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Mineral, Nutritional, and Phytochemical Profile, Total Phenolic Content, and Radical Scavenging Activity of Philippine Bamboo "Bolo" *Gigantochloa levis* (Blanco) Merr. Leaves

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Abstract – The study is a pioneering effort to determine the mineral, nutritional, and phytochemical composition and phenolic content and to determine the free radical scavenging activity of *Gigantochloa levis* (Blanco) Merr, a native bamboo species (locally known as "bolo") in the Philippines. Proximate analysis showed that air-dried *G. levis* leaves contain 15.8% ash, 22.6% crude protein, 1.2% crude fat, 29.3% crude fiber, and 19.7% total sugar. Phytochemical tests indicated the presence of diterpenes, triterpenes, saponins, phenols, tannins, and flavonoids in both the ethanolic and aqueous leaf extracts, while phytosterols were only detected in the ethanolic extract. Folin-Ciocalteu assay determined the total phenolic content in gallic acid equivalents (GAE) to be 85.86 ± 3.71 and 32.32 ± 1.01 mg GAE/100 g dried sample for the ethanolic and aqueous extracts, respectively. The total phenolic content in quercetin equivalents (QE) was 74.44 ± 3.11 and 29.43 ± 0.85 mg QE/100g dried sample for the ethanolic and aqueous extracts, respectively. The radical scavenging activity of the different solvent fractions containing varying concentrations of the extract was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The ethyl acetate and 1-butanol fractions were found to have the highest radical scavenging activity. Mineral analysis via Energy Dispersive X-Ray Spectrometry (EDS) of the ash of *G. levis* leaves showed that Si is the major component, followed by K and Mg. These results point to the potential of *G. levis* leaves as a source of minerals and bioactive compounds with medicinal value.
Keywords – *Gigantochloa levis*, Bamboo, Proximate analysis, Phytochemical screening, Radical scavenging activity

Introduction

Phenolic compounds, which include flavonoids, polyphenols, and tannins, are secondary metabolites that are widely distributed in plants. In some bamboo species, significant amounts of phenolics are present, especially in the shoots, leaves, and branches.[1] Studies concerning bamboo and its phenolic content are mostly limited to China and the local species in that country.[2,3] Prior to this study, there was no literature report on the chemical composition and antioxidant activity of the leaves of *G. levis*. Most studies done on *G. levis* and other bamboo species were concerned with its use as a construction material (tensile strength, elasticity, etc.), as source of pulp for paper production (pulp yield, fiber content, wettability, etc.), and as source of biomass for energy purposes, *e.g.*, viability of bamboo briquettes, calorific value, volatiles and vapor content.[4] The aim of this present study is the analysis of the leaves of *G. levis*, a bamboo species indigenous to the Philippines in terms of the determination of its elemental (mineral), nutritional, and phytochemical profile, total phenolic content, and radical scavenging activity.

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Experimental

Sample collection – *G. levis* leaves were obtained from the *Bambusetum* of the Department of Environment and

Natural Resources-Ecosystems Research Development Bureau (DENR-ERDB) located in Mt. Makiling Forest Reserve, Los Baños, Laguna, Philippines. All bamboo species in the *Bambusetum* including *G. levis* were properly labeled and identified. Bamboo plant materials were air dried for five days to one week prior to extraction.

Nutritional content analysis – Proximate analysis using the procedures adopted from the "Association of Official Analytical Chemists" (AOAC) Official Methods of Analysis (1995) was done to determine the nutritional composition of *G. levis* leaves (ash, crude protein, crude fat, and crude fiber).[5]

Solvent extraction – For ethanolic extracts, powdered air-dried *G. levis* leaves were kept in contact with 70% ethanol in H_2O (soaked) in a stoppered container for 20 minutes with occasional agitation to ensure homogenous mixing. An initial maceration was done followed by clarification (decantation) and addition of fresh solvent to the sample. This was done 3 times with all filtrates pooled together. The pooled extract was concentrated by evaporating ethanol under reduced pressure). The ratio for the volume of solvent to the sample is 3:1 (300 mL solvent to 100 g of *G. levis* leaves). For aqueous extracts, extraction was done by adding hot, boiled distilled water to the large beaker containing the powdered air-dried bamboo material for 15 minutes, and then concentrated by evaporating water under reduced pressure. The same ratio as in the maceration procedure above was used for the decoction procedure.

