Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(1):709-713

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Proximate analysis, phytochemical screening, and total phenolic and flavonoid content of Philippine bamboo *Schizostachyum lumampao*

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ABSTRACT

The chemical composition of the leaves of Schizostachyum lumampao, known as "buho" in the Philippines, was determined for its potential use as herbal tea with potential health benefits. Proximate (nutritional) analysis using standard AOAC methods showed that the air-dried leaves contain 10.0 % moisture, 30.5 % ash, 22.1 % crude protein, 1.6 % crude fat, 28.7 % crude fiber, and 7.2 % total sugar (by difference). Using a variety of reagents for qualitative phytochemical screening, saponins, diterpenes, triterpenes, phenols, tannins, and flavonoids were detected in both the ethanolic and aqueous leaf extracts, while phytosterols were only detected in the ethanolic extract. Using UV-Vis spectrophotometry, the total phenolic content (in GAE) were 76.7 and 13.5 mg gallic acid equivalents per 100 g air-dried sample for the ethanolic and aqueous extracts, respectively. The total flavonoid content of the samples (in QE) were 70.2 and 17.86 mg quercetin equivalents per 100 g air-dried sample for the ethanolic and aqueous extracts, respectively. This preliminary study showed the total amount of phenolics and flavonoids present in buho, the phytochemicals present, and its proximate analysis.

Keywords: bamboo, phenolics, flavonoids, proximate analysis, phytochemical screening

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INTRODUCTION

In Asia, bamboo has been widely cultivated as a fast growing non-timber forest species and used as a construction material because its culm has high strength and versatility. After harvesting the culm, the leaves are simply discarded or burned as fuel. However, dried bamboo leaves had been used by ancient Indians (as stated in the Ayurveda) and Chinese as tea. Dried bamboo leaves are boiled with water and then the residue is filtered before consumption. A previous research study revealed that the medicinal effects of bamboo leaf extracts were attributed to its antioxidant phytochemicals, such as phenolic compounds. These compounds, also known as polyphenols, are secondary metabolites widely distributed in the leaf, branch, trunk, root and fruit of plants [1]. For instance, the bamboo species, "Moso" (*Phyllostachys edulis*), which is harvested in China, had been tested for its antioxidant activity [2]. It showed significant inhibitory effects on superoxide radical, hydroxyl radical, DPPH radical, and ferrous metal-chelating capacities. Korean bamboo (*P. pubescens and P. nigra)* shoots also showed antioxidant activity, and in addition, also inhibited angiotensin converting enzyme which is a potential indicator of its antihypertensive properties [3]. Bamboo leaves of *Phyllostachys nigra* var. *henonis* bamboo from China, have recently been utilized as a source of phenolics and flavonoids that exhibit antioxidant activity [4]. The phenolics of other Chinese bamboos (*Yushania chungii*, *Fargesia robusta*, *Fargesia denudata*, *Fargesia rufa*, *Fargesia scabrida*) were characterized using HPLC and shows that individual species, sampling site, and age affects the phenolic content and identity in bamboo [5]. Flavonoids and phenolics were shown to reduce inflammation, promote overall cardiovascular health and circulation, and even protect against certain kinds of cancer [6,7]. These studies necessitate the chemical characterization (*e.g.*, proximate analysis) and qualitative identification of phenolics and

other bioactive phytochemicals before conducting assays on its biological activity, such as antioxidant activity in living systems, for its use as a human food with potential health benefits.

There is no evidence in the literature that the screening of possible important bioactive phytochemicals and undocumented novel compounds from the leaves of the endemic species [8], *Schizostachyum lumampao* also known as "buho" in the Philippines, had already been studied. Thus, the present study is a pioneering attempt to characterize and quantify phytochemicals present in Philippine bamboo.

EXPERIMENTAL SECTION

Sample collection and preparation: *Schizostachyum lumampao* (buho) bamboo leaves were collected from the *Bambusetum* of the Ecosystems Research and Development Bureau (ERDB) of the Philippine Department of Environment and Natural Resources – (DENR), located in the Mt. Makiling Forest Reserve, Los Baños, Laguna, Philippines. All bamboo species in the ERDB *Bambusetum* are properly labeled and identified by plant taxonomists. The study was conducted from August 2013 to October 2013 at the Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños and at the Wood Chemistry Laboratory, Department of Forest Products and Paper Science, College of Forestry and Natural Resources, University of the Philippines Los Baños. The bamboo leaves were washed and air-dried at room temperature for at least five days prior to solvent extraction.

