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Phytochemical Content and Antioxidant Activity of Boiled and Fresh Ayo *(Tetrastigma harmandii* Planch.*)* Fruits and Leaves

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Abstract *Background and purpose.* One of the underutilized indigenous plants in the Philippines is Ayo, a vine whose fruits and leaves are used by locals as a souring ingredient in native dishes. The study determined the effect of boiling on the phenolic, flavonoid, tannin, and anthocyanidin content of Ayo fruits and leaves. *Method.* The abilities of the samples to act as antioxidants were evaluated by radical scavenging activity assay. *Results*. Boiling significantly decreased ($p < 0.05$) the phytochemicals and antioxidant activity of the samples, except for the anthocyanidin in the fruits ($p > 0.05$). Generally, fruit samples retained higher levels of phytochemicals during cooking than did leaf samples. The changes in the phytochemical content and antioxidant activity of boiled samples might be due to leaching out of the bioactive compounds from their matrix into the boiling water. *Conclusion*. The study revealed that Ayo could be a good source of phytochemicals, and it exhibited a high radical scavenging activity. Greater antioxidants could be obtained by eating the fruits fresh and raw. When boiling is unavoidable as in the case of the leaves, less water and less cooking time are recommended to retain the optimum benefits of the bioactive compounds present in the plant.

Keywords: Ayo, phenols, tannins, flavonoids, antioxidant activity

INTRODUCTION

The importance of daily and adequate fruit and vegetable consumption in the maintenance of health, and the prevention of non-communicable diseases and malnutrition has been extensively researched, documented, and advocated. Low intake of these crops' accounts for approximately 1.7 million (2.8%) deaths worldwide, and it is estimated to cause around 14% of gastrointestinal cancer deaths, 11% of ischemic heart disease deaths, and 9% of stroke deaths. (1). The report of the joint WHO and FAO expert consultation on diet, nutrition, and the prevention of chronic diseases that took place in 2003 recommends a minimum of 400 g or 5 servings of fruits and vegetables per day. Eating a variety of fruits and vegetables regularly as part of a well-balanced diet provides enough of most micronutrients, dietary fibers, and essential non-nutrient substances needed by the body, thereby encouraging weight management, and reducing morbidity and the risk of mortality (2).

Despite the known health benefits of fruits and vegetable, numerous interventions and dietary guidelines promoting consumption, many populations do not meet the recommended amount. The annual average consumption of vegetables globally is 102 kg per capita, with the highest level in Asia and the lowest in Africa and South America (3). In the Philippines, it is only about 111 g per capita per day or 40 kg per capita per year, far below the requirement set by the WHO (4). Factors that contribute to this trend include cultural issues, impediments in the supply chain, urbanization resulting in inaccessibility to nutritious meals, low socio-economic status, and high costs of produce. Households that lack resources have limited dietary choices since they cannot afford the more popular vegetables in the market (3).

The Food and Agriculture Organization recognizes the urgent need to raise public awareness on the role of underutilized indigenous food resources in countering food and nutrition insecurity. There is also a pressing demand to diversify the food base as a response to climate uncertainty. Underutilized crops are tolerant to drought and natural hazards, and resistant to pests and diseases. They address cultural needs, while being less damaging to the environment. Unbeknownst to many, their nutrient content is much higher than the more popular species that are commonly produced and consumed. Thus, they can be tapped to provide palatable and nutritious foods and increase fruit and vegetable consumption at a minimal cost and effort (5).

Ayo (*Tetrastigma harmandii* Planch.) is an underutilized plant that thrives at low to medium elevations in several provinces of the Philippines. It is a slender vine with branchless, coiled tendrils that is harvested from the wild and used locally as a source of food, medicine, and fiber. Its russet-brown, globose fruits grow in clusters and contain a juicy, sour flesh that may be consumed raw or made into preserves. The leaves are likewise sour and can be utilized as a

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condiment to add flavor to dishes. Both fruits and leaves are typically eaten by the natives with fish (6). Ayo is indigenous in the Philippines, but there is limited data about this plant and there is no literature reporting its nutrient and phytochemical content, and antioxidant capacity. It was therefore imperative to undertake research that would raise awareness and expand the knowledge about this plant. The findings of the study will be useful to future researchers who will be interested in the potential impacts of Ayo on nutrition, and in the product development of Ayo as a jam, jelly, or commercial souring ingredient. Finally, the study would contribute to the documentation of the characteristics of underutilized, indigenous crops and their significant roles in preserving cultures, developing, and sustaining local food systems, and protecting human well-being and health. This study was intended to determine the effect of boiling on the phytochemical content and the antioxidant capacity of Ayo (*Tetrastigma harmandii*) fruits and leaves.

