, and bioactive compounds. The powder contained a high protein content, low fat and calorie content, and significant antioxidant and anti-inflammatory activities. The aqueous and ethanolic extracts demonstrated promising results in inhibiting COX-2 activity, indicating their potential for reducing inflammation and pain. This research underscores the importance of exploring underutilized plant resources like ferns for their potential health benefits and sustainable food applications.

To further enhance the application of *D. esculentum powder*, the study recommends improving its flowability through additional processing techniques. Additionally, examining its nutritional composition and shelf-stability as a functional food product is essential. Identifying and characterizing the active compounds present in the extracted treatments can provide a deeper understanding of their bioactive properties. Finally, simulating the phytochemical, antioxidant, and anti-inflammatory properties of the powder in food applications can help assess its potential benefits and inform future product development. Such insights will empower stakeholders in the food and nutrition industry to develop innovative products enriched with *D. esculentum* powder, ultimately promoting consumer health and well-being. For instance, food manufacturers could consider incorporating *D. esculentum* powder into a variety of products, such as protein bars, snacks, and beverages, to enhance their nutritional value and provide additional health benefits.

5. Acknowledgement

The resource and logistic support for this study came from Central Mindanao University, Tuklas Lunas Development Center, Natural Science Research Center (NSRC), Natural Products Research and Development Center (NPRDC), Food Research and Development Center (FRDC), and University of Science & Technology of Southern Philippines.

6. References

Abubakar, A., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1. https://doi.org/10.4103/jpbs.jpbs_175_19

Ahn, J.Y., Kil, D.Y., Kong, C., & Kim, B.G. (2014). Comparison of oven-drying methods for determination of moisture content in feed ingredients. Asian-Australasian Journal of Animal Sciences, 27(11), 1615-1622. https://doi.org/10.5713/ajas.2014.14305

Albuquerque, B.R., Heleno, S.A., Oliveria, M.B.P., Barros, L., & Ferreira, I.C. (2021). Phenolic compounds: Current industrial applications, limitations and future challenges. Food & Function, 12(1), 14-29. https://doi.org/10.1039/D0FO02324H

Alonso-Riaño, P., Sanz Diez, M.T., Blanco, B., Beltrán, S., Trigueros, E., & Benito-Román, O. (2020). Water ultrasound-assisted extraction of Polyphenol compounds

from brewer's spent grain: Kinetic study, extract characterization, and concentration. Antioxidants, 9(3), 265. https://doi.org/10.3390/antiox9030265

Ang, A.M., Sabesaje, R.D., Barbosa, G.B., Cruz, R.Y., Mendez, R.A., & Enot, M.M. (2022). Cyclooxygenase (COX) and 15-Lipoxygenase (15-LOX) inhibitory activity and HPTLC profile of *Asplenium nidus*, *Diplazium esculentum*, and *Drynaria quercifolia* in Bukidnon, Philippines. Indonesian Journal of Pharmacy, 33(2), 215-224. https://doi.org/10.22146/ijp.3975

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. Applied Sciences, 12(4), 2107. https://doi.org/10.3390/app12042107

Aranibar, C., Pigni, N.B., Martinez, M., Aguirre, A., Ribotta, P., Wunderlin, D., & Borneo, R. (2018). Utilization of a partially-deoiled chia flour to improve the nutritional and antioxidant properties of wheat pasta. LWT, 89, 381-387. https://doi.org/10.1016/j.lwt.2017.11.003

Bassey, M.E., Johnny, I.I., Umoh, O.T., & Douglas, F.T. (2020). Phytomedicinal potentials of species of *Nephrolepis* (Schott.). World Journal of Pharmaceutical Research, 9(4), 1400-1410.