Proximate analysis: Air-dried bamboo leaves were analyzed for their moisture, ash, crude fat, crude fiber and crude protein content using standard analytical methods [9].

Solvent extraction: Two solvents were used for extraction: water and ethanol. The bamboo leaves were ground using a blender. For ethanol extraction, the air-dried powdered bamboo leaves were mixed with 80 % ethanol in water for 24 hours with frequent agitation and then filtered. Extraction was done three times and the obtained extracts were pooled. The pooled solution was concentrated by evaporating the solvent under reduced pressure (Stuart RE300/MS rotary evaporator). Water extraction was done by boiling air-dried powdered bamboo material in distilled water for 15 minutes and then cooled.

Phytochemical Screening: The extracts were tested for specific presence of certain phytochemicals. The following lists the procedure and reagents, along with the resulting positive reaction signifying the presence of specific phytochemicals in the screening process [10,11].

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 brown/reddish 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, Na citrate). Formation of orange red precipitate indicates presence of reducing sugars.
3. **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.
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 presence of anthranol glycosides.
5. **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.
6. **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 presence of saponins.
7. **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 presence of diterpenes.
8. **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 presence of triterpenes.
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. **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 presence of phytosterols.
11. **Tannins** (Gelatin test): Two mL of the extracts were treated with few drops of 1 % gelatin solution containing NaCl. Formation of white precipitate indicates presence of tannins.
12. **Flavonoids** (Alkaline reagent test): Two mL of the extracts were treated with few drops of 2 M NaOH. Formation of an intense yellow colored solution, which would then turn colorless when dilute acid was added, signifies the presence of flavonoids.
13. **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 presence of free amino acids.
14. **Proteins** (Nitric acid test): Two mL of the extracts were treated with few drops of concentrated nitric acid. Formation of yellow colored solution indicates presence of proteins.

Total Phenolic and Flavonoid Content: The total phenolic and flavonoid content of the ethanolic and aqueous extract was determined using the Folin-Ciocalteu assay [12].

RESULTS AND DISCUSSION

Proximate analysis: The proximate analysis of buho shows a high amount of ash, crude fiber, and crude protein. The ash content, which is equivalent to the amount of inorganics in the leaves, is about 30 %. The % total sugar was obtained by difference (Figure 1). The values for the proximate analysis of the leaves (reported as mean ± standard deviation, n=3) are as follows: 9.99 ± 0.056 % moisture, 30.49 ± 0.58 % ash, 22.10 ± 1.26 % crude protein, 1.56 ± 0.97 % crude fat, 28.65 ± 0.09 % crude fiber, and 7.21 ± 0.79 % total sugar.

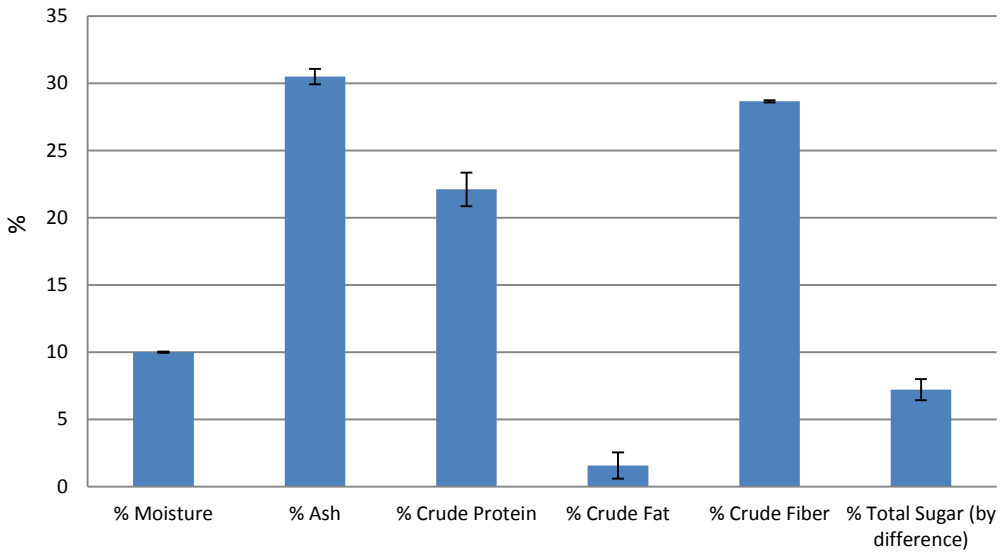

Figure 1. Proximate analysis of buho (*Schizostachyum lumampao*) leaves (n = 3) using standard analytical methods [9]