MATERIALS AND METHODS

Sample preparation

Ripe fruits and young shoots of Ayo were collected from Brgy. Culing West, Cabatuan, Isabela. They were transported to the Bio-Assay Laboratory of the Institute of Human Nutrition and Food at the University of the Philippines-Los Baños, where the study would be conducted. The fruits and the leaves were initially cleaned by washing them thoroughly under running water. The rind of the fruits was peeled off. Using a digital weighing scale, two 200 g fruits and two 200 g leaves were prepared before cooking. A portion of the leaves and the fruits were heated to boiling point in a stock pot for three minutes. The fresh and boiled fruits and leaves were placed in a moisture dish, then oven-dried at 45 ˚C overnight. The dried materials were pulverized into coarse powder using a grinder. The dried, powdered samples were transferred into a plastic vial and stored in a desiccator. An extract was obtained by weighing 50 mg of dried, powdered sample and diluting it with absolute methanol to make a 50 ml solution. The solution was swirled for 10 minutes using a vortex mixer, then percolated through a filter paper. The filtrate was decanted into a test tube, and the residue was discarded.

Total phenolic content

The total phenolic content of the samples was determined according to the Folin-Ciocalteu method(7). A 1 ml aliquot was mixed with 1 ml of freshly prepared Folin-Ciocalteu's phenol reagent. After five minutes, 10 ml of 7% sodium carbonate (Na_2CO_3) solution was added, followed by 13 ml of distilled water. The mixture was shaken thoroughly, then kept in the dark for 90 minutes at 23 ˚C. A set of standard solutions of gallic acid (100, 200, 300, 400, 500 µg/ml) was prepared and analyzed in the same manner as the samples. The absorbance was measured at 760 nm against a blank using a UV-spectrophotometer. The results were expressed as mg gallic acid equivalents (GAE) per g of dried sample.

Flavonoid content

The total flavonoid content of the samples was estimated using the aluminium chloride method (7). A 1 ml aliquot was mixed with 4 ml of distilled water, and subsequently added with 0.30 ml of 10% sodium nitrite ($NaNO_2$). After five minutes, the mixture was added with 0.30 ml of 10% aluminium chloride ($AlCl_3$) solution and 2 ml of 1% sodium hydroxide (NaOH) solution, then it was thoroughly mixed. A set of standard solutions of quercetin (25, 50, 75, 100, 125, 150 µg/ml) was prepared and analyzed using the same procedure described initially. The absorbance was measured at 510 nm against a blank using a UV-spectrophotometer. The results were expressed as mg quercetin equivalents per g of dried sample.

Total tannin content

The total tannin content of the samples was assessed by performing the Folin-Ciocalteu assay (7). A 1 ml aliquot was transferred to a 10 ml volumetric flask, then added with 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu's phenol reagent, and 1 ml of 35% sodium carbonate solution. It was diluted to 10 ml with distilled water and mixed thoroughly. The mixture was kept at room temperature for 30 minutes. A set of standard solutions of catechin (20, 40, 60, 80, 100 µg/ml) was prepared and analyzed in the same manner as the samples. The absorbance was measured at 700 nm against a blank using a UV-spectrophotometer. The results were expressed as mg catechin equivalents per g of dried sample.

Total anthocyanidin content

The total anthocyanidin content of the samples was determined using the vanillin assay (7). A 1 ml aliquot was mixed with 2.5 ml of 1% vanillin and 2.5 ml of 9 M hydrochloric acid (HCl). It was incubated for 30 minutes at 30 ˚C. A set of standard solutions of catechin (100, 150, 200, 250, 300, 350 µg/ml) was prepared and analyzed following the same procedure described initially. The absorbance was measured at 500 nm against a blank using a UV-spectrophotometer. The results were expressed in mg catechin equivalents per g of dried sample.

Antioxidant activity

The antioxidant activity of the samples was evaluated by performing the 1,-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (8). A 1 ml aliquot was diluted with 4 ml of distilled water, then mixed with 1 ml of methanolic solution of DPPH. The mixture was incubated in the dark for 20 minutes, after which the absorbance was measured against a blank at 517 nm. The ability of the samples to scavenge the DPPH radical was calculated using the formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{Absorbance\ of\ control\ -\ Absorbance\ of\ test\ sample\ x\ 100}{Absorbance\ of\ control}$$

Statistical analysis

All functional analyses were carried out in triplicates (n=3). The results were presented as mean ±

standard deviation of three independent observations. Paired sample t-tests were performed to determine whether the differences in the averages were significant. Significance levels were defined at $p < 0.05$.