Phytochemical Screening – Phytochemical screening was used for the detection of the phytochemicals present in *G. levis* leaves. The method used pure and/or mixtures of reagents to signify the presence of specific group of compounds.[6-8]

Total Phenolic Content – Folin-Ciocalteu assay was used to quantify the amounts of phenolics present in the crude aqueous and ethanolic extracts of *G. levis* leaves, using gallic acid and quercetin as standards.[9] The extract (0.2 mL) was mixed with 0.5 mL of Folin-Ciocalteu Reagent (FCR) and 3.0 mL of 10% Na_2CO_3 solution and then the solution was left to stand for 15 minutes at room temperature. Afterwards, 10 mL distilled water was added to dilute the solution. The absorbance of the resulting solution was measured at 725 nm. Standard curves were prepared using standard solutions of gallic acid and quercetin (0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL). A blank solution was prepared using the same conditions and reagents for the preparation of the standard and sample solutions. The absorbance values were read immediately after sample preparation. The blank, samples and standards were kept away from light during analysis. Analysis was done in triplicates.

Radical scavenging activity (DPPH Assay) – Following the partitioning of the 70% ethanol in distilled, deionized H_2O crude extract by liquid-liquid extraction (*n*-hexane, chloroform, ethyl acetate, 1-butanol, and distilled deionized water), the free radical scavenging activity of the different solvent fractions containing varying concentrations of the extract was determined using the DPPH assay.[10] Samples (freeze dried fractions) were prepared in various concentrations (25, 50, 100, 200 µg/mL) in methanol. Fifty (50) µL of each of the prepared reagent concentrations were mixed with 100 µL (10 mg/L) of DPPH solution. The absorbance of the samples was measured at 515 nm after 30 minutes. A control solution was prepared using 50 µL methanol and 100 µL DPPH solution. A reagent blank was prepared using 150 µL methanol only. Gallic acid was used as standard. The assay was done in triplicates.

The DPPH free radical scavenging activity was calculated using the formula:

$$\%\ DPPH\ free\ radical\ scavenging\ activity = \left(1-\frac{A_{sample,515}-A_{blank,515}}{A_{control,515}}\right)\times 100$$

Mineral profile analysis – The elemental profile of the ash of *G. levis* leaves was determined using JEOL JSM-6000 series WDS/EDS (Wavelength dispersive X-Ray spectrometer/Energy dispersive X-Ray spectrometer) coupled to JEOL JSM-6400 Scanning Electron Microscope (SEM) (Japan).

Statistical analysis – All experimental runs (except solvent extractions) were done in triplicates. The mean and sample standard deviation were calculated to measure the amount of dispersion of the resulting data set.

Result and Discussion

Proximate analysis showed that air-dried *G. levis* leaves contains 15.82 ± 0.25% ash, 22.63 ± 1.24% crude protein, 1.18 ± 0.54% crude fat, and 29.32 ± 0.27% crude fiber, and 19.68 1.83% total sugar (Fig. 1). Crude protein, crude fat, crude fiber, and minerals are important in human diet as reported in the literature. In a similar study, the proximate analysis of *Bambusa vulgaris* L. leaves also showed that crude fiber is the major component of the leaves at 37.60%, followed by sugars at 19.73%, 13.13% crude protein, 10.75% ash, and 8.45% crude fat.[11] The results of this study coincide directly with what the