Phytochemical Screening: Qualitative phytochemical screening methods detected the presence of a particular phytochemical in the ethanolic and water extracts. The estimated amount of phytochemicals detected in the samples was based on the intensity of color change observed during screening. Natural products belonging to saponins, diterpenes, triterpenes, phenols, tannins, and flavonoids were shown to be present in both the ethanolic and aqueous extracts. However, phytosterols were only found in the ethanolic extract (Table 1). Saponins, as expected, are very hydrophilic, which dissolve in water more readily; therefore, the saponin test afforded a more visible detection of this group of natural products in the aqueous extract. Diterpenes, having a prominent non-polar part, tends to go with the less polar ethanolic solvent than water alone. Triterpenes are less polar compared to diterpenes, but they are also detected in minimal amounts in both the ethanolic and aqueous extracts. This is due to the presence of some polar functional groups in their structure, such as hydroxyl and carbonyl groups.

Phenols, especially free phenolic acids are detected in higher amounts in the ethanolic extract. Phenols are less soluble in water alone because it cannot break the existing hydrogen bonds between or among phenolic compounds. Tannins and flavonoids, due to their hydroxyl groups, are both detected in the ethanolic and aqueous extracts. Phytosterols are only detected in the ethanolic extract because of the size and polarity of compounds belonging to this group. Phytosterol is soluble in ethanol to a small extent because of some hydroxyl groups present in its

structure. However, phytosterols are not soluble in water because of their large size; solvation in water is not possible. Proteins and amino acids were not detected in the ethanolic and aqueous extracts, although the proximate analysis of the leaves suggested the presence of extractable proteins from the leaves of *S. lumampao*. Both ethanol and water could be more highly polar than proteins to be able to properly solvate the bulkier and less polar proteins present in the leaves.

Cardiac glycosides are known to possess serious toxicity because they could affect the heart and atrial fibrillation [13]. Cyanogenic glycosides on the other hand are toxic because of the neurological effects (Tropical Ataxic Neuropathy) of these compounds [14]. The absence of these toxic plant secondary metabolites in *S. lumampao* leaves makes this plant material a potential and safe herbal tea or supplement. Anthranol glycosides were also not detected in both the ethanolic and aqueous extracts of *S. lumampao.*

Table 1. Qualitative Phytochemical Screening of Ethanolic and Aqueous Extracts of Philippine Bamboo, *Schizostachyum lumampao,* Leaves

Phytochemical Test	Ethanolic Extract	Water 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)	-	-

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

Total Phenolic and Flavonoid Content: The determination of the total phenolic and flavonoid contents of buho leaves was undertaken by adding Folin-Ciocalteu reagent (FCR) to the sample and then measuring the absorbance of the treated sample at 725 nm through UV-Vis spectrophotometry (Table 2). The concentrations of the phenolic and flavonoid extracts were determined from a standard curve of FCR-treated solutions with known gallic acid or quercetin concentrations. The ethanolic extracts contained higher amounts of both the phenolics and the flavonoid components as compared to the aqueous extracts, because of the higher solubility of phenolics in ethanol compared to water.

Table 2. Total Phenolic and Flavonoid Content of *Schizostachyum lumampao* Leaf Extracts

	Ethanolic Extract	Water Extract
Total Phenolic Content (mg GAE/100 g air-dried sample)	76.72 ± 9.06	13.48 ± 4.12
Total Flavonoid Content (mg QE/100 g air-dried sample)	70.24 ± 7.52	17.86 ± 3.42

CONCLUSION

The moisture, ash, crude protein, crude fat, crude fiber, and total sugar content (proximate analysis) of *Schizostachyum lumampao* leaves were determined by using standard analytical methods. Phytochemical screening qualitatively assessed the phytochemicals present in *S. lumampao* leaves. The *S. lumampao* leaf extracts were negative for the toxic compounds such as cardiac and cyanogenic glycosides, which means that the *S. lumampao* leaves are safe for human consumption and possesses great promise as herbal tea. Further studies are being done, especially the determination of bioactivity of the specific phytochemicals in Philippine bamboo species.

Acknowledgments
The study was conducted with the assistance of Sharleen Lobos, Marjhun Ricarte, and Juanito Orozco. The chemicals used in phytochemical screening were provided by Dr. Evelyn B. Rodriguez of the Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños.

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