RESULTS

Total phenolic content

The total phenolic contents of fresh and boiled Ayo were presented in Figure 1. Among the phytochemicals analyzed, phenols had the greatest concentration in all the samples. The phenolic content of the samples ranged from 423.3 mg GAE/g to 551.1 mg GAE/g. The fresh fruits had the highest phenolic content, while the boiled leaves had the lowest phenolic content. After boiling, the phenolic contents of the leaves and the fruits decreased by 23% and 21%, respectively. A two-sample t-test revealed a significant difference ($p < 0.05$) in the phenolic contents of the fresh samples and the boiled samples.

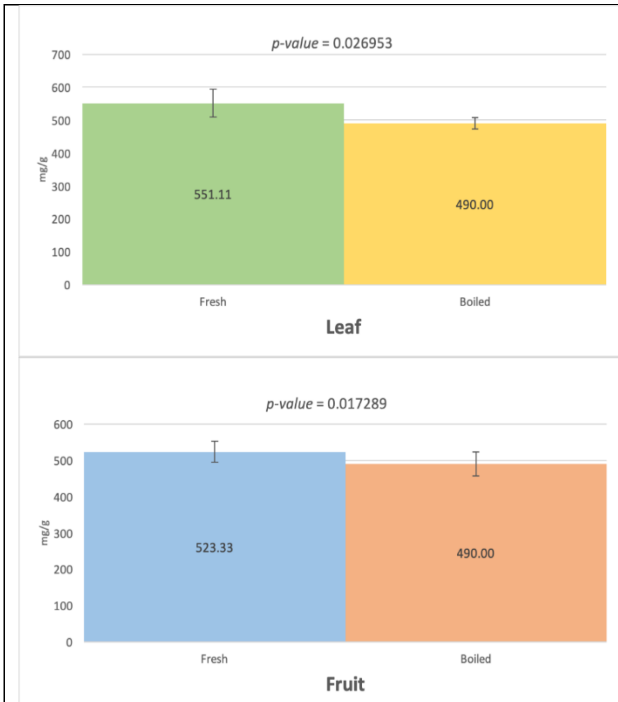

Figure 1. Total phenolic contents of fresh and boiled Ayo

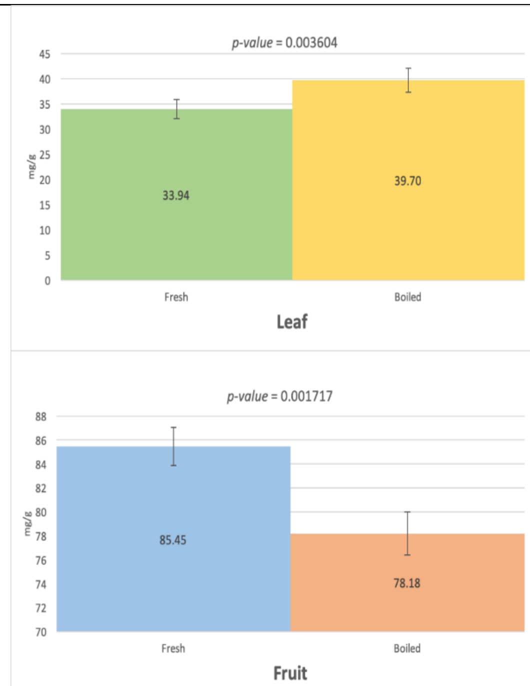

Figure 2. Total tannin contents of fresh and boiled Ayo

Total tannin content

As shown in Figure 2, the tannin concentration of the different samples ranged from 9.09 mg CE/g to 101.82 mg CE/g. Levels of tannins were found to be highest in the fresh fruits and lowest in the boiled leaves. Boiling for three minutes significantly ($p < 0.05$) decreased the tannin contents of the leaves and the fruits by 73% and 23%, respectively.

Flavonoid content

The flavonoid contents of the fruits and leaves before and after cooking were summarized in Figure 3. The flavonoid concentration of the samples varied from 2.17 QE mg/g to 19.90 QE mg/g. The fresh fruits possessed the highest flavonoid levels. On the other hand, the boiled leaves contained the lowest flavonoid levels. The flavonoid concentration of the leaves and the fruits significantly decreased ($p < 0.05$) after boiling.