Brahma, S., Mochahary B., Kalita M., & Goyal, A.K. (2022). Pharmacognostic and physicochemical characterization of potential plants for anti-diabetic herbal formulations. Plant Science Today, 9(sp2), 1-7. https://doi.org/10.14719/pst.1704

Chaudhuri, T.K., & Roy, S. (2020). A comprehensive review on the pharmacological properties of *Diplazium esculentum*, an edible fern. Pharmaceutics and Pharmacology Research, 3(1), 1-9. https://doi.org/10.31579/2693-7247/014

Chai, T.T., Yeoh, L.Y., Ismail, N.M., Ong, H.C., Manan, F.A., & Wong, F.C. (2015). Evaluation of glucosidase inhibitory and cytotoxic potential of five selected edible and medicinal ferns. Tropical Journal of Pharmaceutical Research, 14(3), 449-454.

Dadi, D.W., Emire, S.A., Hagos, A.D., & Eun, J.B. (2019). Effect of ultrasound-assisted extraction of *Moringa stenopetala* leaves on bioactive compounds and their antioxidant activity. Food Technology and Biotechnology, 57(1), 77-86. https://doi .org/10.17113/ftb.57.01.19.5877

Dash, G.K., Khadidi, S.K.J., & Shamsuddin, A.F. (2017). Pharmacognostic studies on *Diplazium esculentum* (retz.) sw. Der Pharmacia Lettre, 9(3), 113-120.

Dela Cruz, R.Y., Ang, A.M.G., Doblas, G.Z., Librando, I.L., Porquis, H.C., Batoctoy, B.C.L.S., Cabresos, C.C., Jacalan, D.R.Y., & Amoroso, V.B. (2017). Phytochemical screening, antioxidant and anti-inflammatory activities of the three fern (*polypodiaceae*) species in Bukidnon, Philippines. Bulletin of Environment, Pharmacology, and Life Sciences, 6(3), 28-33.

Dias, C., Fonseca, A.M.A., Amaro, A.L., Vilas-Boas, A.A., Oliveria, A., Santos, S.A., Silvestre, A.J.D., Rocha, S.M., Isidoro, N., & Pintado, M. (2020). Natural-based antioxidant extracts as potential mitigators of fruit browning. Antioxidants, 9(8), 715. https://doi.org/10.3390/antiox9080715

Feng, P., Weagant, S.D., Grant, M.A., Burkhardt, W., Shellfish, M., & Water, B. (2002). Bacteriological analytical manual (BAM) Chapter 4: Enumeration of *Escherichia coli* and the coliform bacteria. US Food and Drug Administration. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria

Ferreira, A.S., Pereira, L., Canfora, F., Silva, T.H., Coimbra, M.A., & Nunes, C. (2023). Stabilization of natural pigments in ethanolic solutions for food applications: The case study of *Chlorella vulgaris*. Molecules, 28(1), 408. https://doi.org/10.3390/molecules28010408

Food and Drug Administration (FDA). (2022). Revised guidelines for the assessment of microbiological quality of processed foods. Retrieved from: https://www.fda.gov.ph/wp-content/uploads/2022/12/FDA-Circular-No.2022-12-2.pdf

Food and Nutrition Research Institute (FNRI). (2018). Philippine dietary reference intake. Retrieved from https://www.fnri.dost.gov.ph/images/images/news/PDRI-2018.pdf.

González-Burgos, E., Ureña-Vacas, I., Sánchez, M., & Gómez-Serranillos, M.P. (2021). Nutritional value of *Moringa oleifera* Lam. leaf powder extracts and their neuroprotective effects via antioxidative and mitochondrial regulation. Nutrients, 13(7), 2203. https://doi.org/10.3390/nu13072203

Gupta, S.M., Ballabh, B., Yadav, P.K., Agarwal, A., & Bala, M. (2020). Nutrients analysis of *Diplazium esculentum*: Underutilized wild wetland pteridophytes ensure food and nutritional security. ACTA Scientific Nutritional Health, 4(11), 46-49.

Harris, G.K., & Marshall, M.R. (2017). Ash analysis. In S. S. Nielsen (Ed.), Food analysis (pp. 287-297). Cham, Switzerland: Springer.

Hermann, J.R. (2019). Protein and the body. Retrieved from https://extension.okstate.edu/fact-sheets/print-publications/t/protein-and-the-body-t-3163.pdf

Horwitz, W. (2005). Official methods of analysis of AOAC international. Maryland, United States: AOAC International.

