

## The Effect of Variety and Fertilizer Application on Functional Properties of ‘Mukunuwenna’ (*Alternanthera sessilis*)

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### ABSTRACT

The study investigated the functional properties, such as antioxidant activity, phenolic content and ascorbic acid content of the popular leafy vegetable, *Alternanthera sessilis* (‘Mukunuwenna’). Two varieties such as Piliyandala and Colombo Selection grown under four different fertilizer application types namely, Inorganic fertilizer mixture recommended by the Department of Agriculture (Urea 9 kg/1000 m<sup>2</sup>, MOP 13.5 kg/1000 m<sup>2</sup>, TSP 10 kg/1000 m<sup>2</sup>), Integrated treatment [mixture of inorganic (Urea, MOP and TSP) and organic fertilizer (cattle manure and gliricidia)], Organic fertilizer (cattle manure and gliricidia) and Home-scale cultivation (No fertilizer) were used. Antioxidant activity (AOA) of the methanolic leaf extract was determined by using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) Radical Scavenging Assay. Phenolic content (PC) and ascorbic acid content (AAC; IC<sub>50</sub> value) were

measured by the Folin-Ciocalteu method and 2, 6-Dichlorophenol-indophenol titration method, respectively. A 2-factor factorial experiment was used in the study. Varieties Piliyandala (7.92 mg/mL) and Colombo Selection (9.50 mg/mL) did not differ in their AOA while variety Colombo Selection recorded a higher phenolic content (472.67 mg GAE/100 g) compared to Piliyandala (343.83 mg GAE/100 g). Ascorbic acid contents of Piliyandala and Colombo Selection varieties were 11.48 and 12.44 mg/100 mg, respectively. Home-scale cultivation and organic fertilizer treatment resulted in significantly higher ( $p < 0.05$ ) AOA of 3.364 and 8.034 mg/mL and PC of 410.87 and 404.99 mg GAE/100 g, respectively. Organic fertilizer application significantly increased ( $p < 0.05$ ) the antioxidant activity of variety Piliyandala but not in Colombo Selection. Results indicated that, *A. sessilis* grown with inorganic fertilizer and a mixture of inorganic and organic fertilizer contained significantly lower antioxidant levels and phenolic contents compared to those grown organically and in home-scale without fertilizer application.

**Keywords:** *Alternanthera sessilis*, Antioxidant activity, Ascorbic acid, Fertilizer, Phenolics

### INTRODUCTION

Fruits and vegetables contain natural antioxidants which help to minimize the risks of cardiovascular diseases and cancers (Renaud *et al.*, 1998; Temple, 2000). Currently, research is focused towards identification of natural antioxidants from herbal sources because the synthetic antioxidants such as butylated

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hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are shown to be carcinogenic (Moussa *et al.*, 2011). There are two major categories of antioxidants namely, enzymatic and non – enzymatic. Superoxide dismutase, catalase and glutathione peroxidase, which are produced endogenously, are enzymatic antioxidants. Tocopherols, carotenoids, ascorbic acid, flavonoids and tannins are non-enzymatic antioxidants which are obtained from natural sources (Lee *et al.*, 2004). Vitamins, phenolics and carotenoids are the three major groups of natural antioxidants in fruits and vegetables. Ascorbic acid and phenolics are hydrophilic antioxidants while carotenoids are lipophilic antioxidants (Halliwell, 1996). Polyphenol compounds are contained in edible and non-edible plants and they are reported to have multiple beneficial effects on human health (Kahkonen *et al.*, 1999).

Essential dietary antioxidant compounds are supplied by many leafy vegetables which are naturally high in antioxidant properties (Gunathilake and Ranaweera, 2016). However, management practices such as fertilizer application (e.g. source and rate of fertilizer) directly influence the composition and quality of bioactive compounds in plants (Amujoyegbe *et al.*, 2007). Nitrogen fertilizers at high rates tend to decrease the vitamin C content in many horticultural crops (Seung and Adel, 2000). Fertilizer applications have also affected the antioxidant activity of medicinal plants (Hassan *et al.*, 2005).

Farmers tend to use inorganic fertilizers for leafy vegetables in order to obtain a vigorous and rapid foliage growth. Nevertheless, in the context of consumer safety and environmental pollution, chemical fertilizer usage is not accepted in leafy vegetable cultivation. Externally applied synthetic fertilizers result in chemical residues and can alter the antioxidant profile of plants. On the other hand, organic fertilizer application is encouraged in leafy vegetable production. Compost made with chicken manure enhanced the antioxidant properties of tomato while compost as a soil supplement increased the bioactive compounds in strawberry (Wang and Lin, 2003; Toor *et al.*, 2006).

