Phytochemical Profiles and Antioxidant Activity of Selected Indigenous Vegetables in Northern Mindanao, Philippines

Renee P. Baang, Romeo M. Del Rosario, Nenita D. Palmes

***Abstract*—**The crude methanol extracts of five indigenous vegetables namely, *Amarathus tricolor, Basella rubra* L., *Chochurus olitorius* L., *Ipomea batatas,* and *Momordica chuchinensis* L., were examined for their phytochemical profile and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The values for DPPH radical scavenging activity ranged from 7.6-89.53% with *B. rubra* and *I. batatas* having the lowest and highest values, respectively. The total flavonoid content of all five indigenous vegetables ranged from 74.65-277.3 mg quercetin equivalent per gram of dried vegetable material while the total phenolic content ranged from 1.93-6.15 mg gallic acid equivalent per gram dried material. Phytochemical screening revealed the presence of steroids, flavonoids, saponins, tannins, carbohydrates and reducing sugars, which may also be associated with the antioxidant activity shown by these indigenous vegetables.

***Keywords*—**Antioxidant, DPPH radical scavenging activity, Philippine indigenous vegetables, phytochemical screening.

I. Introduction

INDIGENOUS vegetables are a potential source of micronutrients and phytochemicals, including antioxidants [1]. Antioxidants have been studied to inhibit oxidative processes in human body and food products. Antioxidant compounds (phytochemicals) such as phenolics may help safeguard cellular systems from oxidative damage. Phytochemicals are biologically active secondary metabolites present in plants in smaller quantities that include alkaloids, flavonoids, steroids, tannins and many others. The awareness of the importance of indigenous vegetables not yet identified and characterized in the locality, can lead to a deeper interest in finding out how to propagate them and discover their potential as phytochemicals. Knowledge of the antioxidant activity of indigenous vegetables can help identify materials with good potential.

Recent research on indigenous vegetables has identified and preserved a wide range of germplasm within the Philippines, and assessed consumer attitudes to its consumption [2]. Indigenous vegetables are now recognized due to their vital role in alleviating 'hidden hunger' -i.e., the lack of minerals, vitamins, and micronutrient in the diet. A botanical characterization of Northern Philippines' indigenous vegetables documented 49 species of plants belonging to 23 families, which are eaten as food, utilizing mostly the young shoots or tops [3]. However, it was noted that indigenous wild plants of the area will be "lost unless work in raising awareness of these species is taken up and their nutritional and medicinal benefits are truly valued not just by isolated communities but all across the northern Philippines" [4].

Crude plants extracts could be assayed and the positive fractions then evaluated for phytochemicals [5]. The DPPH radical scavenging assay is popular in natural antioxidant studies because it is simple and highly sensitive. This procedure operates on the concept that an antioxidant is a hydrogen donor. The antioxidant effect is proportionate to the disappearance of $DPPH^{-}$ organic nitrogen radical) in the samples [6]. Numerous studies on antioxidants present in fruits and vegetables, cereals and beans, spices and herbs have been conducted using the DPPH assay. More efforts have been exerted in investigating natural resources as possible source of active antioxidants compounds [7]. However, scientific information to support the antioxidant properties of Philippine indigenous vegetables is largely unexplored.

Five indigenous vegetables of interest to the authors include *Amarathus tricolor, Basella rubra* L., *Chochurus olitor* L., *Ipomea batatas* and *Momordica chuchinensis* L.

Alugbati (*Basella rubra* L.) is a succulent, branched, smooth, twining, herbaceous vine. The stems are green or purplish. The leaves are somewhat fleshy, ovate or heart-shaped. It is widely cultivated in all regions of the country particularly in Mindanao. Alugbati is commonly grown for its young shoots which make a good mucilaginous vegetable, used in stews or soup; or sometimes as a green salad. Its fruits seem to have been earlier used for dyeing purposes in China. The red fruit juice can be used as ink, cosmetic and for coloring foods. The young leaves can be used as laxative and the pulped leaves to poultice sores. Shoots of alugbati per 100 grams (g) edible portion contain water (91 g), protein (2.1 g), fat (0.3 g) carbohydrates (3.9 g) and fiber (1.3 g). The energy value is approximately 112 kJ/100 g. The protein content is relatively low compared to other greens. The vitamin and mineral contents vary widely as follows: vitamin A, 1686-6390 IU; vitamin C, 29-166 mg; calcium (Ca), 16-117 mg; and iron (Fe), 1.2-3.1 milligrams per 100 gram edible portion [8].