Huang, Y.L., Liao, H.F., Hung, C.Y., Lin, Y.Y., & Lin, Y.C. (2020). Fern extracts inhibit the growth of pathogenic bacteria in vitro. BMC Complementary Medicine and Therapies, 20(1), 1-8. https://doi.org/10.1186/s12906-020-02929-3.

Hui, C.K., Majid, N.I., Zainol, M.K.M., Mohamad, H., & Zin, Z.M. (2018). Preliminary phytochemical screening and effect of hot water extraction conditions on phenolic contents and antioxidant capacities of *Morinda citrifolia* leaf. Malaysian Applied Biology, 47(4), 13-24.

Joshi, N., Bains, K., & Kaur, H. (2019). Optimization of drying time and temperature for preparation of antioxidant rich vegetable powders from unconventional leafy greens. Chemical Science Review and Letters, 8(29), 70-78.

Junejo, J.A., Gogoi, G., Islam, J., Rudrapal, M., Mondal, P., Hazarika, H., & Zaman, K. (2018). Exploration of antioxidant, antidiabetic and hepatoprotective activity of

Diplazium esculentum – A wild edible plant from Northeastern India. Future Journal of Pharmaceutical Sciences, 4(1), 93-101. https://doi.org/10.1016/j.fjps.2017.10.005

Khoza, B.S., Chimuka, L., Mukwevho, E., Steenkamp, P.A., & Madala, N.E. (2014). The effect of temperature on pressurized hot water extraction of pharmacologically important metabolites as analyzed by UPLC-qTOF-MS and PCA. Evidence-Based Complementary and Alternative Medicine, 2014(1), 914759. https://doi.org/10.1155/2014/914759

Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. Technical Paper on Postharvest Losses, Action Contre la Faim (ACF), 25, 1-25.

Koniyo, Y., Lumenta, C., Olii, A.H., Mantiri, R.O.S.E., & Pasisingi, N. (2021). Nutrition of local wild edible fern (*Diplazium esculentum*) leaves. IOP Conference Series: Earth and Environmental Science, 637, 012008.

Li, H., & Liu, F. (2015). The chemistry of sour taste and the strategy to reduce the sour taste of beer. Food Chemistry, 185, 200-204. https://doi.org/10.1016/j.foodchem.2015.03.135

Ma, F., Wang, R., Li, X., Kang, W., Bell, A. E., Zhao, D., Liu, X., & Chen, W. (2020). Physical properties of mucilage polysaccharides from *Dioscorea opposita* Thunb. Food Chemistry, 311, 126039. https://doi.org/10.1016/j.foodchem.2019.126039

Majid, H., & Rining, H.A. (2018). The effect of drying techniques on the antioxidant capacity, flavonoids and phenolic content of fermented local cocoa bean. Journal of Advanced Research in Applied Mechanics, 47(1), 11-19.

Maturin, L., & Peeler, J. (2001). Bacteriological analytical manual (BAM) Chapter 3: Aerobic Plate count. US Food and Drug Administration. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobic-plate-count

Mistriyani, Riyanto, S., & Rohman, A. (2018). Antioxidant activities of Rambutan (*Nephelium lappaceum* L) peel in vitro. Food Research, 2(1), 119-123. https://doi.org/10.26656/fr.2017.2(1).150

Pasrija, D., & Anandharamakrishnan, C.J.F.B.T. (2015). Techniques for extraction of green tea polyphenols: A review. Food and Bioprocess Technology, 8, 935-950. https://doi.org/10.1007/s11947-015-1479-y

Pietrak, A., Łopusiewicz, Ł., & Salachna, P. (2022). Growth, leaf pigment content, and antioxidant potential of ferns grown in peat substrate amended with camelina press cake. Agronomy, 12(12), 3100. https://doi.org/10.3390/agronomy12123100

Pradhan, S., Manivannan, S., & Tamang, J.P. (2015). Proximate, mineral composition and antioxidant properties of some wild leafy vegetables. Journal of Scientific and Industrial Research, 74, 155-159.

