

Nutritional Analysis, Phytochemical Screening, and Total Phenolic Content of *Basella alba* Leaves from the Philippines

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ABSTRACT

Basella alba, known locally in the Philippines as “Alugbati” is an underutilized crop having potential health benefits. Proximate (nutritional) analysis using standard methods showed that the dried leaves contain 15.49 ± 0.36 % ash (minerals), 1.58 ± 0.08 % crude fat, 7.23 ± 0.17 % crude fiber, 17.55 ± 0.08 % crude protein, and 50.62 ± 0.30 % total carbohydrates. The presence of phytochemicals such as saponins, diterpenes, phenols, tannins, and flavonoids in both ethanolic and aqueous extracts, alkaloids in aqueous extract, and cardiac glycosides in ethanolic extract was known through qualitative phytochemical screening of *B. alba* leaves extracts. Using the Folin-Ciocalteu method, the total phenolic contents were found to be 93.89 and 85.13 mg gallic acid equivalents (GAE)/g extract for ethanol and aqueous extracts, respectively and 100.18 and 90.80 mg quercetin equivalents (QE)/g extract for ethanol and aqueous extracts, respectively. The phytochemicals detected in the aqueous and ethanolic extracts contributes to the anticancer, antioxidant, and anti-inflammatory properties of *B. alba* as described in literature.

Keywords: Proximate analysis, phytochemical screening, total phenolic content, *Basella alba*

INTRODUCTION

In response to the growing trend in consumer preferences for natural and minimally processed foods, many researches today focus broadly on the bioactive compounds of plants for their potential use in the food industry and in other related fields. Natural foods certainly provide numerous health benefits¹. In fact, a number of studies suggested that fruits and vegetables consumption is strongly associated with reduced risk of cardiovascular diseases, stroke, and cancer caused by oxidative stress². Extensive investigation of most fruits and vegetables led to the attribution of their protective effects against severe human diseases to the presence of vitamin C and E, β -carotene, and phytochemicals. Phytochemicals are bioactive non-nutrient plant compounds that have shown wide range of biological effects which include anti-inflammatory, anti-microbial, vasodilatory actions, and antioxidant effects³.

Many nutritious, inexpensive and available all year-round vegetables and herbs are found in the Philippines. One of the indigenous vegetables that the Bureau of Plant Industry of the Philippine government promotes for consumption is *Basella alba*, locally known as “Alugbati”. It is a succulent, branched, herbaceous vine with heart-shaped leaves and purplish stems bearing green to dark red fruits. *Basella alba* is also called Malabar spinach or Ceylon spinach since it is usually used as a substitute for spinach. It is a good source of fiber, vitamins A, B, and C, iron, calcium, and saponins. Saponins act as phytochemicals, having the

ability to fight cancer and cardiovascular diseases. *Basella alba* is also used in treating headache, inflammation, and ulcer⁴.

Basella alba is a good source of vitamins A, C and B9, thiamine, riboflavin, niacin, minerals like calcium, magnesium and iron, and several antioxidants. It also contains essential amino acids such as arginine, isoleucine, leucine, lysine, threonine, and tryptophan and a low percentage of soluble oxalates. Different drying methods and storage resulted in a considerable reduction in calcium, magnesium, sodium, iron, manganese, and zinc. *Basella* saponins, peptide, and phenolic compounds were shown to be present in *Basella alba*. The leaves contain carotenoids, organic acids, water soluble polysaccharides, bioflavonoid, betacyanin, and vitamin K. Also, triterpene oligoglycosides basella saponins A, B, C, and D, betavulgaroside I, momordins IIb and IIc have been isolated from the plant⁵. From the same study, it was found out that the anticancer, antioxidant and anti-inflammatory properties of *Basella alba* were attributed to the presence of β -sitosterol and lupeol. The present study was aimed to determine the proximate composition; to qualitatively assess the phytochemical profile of the ethanolic and aqueous extracts; and to determine the total phenolics and flavonoid content of *Basella alba* leaves from the Philippines.

MATERIALS AND METHODS

Collection and Preparation of Samples

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Table 1: Proximate analysis of *Basella alba* leaves.

Constituent	Percentage (%)*
Ash (Minerals)	15.49 ± 0.36
Crude Fat	1.58 ± 0.08
Crude Fiber	7.23 ± 0.17
Crude Protein	17.55 ± 0.08
Total Carbohydrates	50.62 ± 0.30

* Percentage compositions are on a dry weight basis available on three replicates.

Table 2: Qualitative screening of ethanol and aqueous extracts of *Basella alba* leaves.

Phytochemical Test	Ethanol Extract	Aqueous Extract
Alkaloids	-	++
Carbohydrates	-	-
Cardiac Glycosides	+	-
Anthranol Glycosides	-	-
Cyanogenic Glycosides	-	-
Saponins	++	-
Diterpenes	++	+
Triterpenes	-	-
Phenols	++	+
Tannins	+	+
Flavonoids	+	+

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

Basella alba leaves were collected from the Agricultural Systems Cluster, College of Agriculture, University of the Philippines Los Baños. The plant samples were identified and authenticated by specialists from the Food Science Cluster, College of Agriculture, University of the Philippines Los Baños. Two sample sets of *Basella alba* leaves (1.065 kg and 1.350 kg) were washed separately and they were dried in a cabinet dryer at 55°C for 10 hours and at 40°C for 48 hours, respectively. The dried leaves were ground using an osterizer and stored in airtight containers. The latter was used for phytochemical screening and total phenolics and flavonoid content determination while the former was used for proximate analysis.