*Alternanthera sessilis* (Mukunuwenna; Family Amaranthaceae) is a vegetable popular in South East Asia and is one of the most widely consumed leafy vegetables in Sri Lanka. It is also known as ‘sessile joy weed’ and is found in warm humid regions of the world (Arshad *et al.*, 2011). This plant has medicinal properties and research has shown that it is a potential source of natural antioxidants (Walter *et al.*, 2014; Gunathilake and Ranaweera, 2016). The objective of the present study was to evaluate the variation in antioxidant activity, phenolic content and ascorbic acid content of *A. sessilis* as affected by the cultivar and different fertilizer types.

## **MATERIALS AND METHODS**

### ***Experimental Material and Treatments***

The fresh mature leaf samples of

*Alternanthera sessilis* cultivars 'Piliyandala' and 'Colombo Selection' were used for the chemical analyses. The samples were obtained from cultivations which were maintained under four different fertilizer application types. The four fertilizer treatments were: Inorganic fertilizer mixture recommended by the Department of Agriculture (T1; Urea 9 kg/1000 m<sup>2</sup>, MOP – 13.5 kg/1000 m<sup>2</sup>, TSP 10 kg/1000 m<sup>2</sup>), Integrated treatment [T2; mixture of inorganic (Urea 9 kg/1000 m<sup>2</sup>, MOP – 13.5 kg/1000 m<sup>2</sup>, TSP 10 kg/1000 m<sup>2</sup>), Organic fertilizer (T3; cattle manure and gliricidia 10 mt/ha) and Home-scale cultivation (T4: no application of fertilizer). For Inorganic, Organic and Home-scale fertilizer treatments, the two varieties were cultivated in the field and for the integrated treatment, samples were obtained from commercial growers in Gampaha, Western Province, Sri Lanka.

Chemical analyses were performed at the Food Research Unit, Department of Agriculture, Gannoruwa, Sri Lanka. Plants were washed with distilled water and extracts were obtained as described under each method. All reagents (Sigma, USA) used were of Analytical Reagent grade.

#### **Determination of Antioxidant Capacity by DPPH Radical Scavenging Assay**

DPPH (2, 2-diphenyl-1-picrylhydrazil) scavenging activity was evaluated using a spectrophotometric method described by Abdelrahmann *et al.* (2014) with minor modifications. Plant parts were air dried for 3 – 4 days and powdered by using a grinder.

Leaf sample of 0.75 g was mixed with 80% methanol and kept in an electrical shaker for 3 h. Extracts were filtered using Whatman No. 1 filter paper. Then, 0.5 mL of 80% methanol and 0.5 mL of plant extract were added and mixed well. Five concentrations from each sample were prepared and 2.5 mL of DPPH solution (3.94 mg of DPPH powder mixed with 100 mL of absolute methanol) was added to obtain a dilution series. Final optimum volume was reached to 3 mL of each sample and was analyzed in triplicate. As the blank solution, 0.5 mL of 80% methanol and 2.5 mL of DPPH solution mixture was used and 0.5 mL of 80% methanol and 2.5 mL of absolute methanol mixture was used as the control solution. Samples were incubated at room temperature for 1 h in dark. Absorbance was measured at 517 nm using UV – Visible spectrophotometer (Shimadzu UV mini 1240, Japan).

Percentage of DPPH radical scavenging activity was determined in all five concentrations (0.94 mg/mL – 15 mg/mL) using equations (1) and (2).

$$A_S = A_B - A \dots \dots \dots (1)$$

$$ASA = \left( \frac{A_0 - A_S}{A_0} \right) \times 100\% \dots \dots \dots (2)$$

where,  
ASA=Percentage Antioxidant Scavenging Activity,  
A=Absorbance of DPPH in the presence of plant extract,  
A<sub>o</sub>=Absorbance of DPPH of the control sample,  
A<sub>B</sub>=Absorbance of DPPH of the blank

sample and

$A_s$  = Absorbance of DPPH of the Blank sample - Absorbance of DPPH in the presence of plant extract.

Results were expressed as  $IC_{50}$  value which denotes the concentration of the sample required to scavenge 50% of DPPH radicals.