R.P. Baang is currently taking up Ph.D. in Science Education major in Chemistry at the Mindanao University of Science and Technology, Cagayan de Oro City, Philippines (e-mail: reneeapb@gmail.com).

R. M. del Rosario is the Head, Center for Research in Advanced Science at the Mindanao University of Science and Technology, Cagayan de Oro City, Philippines (e-mail: rmdelr@yahoo.com).

N.D. Palmes is the VP–Research, Extension, Development and Educational Advancement Services at the Mindanao University of Science and Technology, Cagayan de Oro City, Philippines.

Kulitis (*Amaranthus tricolor* L.) is an annual leafy vegetable belonging to the Amaranthaceae or amaranth family the plant is annual, erect, strongly branching up to 2.5 cm tall, and with branched taproot. The leaves are alternate, with long petioles, simple and entire. Flowers are borne in axillary clusters; upper clusters are often leafless. A spiny relative of kulitis called uray (*A. spinosus* L) is a common vegetable in some regions in the Philippines. Though spiny, it makes excellent greens when used in the same way as spinach. Amaranthus weeds are used as fodder (pigweed). Vegetable amaranths have medicinal properties good for young children, lactating mothers and patients with fever, hemorrhage, anemia or kidney problems.

Ampalayang ligaw (*Momordica chuchinensis* L.) is a monoecious, annual vine having a five-ridged stem and simple tendrils. The leaves are simple, pellucidly dotted, palmately veined; petiole is 1-7 cm long; leaf-blade broadly ovate-reniform or subobicular in outline leaves obovate and sinuate-lobulate or sinuate-toothed. Fruits, young shoot and flowers are used as flavoring; the leaves as leafy vegetable; and the pulpy arils as sweets. Ampalayang ligaw may be canned, pickled or dehydrated. To reduce the bitter taste, the fruits can be blanched or soaked in saltwater before cooking.

Ampalayang ligaw has been recommended by the Department of Health (DOH) as one of the best supplements to prevent liver problems and diabetes. It is also used to treat skin diseases, as a parasiticide, antipyretic and purgative. The edible portion of ampalayang ligaw fruits is about 95%. A 100 g edible fruit contains (83-92 g), protein (1.5-2 g), carbohydrates (4-10.5 g), and fiber (0.8-1.7 g). The energy value is 105-250 kJ/100 g. compared with other cucurbits, it is high in minerals and vitamins including Calcium (20-23 mg), Iron (1.8-2 mg), Phosphorous (38-70 mg), and Vitamin C (88-96 mg). Every 100 g of edible leaves contain water (82-86 g), protein (2.3 g), fat (0.1 g), carbohydrates (17 g), and fiber (0.8 g) [9].

Kamote (*Ipomea batatas*) is a spreading, prostrate, herbaceous, smooth or somewhat hairy vine. Stems or runners, sprawling several meters long, take root when in contact with soil. Leaves are ovate to oblong-ovate, 6 to 14 centimeters long, somewhat entire, with alternate heart-shaped or palmately lobed leaves, pointed at the tip, and heart-shaped at the base. Leaves and roots are edible [10].

According to the index of nutritional quality, sweet potato leaves are good sources of protein, fiber, and minerals, especially K, P, Ca, Mg, Fe, Mn, and Cu. The correlation coefficient between antioxidant activity and total polyphenol content was the highest (0.76032, $p<0.0001$), indicating that polyphenols are important antioxidants in sweet potato leaves. Sweet potato leaves, which contain several nutrients and bioactive compounds, should be consumed as leafy vegetables to reduce malnutrition, especially in developing countries [11].