Solvent Extraction

Ethanol and water were the solvents used for extraction. Dried sample (50 g) was mixed with 150 mL ethanol, placed in a shaker for two hours and then filtered. The process was done three times and the collected extracts were pooled. The extract was concentrated by evaporating ethanol under reduced pressure at 50°C using a rotary evaporator. The semisolid residue was then dissolved with 100 mL ethanol and stored in an amber bottle at refrigeration temperature. Water extraction was done by mixing 50 g of dried sample with boiling distilled water. It was allowed to stand for 15 minutes, cooled and then filtered. The extract was also stored in an amber bottle at refrigeration temperature prior to analyses.

Proximate (Nutritional) Analysis

The standard procedures and protocols for the content determination of ash (minerals), crude fat, crude fiber, crude protein, and total carbohydrates were adapted from

the "Association of Official Analytical Chemists" (AOAC) Official Methods of Analysis (1995)⁶.

Phytochemical Screening

Qualitative phytochemical screening was done by using sets of reagents and mixtures for the detection of specific phytochemical groups present in *Basella alba* aqueous and ethanolic extracts^{7,8}.

Total Phenolic Content Determination

Folin-Ciocalteu Assay was employed in the determination of the total phenolic content of crude ethanol and aqueous extracts⁹. The extracts (0.1 mL) were mixed with 0.25 mL of Folin-Ciocalteu Reagent (FCR) and 1.5 mL of 10% Na₂CO₃ and then the resulting solutions were left to stand for 15 minutes at room temperature. Afterwards, they were diluted to 5 mL and the absorbance was read immediately at 725 nm. Calibration 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 also prepared employing the same conditions and reagents for the preparation of the standard and sample solutions.

RESULTS AND DISCUSSION

The nutritional composition of the dried *Basella alba* leaves (Table 1) shows relatively high amounts of ash (minerals) because on a moisture-free basis, it may be less than 2 % or greater than 10 %¹⁰. As compared to other vegetables, *Basella alba* leaves has higher crude protein. This justifies its use as a source of protein for the alleviation of "Protein Energy Malnutrition". When combined satisfactorily with other foods through dietary supplementation with proteins from cereals and legumes, it can be of high biological value and meet the protein needs of malnourished children and adults. Its low level of crude fat makes it helpful to individuals suffering from overweight and obesity. The leaves also have appreciable amount of crude fiber and sugars. High crude fiber content is quite beneficial as it helps in the enhancement of gastrointestinal function, prevention of constipation because of their ability to absorb water, and reduction of the incidence of metabolic diseases¹¹.

Phytochemical screening involves color changes in knowing the presence of certain phytochemicals. Table 2 shows the results of the qualitative phytochemical screening done on the ethanol and aqueous extracts of *Basella alba* leaves. Saponins, diterpenes, phenols, tannins, and flavonoids were shown to be present in both ethanol and aqueous extracts but are generally present in higher amounts in the ethanol extract. However, alkaloids were only present in the aqueous extract while cardiac glycosides were only present in the ethanol extract. These indicate that more of the active components of the leaves are more soluble in ethanol. Saponins are hydrophilic and are readily soluble in water and ethanol. Diterpenes are made of unsaturated hydrocarbons, making them more soluble in less polar ethanol solvent. Phenols are less soluble in water because the existing strong hydrogen bonds between or among water molecules are difficult to break. The structure of phenols having a bulky phenyl ring also contributes to its less solubility with water. The hydroxyl groups present in tannins and flavonoids are the

Table 3: Total phenolic content of *Basella alba* leaves extracts.

Chemical Test	Ethanol Extract	Aqueous Extract
Phenolic equivalents (mg GAE/g extract)	93.89 ± 2.97	85.13 ± 4.96
Flavonoid equivalents (mg QE/g extract)	100.18 ± 3.20	90.80 ± 5.35

ones responsible for their detection in both ethanol and aqueous extracts⁸. The presence of cardiac glycosides in the ethanol extract can be undesirable because of their potential toxicity. Cardiac glycosides that have long half-lives accumulate in the body which further increases the danger of toxicity. However, those that are detoxified immediately by the body are of mild potency¹². Wide consumption of *Basella alba* in Nigeria and India is an indicator that *Basella alba* do not possess serious toxicity.

Folin-Ciocalteu colorimetric method was used in determining the total phenolic content of *Basella alba* leaves. Standard curves of solutions with known concentrations of gallic acid and quercetin were used to estimate the concentrations of phenolics and flavonoid components of the sample extracts, respectively.

Knowing the concentrations of the sample extracts with the use of the standard curves, total phenolic content in terms of gallic acid equivalents (GAE) and quercetin equivalents (QE) were calculated as shown in Table 3. The ethanol extracts contained higher amounts of both the phenolics and flavonoid components as compared to the water extracts because of their higher solubility in ethanol. Phenolic substances are responsible for the antioxidant activity of plant materials which is gaining much interest these days because of their known health benefits.

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