#### ***Determination of Ascorbic Acid Content***

Leaf extracts for ascorbic acid analysis were obtained by homogenizing 2 g of fresh plant parts in 20 mL of 3%  $HPO_3$  and the extract was filtered using a muslin cloth (Ranganna, 1986). Standardized ascorbic acid prepared by 100 mg of ascorbic acid was diluted with the 100 mL of 3%  $HPO_3$  solution. Ten milliliters of that solution was again diluted with the 10 mL of 3%  $HPO_3$  solution. Dye solution was prepared by dissolving 50 mg of sodium salt of 2,6 – Dichlorophenol-indophenol in 150 mL of hot glass distilled water containing 42 mg of sodium bicarbonate. The solution was cooled and diluted with 200 mL of distilled water. Ascorbic acid standard was standardized every day. For standardization of dye: 5 mL of ascorbic acid solution was mixed with 5 mL of 3%  $HPO_3$  solution and titrated with the dye solution until a pink colour which persisted for 15 s was obtained. Five milliliters of plant leaf extract were titrated with the standard dye to a pink colour end point which persisted for at least 15 s. Ascorbic acid content was calculated using equations (3) and (4),

$$Dye\ factor = 0.5 / titre \dots\dots\dots (3)$$

$$ACC = \frac{Titre \times Dye\ factor \times volume\ made\ up}{\left( \frac{Aliquot\ of\ extract\ taken\ for\ estimation}{Weight\ or\ volume\ of\ sample\ taken\ for\ estimation} \right) \times 100\%} \quad (4)$$

where,

AAC = Ascorbic Acid Content (mg per 100g or mL).

#### ***Determination of Total Phenolic Content***

Total phenolic content was determined by the Folin-Ciocalteu method, which was adapted from Swain and Hillis (1959). A powdered leaf sample of 0.5 g was mixed with 100 mL of absolute methanol and kept in an electrical shaker for 24 h. Extracts were filtered using Whatman No. 1 filter paper. The 150  $\mu$ L of plant extract, 150  $\mu$ L of diluted Folin Ciocalteu reagent (3 mL of Folin Ciocalteu reagent diluted with 50 mL of distilled water) and 2400  $\mu$ L of distilled water were added and mixed using a vortex. The mixture was allowed to react for 3 min and 300  $\mu$ L of 1 M  $Na_2CO_3$  solution was added and mixed. Final optimum volume was made to 3000  $\mu$ L of each sample. The samples were incubated at room temperature (27°C) in dark for 2 h. Results were expressed in Gallic acid equivalents (GAE; mg / mL) using the standard curve. Dilution series was prepared using Gallic acid solution and distilled water. Blank sample was prepared using 2550  $\mu$ L of distilled water and 150  $\mu$ L of diluted Folin Ciocalteu reagent. Blank sample and dilution series were mixed using vortex. The mixture was allowed to react for 3 min and 300  $\mu$ L of 1M  $Na_2CO_3$  solution was added and mixed. The solution was

incubated at room temperature (20°C–22°C) in dark for 2 hours and the absorbance was measured at 725 nm using UV – Vis Spectrophotometer (Shimadzu-UV-120-02, United Kingdom).

### ***Experimental Design and Statistical Analysis***

Experimental setup was a 2 x 4 Factorial Design with main factors of cultivars and fertilizer application. Chemical analyses were performed in triplicate samples from each treatment (n = 3). Data were subject to one way Analysis of Variance (ANOVA) and statistical analysis was carried out using SAS Version 9.4 statistical software.

## **RESULTS AND DISCUSSION**

### ***Antioxidant Activity of *Alternanthera sessilis* Leaf Extract***

The DPPH Radical scavenging assay is a widely used method to measure the antioxidant capacity of plant parts. In this assay, the DPPH reagent provides DPPH radicals into the medium. When the medium contains antioxidants, they stabilize DPPH radicals similar to the way that antioxidants neutralize harmful free radicals in human body. IC<sub>50</sub> value or the 50% scavenging activity is the concentration of the sample extract needed to react with 50% of DPPH radicals. IC<sub>50</sub> value is less when there are high number of antioxidants present in the medium and vice versa (Huang *et al.*, 2005).