Saluyot (*Corchurus olitorious* L.) is an erect, glabrous, annual plant or shrub, growing up to 2 meters high. The leaves are broad-ovate, lanceolate, toothed margins. Jute or saluyot is often grown near riverbanks. It is famous for its sturdy, natural fiber but there are cultivars that are grown as a leafy vegetable. The young leaves are used fresh or dried. They can be stored after drying and used during periods of scarcity. The leaves become slimy when cooked, a trait of this crop which is highly appreciated. Saluyot per 100 g of edible portion, contains water (83 g), protein (6.5 g), fat (1.0 g) carbohydrate (7.5 g), fiber (2.0 g), ash (2.0 g), Ca (488 g), P (114 mg), Fe (11.6 mg), beta carotene (7325 mg), vitamin A (1221 mg), thiamin (0.15 mg), riboflavin (0.28 mg), niacin (1.5 mg), and vitamin C (95 mg). The energy value is 65 kcal/100 grams [12].

II. Materials and Methods

A. Chemicals and Reagents

All chemicals and reagents used (various brands) were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was purchased from Sigma-Aldrich.

B. Identification of Indigenous Vegetable Species

A sampling of popular indigenous vegetable in the localities of Northern Mindanao, Philippines was carried out through a survey. The identification of vegetable species was based on identification methods using plant leaf characteristics which include the leaf type, leaf margin, leaf shape, leaf attachment and leaf arrangement [13]. The scientific names of the vegetable species were identified from the library of botanical plants in the Philippines and Co's digital flora and fauna.

C. Sample Preparation and Phytochemical Screening

Fresh leaf and young stalk samples of five selected indigenous vegetables were obtained from small farms and markets in Northern Mindanao like Cogon Market in Cagayan de Oro City, backyard farm in Baungon, Bukidnon, Balingasag, Misamis Oriental and Iligan City, Philippines. The leaves and stalks were sorted, air-dried and milled, placed in air-tight bottles and stored under controlled temperatures. At the point of analysis, a 1:1:1:1 (mass/mass) homogenous mixture of the milled leaves and stalks was prepared by homogenizing 20g of each together. Phytochemical analysis was done using common standard methods to determine the following: alkaloids, anthraquinones, carbohydrates, glycosides, reducing sugar, saponins, steroids, and tannins.

D. Extract Preparation

Twenty (20) grams of pounded vegetable materials were soaked with 300 mL of methanol for 12 hours with occasional shaking. This mixture was filtered and the filtrate set aside. The residue was soaked with another 300 mL of methanol for one hour and the filtrate collected. The residue was soaked again with 300 mL methanol, and filtered. The filtrates were then combined and concentrated to about 100 mL by using the rotary evaporator. The crude extract concentrate was subjected to further analysis of solid content via oven. Then based on the resulting extract concentration, a solution of 1 mg extract per mL was prepared for the subsequent tests.

E. Total Phenolics

The total phenolics was determined by spectrophotometric analysis using Folin Ciocalteu method and expressed in terms of gallic acid equivalent (GAE) adopted from the parallel study conducted by [14].

F. Total Flavonoids

The total flavonoids analysis was calculated from a quercetin calibration curve expressed in terms of mg quercetin per gram dried material adopted with slight modification from the protocol used by [15].

G. DPPH Radical Scavenging Activity

The free radical scavenging capacity of the extracts was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH solution (0.006% w/v) was prepared in 95% methanol. The methanol extract of the leaves and stalks from the five vegetables were mixed with 95% methanol to prepare the stock solution (1 mg/mL). Freshly prepared DPPH solution was placed in test tubes and extracts were added by serial dilutions (100-1000 µg) to every test tube so that the final volume is 2 mL, and discoloration were measured at 517 nm after incubation for 30 minutes in the dark (Themo UV1 spectrophotometer). Measurements were recorded in three trials. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (1 mg/mL). The control sample was prepared containing the same volume without any extract and 95% methanol. This was used as the blank. The percentage scavenged DPPH free radical was calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0-A_1)/ A_0] \times 100$$

where, A_0 and A_1 is the absorbance of the control and test sample, respectively.

H. Statistical Analysis

Experimental results were reported as mean ± standard deviation of triplicate measurements. The test for significant relationship was evaluated using Pearson-product moment correlation.