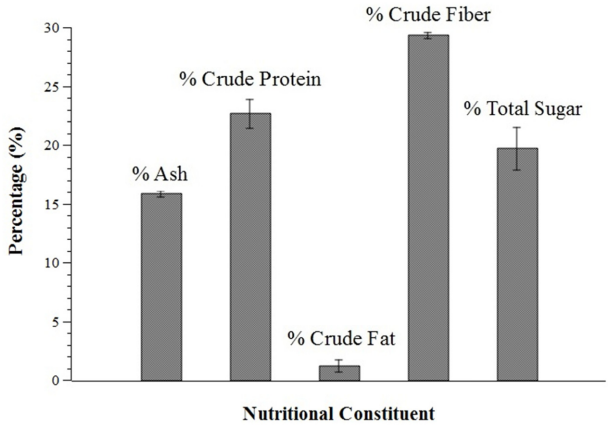

Fig. 1. Nutritional constituents of *Gigantochloa levis* (Blanco) Merr. leaves. Error bars represent measured standard deviation.

Table 1. Qualitative phytochemical screening of ethanolic and aqueous extracts of *Gigantochloa levis* (Blanco) Merr. leaves

Phytochemical test	Ethanolic extract	Aqueous extract
Alkaloids (Wagner's test)	–	–
Carbohydrates - reducing sugars (Benedict's test)	–	–
Cardiac glycosides (Legal's test)	–	–
Anthranol glycosides (Modified Borntrager's test)	–	–
Cyanogenic glycosides (Picrate paper test)	–	–
Saponins (Froth test)	+	++
Diterpenes (Copper acetate test)	++	+
Triterpenes (Salkowski's test)	+	+
Phenols (Ferric chloride test)	++	+
Phytosterols (Liebermann - Burchard's test)	+	–
Tannins (Gelatin test)	+	+
Flavonoids (Alkaline reagent test)	+	+
Amino acids (Ninhydrin test)	–	–
Proteins (Nitric acid test)	–	–

Legend: (–) not detected/present, (+) present in low amounts, (++) present in high amounts

literature data shows. *Bambusa vulgaris* L. leaves is being used as a beverage, fortified with orange and pineapple juices, in the locality in which the study was undertaken.

Both the ethanolic and aqueous extracts were tested for their phytochemical profile. Phytochemical tests indicated the presence of diterpenes, triterpenes, saponins, phenols, tannins, and flavonoids in both the ethanolic and aqueous leaf extracts, while phytosterols were only detected in the ethanolic extract. Table 1 shows the chemical tests done for the phytochemical screening of both the ethanolic and aqueous extracts of *G. levis* leaves. Both the ethanolic and aqueous extracts show no positive indication when it comes to cardiac and cyanogenic glycosides. Both these glycoside groups are considered toxic to humans to a certain extent, especially cyanogenic glycosides which can cause neurological damage.[12]A previous study[13], involving *Gigantochloa manggong,*a bamboo species from Indonesia reported the detection of flavonoids, triterpenes, and saponins in 70% ethanol in water leaf extract. Furthermore, alkaloids were detected but the presence of phenolics and tannins were found to be negative.

Table 2. Total phenolic contents (TPC) of the extracts of *Gigantochloa levis* (Blanco) Merr. leaves in terms of gallic acid and quercetin equivalents

	Ethanolic extract	Aqueous extract
Gallic acid equivalents (mg GAE/100 g air-dried sample)	85.86 ± 3.71	32.32 ± 1.01
Quercetin equivalents (mg QE/100 g air-dried sample)	74.44 ± 3.11	29.43 ± 0.85

Folin-Ciocalteu assay determined the total phenolic content expressed in GAE to be 85.86 ± 3.71 mg GAE/ 100 g dried sample for the ethanolic extract and 32.32 ± 1.01 mg GAE/100 g dried sample for the aqueous extract. The total phenolic content expressed in QE was found to be 74.44 ± 3.11 for the ethanolic extract and 29.43 ± 0.85 mg QE/100 g dried sample for the aqueous extract (Table 2).