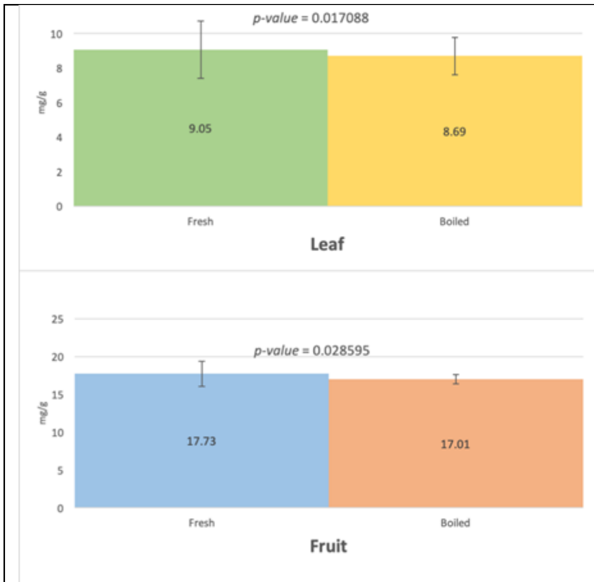

Figure 3. Total flavonoid contents of fresh and boiled Ayo

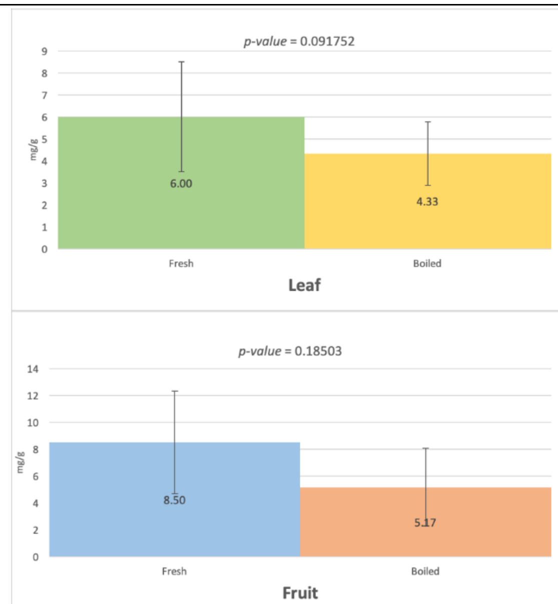

Figure 4. Total anthocyanidin contents of fresh and boiled Ayo

Total anthocyanidin content
Anthocyanidin had the lowest concentration in all the samples. The results presented in Figure 4 suggest that the highest anthocyanidin content was observed in the fresh fruits with 9.33 ± 3.82 mg CE/g dry weight, while the lowest was observed in the boiled leaves with 2.67 ± 1.44 mg CE/g dry weight. After the application of heat, the anthocyanidin content of the leaves and the fruits were reduced by 55% and 44%, respectively. There was a significant difference ($p < 0.05$) in the anthocyanidin content of the leaves before and after cooking, whereas there was no significant difference ($p > 0.05$) in the anthocyanidin content of the fresh and the boiled fruits.

Antioxidant activity
The antioxidant activity of the samples was shown in Figure 5. The greatest radical scavenging activity (94.24 ± 0.08 %) was demonstrated by the fresh fruits, while the least radical scavenging activity (92.89 ± 0.03 %) was exhibited by the boiled leaves. There was a significant difference ($p < 0.05$) in the antioxidant activity of the fresh and boiled samples. The fresh samples had a greater radical scavenging activity compared to the boiled samples. The antioxidant activity may be classified as high (>70%), intermediate (40-70%) or low (<40%) levels of inhibition (9). There was a significant difference in the radical scavenging activity between the fresh leaves and the fresh fruits, and between the boiled leaves and the boiled fruits. Nevertheless, the antioxidant capacities of the four samples were classified as high.