TABLE I
RESULTS OF PHYTOCHEMICAL SCREENING

Phytochemicals	*A. tricolor*	*B. rubra*	*C. olitorius*.	*I. batatas*	*M. chuchinensis*
Alkaloids	+	+	+	+	+
Anthraquinones	-	-	-	-	-
Carbohydrates	+	+	+	+	+
Glycosides	-	-	-	-	-
Reducing sugars	+	+	+	+	-
Saponins	+	+	+	+	+
Steroids	+	+	+	+	+
Tannins	+	+	+	+	+

+ = Present; - = Absent

III. RESULTS

A. Phytochemical Screening

Phytochemical screening on the crude methanol extracts of five indigenous vegetables namely: *A. tricolor, B. rubra, C. olitorius, I. batatas, M. chuchinensis* was carried out using standard method. Results (Table I) revealed the presence of secondary metabolites such as carbohydrates, tannins, saponins, alkaloids and steroids. Reducing sugars were present in all extracts except *M. charantia* while anthraquinones and glycosides were absent from all crude extracts of the vegetable samples.

B. Total Phenolics

Results (Table II) revealed that *C. olitorius* had the highest total phenolic content with an equivalent value of 6.15±0.5 mg gallic acid equivalent (GAE) per gram of dried material, followed by M. *charantia* (2.78±0.1 GAE mg per gram), *A. tricolor* (2.75±0.2 GAE mg per gram), *B. rubra* (2.19±0.1 GAE mg per gram), and *I. batatas* (1.93±0.1 GAE mg per gram).

C. Total Flavonoids

Results (Table II) indicated that *I. batatas* had the highest total flavonoids with an equivalent value of 277.30±11 mg uercetin per gram dried material. *A. tricolor* ranked second, *B. rubra* ranked third, *C. olitorius* was fourth and *M. charantia* ranked fifth with total flavonoid contents of 147.51±7, 123.91±5, 86.92±2 and 74.65±4 mg quercetin per gram dried material, respectively.

D. DPPH radical Scavenging Activity

The results of DPPH free radical scavenging activity on the five crude methanol extracts are shown on Table III. The highest radical scavenging activity (EC_{50}, 1000 µg per mL) was shown by I. *batata*s (89.53%) followed by *C. olitorius* (63.76%), *M. charantia* (20.33%), *A. tricolor* (17.32%), and *B. rubra* (7.6%).

TABLE II
TOTAL PHENOLIC AND FLAVONOID CONTENT IN FIVE INDIGENOUS VEGETABLES CRUDE METHANOL EXTRACTS

Botanical Name	Family	Plant part used	Total phenolics content* (in GAE mg per gram)	Total flavonoids content (in quercetin mg per gram)
Amarathus tricolor	Amaranth	Leaves and stalks	2.75±0.2	147.51±7.0
Basella rubra L.	Basellaceae	Leaves and stalks	2.19±0.1	123.91±5.0
Chochurus olitorius L.	Tiliaceae	Leaves and stalks	6.15±0.5	86.92±2.0
Ipomea batatas	Convolulaceae	Leaves and stalks	1.93±0.1	277.3±11.0
Momordica chuchinensis L.	Cucurbitaceae	Leaves and stalks	2.78±0.1	74.65±4.0

*Data are expressed as the mean of triplicate ±SD.

TABLE III
DPPH FREE RADICAL SCAVENGING ACTIVITY IN FIVE INDIGENOUS VEGETABLES CRUDE METHANOL EXTRACTS

BOTANICAL NAME	% Inhibition*
Amarathus tricolor	17.32±3.0
Basella rubra L.	7.60±1.6
Chochurus olitorius L.	63.76±4.1
Ipomea batatas	89.53±0.2
Momordica chuchinensis L.	20.33±0.7

*Data are expressed as the mean of triplicate ±SD.

IV. DISCUSSION

A. Phytochemical Screening

Indigenous vegetables can be considered sources of essential nutrients and chemical substances for good nutrition and disease prevention. In order to promote these plants, it is necessary that their compositions be known, especially in terms of nutrients and phytochemicals with health-promoting effects.

Saponins which are present in all of the crude plant extracts examined are considered as health-benefitting bioactive compounds. Research studies demonstrated the advantageous effects of saponins on cancer, cholesterol levels in the blood, bone health and immune system. The non-sugar component of the molecular structure of saponins has antioxidant activity and found to lower the risk of cancer and heart diseases [16].