According to the results presented in Table 1, there was no significant effect of

cultivar ( $p>0.05$ ) on the antioxidant capacity of *Alternanthera sessilis* in the present study. The mean antioxidant activities (IC<sub>50</sub> value) of cultivars Piliyandala and Colombo Selection across different fertilizer treatments were 7.92 mg/mL and 9.50 mg/mL, respectively. In a previous study, the DPPH radical scavenging activity (% inhibition) of the methanolic extract of *A. sessilis* has been reported as 4.37% (Gunathilake and Ranaweera, 2016). However their unit of measurement was different.

On the other hand, the fertilizer treatment, as a main factor across the two cultivars, showed significant effects ( $p<0.05$ ) on antioxidant capacity of the leaf extract of *A. sessilis* (Table 1). A significantly higher antioxidant capacity (*i.e.* lowest IC<sub>50</sub> value) was shown by plants from home-scale cultivation (3.36 mg/mL) for which no fertilizer application was done, followed by the plants from organic fertilizer applied field (8.03 mg/mL). Leaf extracts of *A. sessilis* plants grown only with inorganic fertilizer showed the least antioxidant capacity of 14.23 mg/mL (Table 1).

There was a significant ( $p<0.05$ ) interaction effect (variety x fertilizer treatment) on antioxidant capacity of *A. sessilis* (Table 1). The highest antioxidant capacities were recorded in home-scale cultivations of both Piliyandala (1.08 mg/mL) and Colombo Selection (2.79 mg/mL) and also in inorganic fertilizer applied plants of variety Piliyandala as 1.46 mg/mL. The lowest antioxidant activity

**Table 1.** Antioxidant activity, phenolic and ascorbic acid contents of *Alternanthera sessilis* varieties under different fertilizer applications

Variety and Fertilizer application	Antioxidant activity (IC <sub>50</sub> value; mg/mL)	Phenolic content (mg GAE/100 g)	Ascorbic acid content (mg/100 mg)
Variety ( <i>n</i> =12)	p>0.05	p<0.05	p>0.05
Piliyandala (P)	7.92±5.09	343.83±85.71 <sup>b</sup>	11.48±8.92
Colombo Selection (C)	9.50±4.56	472.67±102.06 <sup>a</sup>	12.44±7.40
Fertilizer ( <i>n</i> =12)	p<0.05	p<0.05	p>0.05
Inorganic fertilizer	14.23±2.31 <sup>a</sup>	353.63±65.98 <sup>b</sup>	13.45±4.88
Integration	10.56±3.42 <sup>b</sup>	350.84±67.94 <sup>b</sup>	11.45±2.97
Organic fertilizer	8.034±2.48 <sup>b</sup> <sup>c</sup>	404.99±75.87 <sup>a</sup>	15.09±3.37
Home-scale	3.364±1.76 <sup>d</sup>	410.87±105.34 <sup>a</sup>	7.83±7.87
Variety x Fertilizer ( <i>n</i> =3)	p<0.05	p<0.05	p>0.05
P x Inorganic fertilizer	1.46±1.09 <sup>d</sup>	403.67±75.22 <sup>b</sup>	13.45±7.18
P x Integration	11.62±11.62 <sup>b</sup>	537.67±92.44 <sup>a</sup>	11.45±8.17
P x Organic fertilizer	17.50±1.09 <sup>a</sup>	333.33±88.84 <sup>b</sup>	15.09±11.83
P x Home-scale	1.08±1.09 <sup>d</sup>	358.33±83.97 <sup>b</sup>	7.83±1.78
C x Inorganic fertilizer	13.41±1.09 <sup>b</sup>	403.67±75.22 <sup>b</sup>	13.45±7.18
C x Integration	14.31±1.09 <sup>ab</sup>	537.67±92.44 <sup>a</sup>	11.45±8.17
C x Organic fertilizer	7.48±1.09 <sup>c</sup>	333.33±88.84 <sup>b</sup>	15.09±11.83
C x Home-scale	2.79±1.09 <sup>d</sup>	358.33±83.97 <sup>b</sup>	7.83±1.78

**Note:** Values are mean ± Standard Deviation. Means within a column, within each main factor and interaction factor, followed by different superscript letters are significantly different at  $\alpha=0.05$  by LSD test. GAE- Gallic acid equivalents.

(highest IC<sub>50</sub> value) was shown by the organic fertilizer treated Piliyandala variety (17.50 mg/mL). However, it was not significantly different ( $p<0.05$  at 95% confident interval) from variety Colombo Selection treated with a mixture of organic and inorganic fertilizers (Integration; 14.31 mg/mL). Variety Colombo Selection obtained from organic fertilizer applied fields also showed a relatively high antioxidant activity (7.48 mg/mL).