Putri, R., Yahya, A., Adam, N., & Abd Aziz, S. (2015). Correlation of moisture content to selected mechanical properties of rice grain sample. International Journal on Advanced Science, Engineering and Information Technology, 5(5), 264-267.

Riaz, A., Khan, M.S., Saeed, M., Kamboh, A.A., Khan, R.U., Farooq, Z., & Farid, M. U. (2022). Importance of Azolla plant in poultry production. World's Poultry Science Journal, 78(3), 789-802.

Saragih, B., Prakoso, H.T., Rahmadi, A., Emmawati, A., & Kurniadinata, O.F. (2017). Phytochemicals, quality and glycemic response fern red herbal (*Stenochlaena palustris*). Proceedings of the Asian Academic Society International Conference (AASIC). Khon Kaen, Thailand.

Sareen, B., Bhattacharya, A., & Srivatsan, V. (2021). Nutritional characterization and chemical composition of *Diplazium maximum* (D. Don) C. Chr. Journal of Food Science and Technology, 58, 844-854.

Sharma, D.K., Dave, R.S., & Shah, K.R. (2020). Proximate analysis and evolution of energy value from leaves and stem of sword fern: *Nephrolepis exaltata*. International Research Journal Pure Applied Chemistry, 21(7), 10-17.

Singh, B., Suri, K., Shevkani, K., Kaur, A., Kaur, A., & Singh, N. (2018). Enzymatic browning of fruit and vegetables: A review. Enzymes in Food Technology: Improvements and Innovations, 63-78.

Smita, M., Bashir, M., & Haripriya, S. (2019). Physicochemical and functional properties of peeled and unpeeled coconut haustorium flours. Journal of Food Measurement and Characterization, 13(1), 61-69.

Speer, H., D'cunha, N.M., Botek, M., Mckune, A.J., Sergi, D., Georgousopouou, E., & Naumovski, N. (2019). The effects of dietary polyphenols on circulating cardiovascular biomarkers and iron status: A systematic review. Nutrition and metabolic insights, 12, 1-12. https://doi.org/10.1177/1178638819882739

Tambunan, A.P., Bahtiar, A., & Tjandrawinata, R.R. (2017). Influence of extraction parameters on the yield, phytochemical, TLC-densitometric quantification of quercetin, and LC-MS profile, and how to standardize different batches for long term from *Ageratum conyoides* L. leaves. Pharmacognosy Journal, 9(6), 767-774.

Tapia, M.S., Alzamora, S.M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. In Barbosa-Cánovas, G.V., Fontana, A.J., Jr., Schmidt, S.J., & Labuza, T.P. (Eds.), Water activity in foods: Fundamentals and applications (2nd ed., pp. 323-355). New Jersey, United States: Wiley Blackwell.

Tongco, J.V.V., Villaber, R.A.P., Aguda, R.M., & Razal, R.A. (2014). Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from Philippines. Journal of Chemical and Pharmaceutical Research, 6(8), 238-242.

Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., & Bandler, R. (2001). Bacteriological analytical manual (BAM) Chapter 18: Yeasts, molds and mycotoxins.

US Food and Drug Administration. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins

Vyas, S., Kachhwaha, S., & Kothari, S. (2015). Comparative analysis of phenolic contents and total antioxidant capacity of moringa oleifera Lam. Pharmacognosy Journal, 07(01), 44-51. https://doi.org/10.5530/pj.2015.1.5

World Health Organization. (2018). *E. coli*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/e-coli

Zihad, S.N., Gupt, Y., Uddin, S.J., Islam, M.T., Alam, M.R., Aziz, S., Hossain, M., Shilpi, J.A., Nahar, L., & Sarker, S.D. (2019). Nutritional value, micronutrient and antioxidant capacity of some green leafy vegetables commonly used by southern coastal people of Bangladesh. Heliyon, 5(11), e02768. https://doi.org/10.1016/j.heliyon.2019.e02768