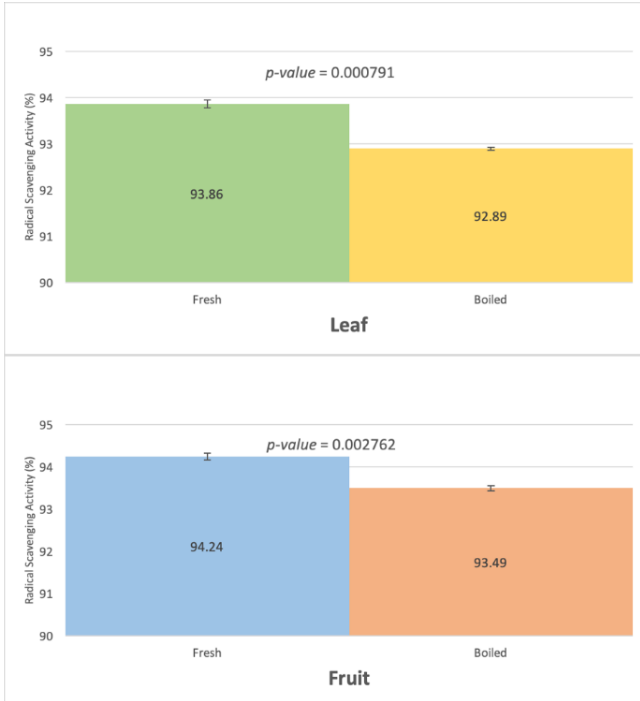

Figure 5. Antioxidant activity of fresh and boiled Ayo

DISCUSSION

Polyphenols are a large group of secondary metabolites present in significant amounts and widely distributed in the plant kingdom, with more than 8,000 structures found in different species. They include simple phenols, flavonoids, tannins, and anthocyanidins, which were the phytochemicals involved in this study. They are synthesized through the phenylpropanoid and polyketide pathways in response to physiological stimuli, stress, ultraviolet radiation, or aggression by pathogens (10). The antioxidant properties of polyphenolic compounds are attributed to the inherent propensity of their hydroxyl groups to dismutate radicals by donating their hydrogen atoms (11). The free radical scavenging activity of the whole molecule is defined by the number of hydroxyl groups it contains (12). Polyphenols are highly reactive and good substrates for different enzymes including glycosidases, esterases, peroxidases, and polyphenoloxidases. They can undergo degradation and polymerization through enzymatic and non-enzymatic reactions during post-harvest storage or food processing, which may result in structural changes and a decrease in their biological activities. The behavior of polyphenols after processing is influenced by several factors such as concentration, type of heat treatment applied, location inside the cell, and interactions with other components of the food (13). The difference in the polyphenolic contents of the fresh and boiled samples in this study could be explained by the fact that polyphenols are polar compounds and leaching occurred during boiling. Polyphenols are also thermo-liable, which means that the greater the amount of water used in cooking, the greater the loss of these compounds (14). Cooking the samples prompted the phenolic compounds to move from uniform distribution in the vacuoles and localize around the cell walls. It also caused the cell-wall components to soften and rupture, which enabled the water-soluble phenolic compounds to be released from the matrix and be dissolved in water (13). According to research on almond skin, the polyphenols decreased during the blanching process, while these compounds increased in the water (15).

Many researches positively correlated the radical scavenging effect with the total amount of phenolic compounds (11); (16); (17). Hence, the decline in antioxidant activity of the boiled samples in this study was a consequence of the leaching process, and structural changes or thermal degradation of phenols, flavonoids, tannins, and anthocyanidins (12). The greater reduction of antioxidant activity in boiled leaves than in boiled fruits could also be due to the larger surface area of the leaves in contact with the water (18). In a study reported, radical scavenging activity of colored peppers considerably decreased ($p < 0.05$) to below 77% of its initial level after boiling for five minutes. It was further reduced to 64% when the boiling time was prolonged to 30 minutes (19). The cooking water was also analyzed, and it was found that there was no significant difference ($p > 0.05$) between the RSA of the raw pepper samples, and the sum of the RSA values in cooked tissues and cooking water after five minutes of boiling. The researchers noted that this was an indication that the antioxidant compounds could have leached into the boiling water.

CONCLUSION

The study determined the effect of boiling on the phenolic, flavonoid, tannin, and anthocyanidin content, and antioxidant activity of Ayo fruits and leaves. The highest concentration for each of the four phytochemicals was consistently observed in the fresh fruits, whereas the lowest amount was invariably noted in the boiled leaves. There was a significant loss ($p < 0.05$) of phytochemical content in the samples after boiling, except for the anthocyanidin in fruits. The antioxidant activity also significantly decreased ($p < 0.05$) in the boiled samples which might be a result of the leaching of the thermo-liable and water-soluble bioactive compounds into the boiling water. In conclusion, maximum antioxidants could be obtained by eating fresh Ayo fruits. However, when boiling is unavoidable as in the case of the leaves, less water and less cooking time are suggested to retain the optimum benefits of the bioactive compounds present in the plant. The water used for boiling could also be consumed in addition to Ayo because soluble bioactive compounds would be present in the boiling water. This study provided some basis for the consumption of Ayo and its possible uses as a functional food or ingredient. Nevertheless, more studies on its proximate composition and vitamin content should be done to obtain comprehensive nutritional properties of this plant.

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