Tannins are complex organic, non-nitrogenous plant products, which generally have astringent properties and bitter taste; used medicinally as antidiarrheal, hemostatic, and antihemorroidal compounds. Generally, tannins are antimicrobial and antioxidant agents [17]. At low concentration tannins can inhibit the growth of microorganisms and at higher concentration, act as an antifungal agent through coagulation of microorganism's protoplasm [18]. The presence of tannins in all five samples would justify the therapeutic property of the crude extracts. The above-mentioned results show that the five indigenous vegetables examined may be rich sources of phytochemicals particularly tannins, steroids, saponins, which could be additionally evaluated for biological activities.

B. Total Phenolics

The amount of total phenolics in the indigenous vegetables is ranked in the following order: *C. olitorius* > *M. chuchinensis* > *A. tricolor* > *B. rubra* > *I. batatas*. Phenolic compounds are widespread bioactive compounds generally present in higher plants [19]. Acting as oxidation terminators, phenolic compounds scavenge radicals to form resonance stabilized radicals [20]. In the present study, the total phenolic content was lower compared to total flavonoids content. The lower values could be due to the unknown phenolic compounds that could not be quantified. The Folin-Ciocalteu phenol reagent was utilized to obtain an estimate of the amount of phenolic compounds in the extract. However, as reported by various researchers, the assay has poor specificity not only to polyphenols but to other substance that could be oxidized by the Folin reagent [21], [22]. The low specificity of the assay could explain the relatively weak correlation between total polyphenol contents and the antioxidant activity of I. batatas (r=0.242). A study by Babbar et al. concluded that phenolic compounds alone are not fully responsible for the antioxidant activity of plants. Other constituents such as carotenoids, terpenes, ascorbates, reducing carbohydrates, tocopherols and the synergistic effect between these compounds may contribute to the total antioxidant activity [23].

C. Total Flavonoids

Flavonoids as naturally occurring secondary metabolites in plants are thought to have positive effects on human health. Plants with flavonoids have been reported to have antiviral, antitumor, and antioxidant properties. These compounds are considered as powerful antioxidants because they exhibited inhibitory properties against carcinogenesis in a number of in vivo studies [24]. Flavonoid has been confirmed to effectively scavenge most oxidizing molecule such as singlet oxygen and various free radicals linked in chronic diseases [25]. The total flavonoids content of the vegetables is ranked from highest to lowest: *I. batatas* > *A. tricolor* > *B. rubra* > *C. olitorius* >*M. chuchinensis*. This is in agreement with the previous study on *I. batatas* leaves by Seow-Mun Hue, et al. [26] which reported flavonoids content that ranged from 96 to 263.5 µg/g. From the results, it is suggested that flavonoids may be one major contributor of the antioxidant activity of the crude extracts as the total flavonoids content and radical scavenging activity are significantly correlated, (r=0.629). This confirms the findings of [27] which reported strong positive correlation between total phenolic content and antioxidant activity that seems to be an emerging trend in various plant species.

D. DPPH Radical Scavenging Activity

One way of estimating antioxidant activity is by the use of the stable free radical DPPH [28], [29]. Although antioxidant activities are influenced by many factors; the scavenging effects of plant extracts has gained popularity in natural product research. Results show that the crude methanol extracts show appreciable free radical scavenging activity that ranged from 7.6% to 89.53% with *I. batatas* showing the most promising antiradical activity.

The high scavenging activity of *I. batata*s leaves is in agreement with the study conducted by [30], in which sweet potato ranked first with highest DPPH radical scavenging activity among the commonly consumed vegetables in Taiwan. The antioxidant activity exhibited could be attributed to the presence of different phenolic compounds such as flavonoids and to the synergistic effects of different phytochemicals, which would explain the moderately strong correlation (r=0.629) between total flavonoids content and % DPPH radical scavenging activity.

V. Conclusion

The five indigenous vegetables exhibited antioxidant activity by inhibiting DPPH. In addition, these vegetables, while rather low in total phenolics, are appreciably high in total flavonoids. The results of this study show that these vegetables can be used as accessible sources of natural antioxidant.

The identification of indigenous vegetable food sources rich in phytochemicals and bioactive compounds could provide scientific evidence to increase consumption and promotion of sustainable indigenous vegetable diet in the different localities in Northern Mindanao, Philippines.

A confirmatory study can also be done by testing the antioxidant activities of the vegetables *in vivo*.

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