It is well known today that many diseases are caused by oxidative stresses due to free radicals or reactive oxygen species (ROS). These cell-damaging species need to be sufficiently neutralized or balanced with endogenous antioxidative compounds. Cells are naturally capable of combating free radicals and ROS by developing antioxidant mechanisms to quench these reactive species. One such way is the production of superoxide dismutase by the body to react with free radicals and ROS in vivo. Using 70% ethanol in distilled, deionized H_2O afforded a freeze dried extract yield of 15.45% from the initial 300 g (oven dried basis, as calculated from the experimental moisture content of 11.50%) of *G. levis* leaves. Subsequent liquid-liquid extraction using solvents of different polarity afforded the following yield values (freeze dried); *n*-hexane fraction, 10.54%; chloroform fraction, 23.02%; ethyl acetate fraction, 1.70%; 1-butanol fraction, 21.18%; and water fraction, 42.90% with an overall yield of 99.34%. The ethyl acetate and 1-butanol fractions were found to have the highest free radical scavenging activity using the DPPH assay (Fig. 2). Polar phenolic and flavonoid compounds are believed to be found in the ethyl acetate and 1-butanol fractions of the crude ethanolic extract of *G. levis.*

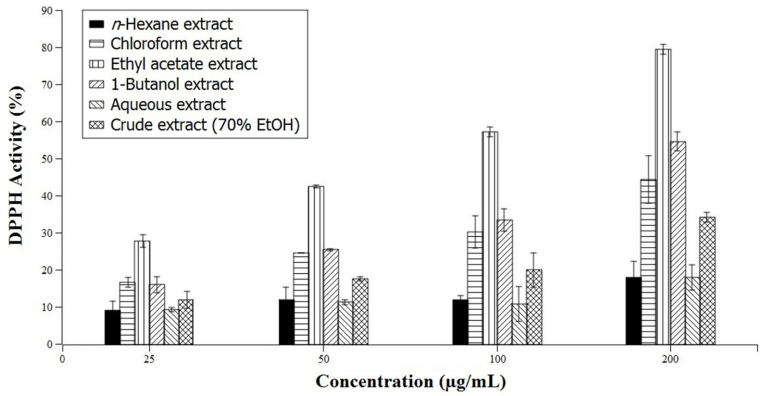

Fig. 2. DPPH scavenging activity of the solvent fractions and crude extract of *Gigantochloa levis* (Blanco) Merr. leaves compared to gallic acid as standard (100%) in varying concentrations. Error bars represent measured standard deviation.

Table 3. Mineral composition of recovered inorganic ash from *Gigantochloa levis* (Blanco) Merr. leaves

Elements	Atomic %	SD
Na	1.19	0.12
Mg	7.06	0.21
Al	0.19	0.29
Si	74.48	0.18
P	2.55	0.32
S	0.38	0.06
Cl	0.88	0.04
K	9.60	0.11
Ca	3.22	0.22
Mn	0.10	0.03
Fe	0.33	0.15
Total	100.00	0.00

Note: Oxygen is negligible since it is available in the sample as oxides

The spectrum clearly shows which elements are abundant and which are not because the relative amounts of the atoms that produced an X-ray is detected by EDS. In the spectrum, it is clear that silicon is the most abundant, followed by potassium and magnesium. The oxygen detected in the spectrum is not included in the calculations because it is found in the samples in the form of oxides. Using EDS analysis for the determination of the mineral profile of the ash of *G. levis* leaves, it was observed that Silicon was the major component at 74.48 ± 0.18%, followed by Potassium and Magnesium at 9.60 ± 0.11% and 7.06 ± 0.21%, respectively (Table 3).

The results of the present study show the potential of *G. levis* leaves as a source of minerals and bioactive compounds with medicinal value.

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