In a previous study, antioxidant activity of *A. sessilis* has been estimated for plant

extract of different solvents by Phosphomolybdate method and DPPH assay. The DPPH radical scavenging activity of methanol extracts (IC<sub>50</sub> value) of an Indian *A. sessilis* variety was reported as 587.093  $\mu$ g/mL (0.587 mg/mL; Yadav *et al.*, 2011) which was relatively higher than the values shown in the present study.

#### ***Ascorbic Acid Content of Alternanthera sessilis Leaf Extract***

The effects of main factors (variety or fertilizer treatment) or their interactions on

the ascorbic acid content of *A. sessilis* were not significant ( $p > 0.05$ ; Table 1). The mean ascorbic acid contents of varieties Piliyandala and Colombo Selection across four fertilizer treatments were 11.48 and 12.44 mg/100 mg, respectively. Irrespective of the variety, plants from organic fertilizer applied field showed the highest ascorbic acid content of 15.09 mg/100 mg although it was not significantly different from others. Plants grown in home-scale showed comparatively lower ascorbic acid levels (7.83 mg/100 mg).

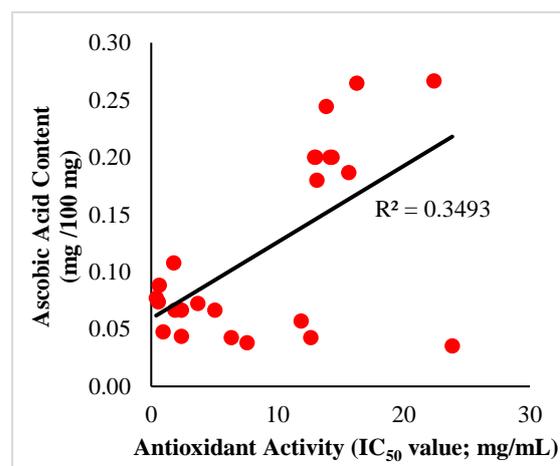
A study by Hassan *et al.* (2012) indicated that both inorganic and organic fertilizer application resulted in an increased ascorbic acid content of the leafy herb *Cosmos caudatus* compared to zero fertilizer application. In the same study, application of organic fertilizer increased the ascorbic acid content of *C. caudatus* leaf extract relative to inorganic fertilizers.

#### ***Phenolic Content of Alternanthera sessilis Leaf Extract***

The phenolic content of *A. sessilis* was significantly influenced by the variety, fertilizer treatment and their interactions (variety x fertilizer treatment; Table 1). Variety Colombo Selection showed a higher phenolic content of 472.67 mg GAE/100 g compared to that of variety Piliyandala (343.83 mg GAE/100 g). Across the two varieties, significantly higher phenolic contents were reported in plants raised in home-scale (410.87 mg GAE/100 g) and with organic fertilizer treatment (404.49 mg GAE/100 g)

compared to inorganic fertilizer and integration treatments (Table 1).

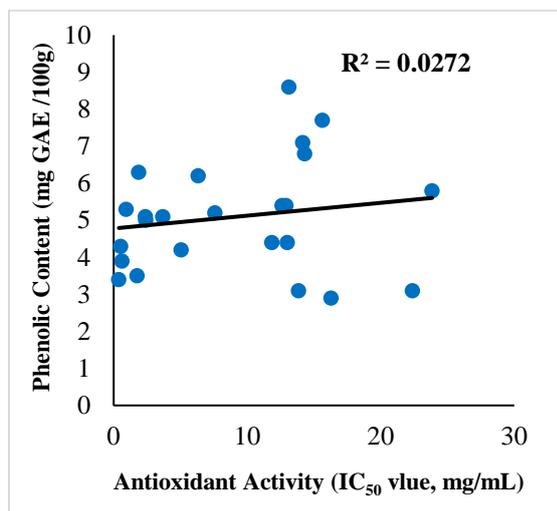
Phenolic compounds were found to be the major contributors to antioxidant capacity in many leafy vegetable species (Gunathilake and Ranaweera, 2016). In a study done in India, the phenolic content of the methanolic extract of leaves and stems of *A. sessilis* was 140.4 mg GAE/100 g which was lower than the values reported in current study (Yadav *et al.*, 2011). However, the fertilizer regime or the growing conditions were not reported in the study. A positive relationship was observed between antioxidant activity and ascorbic acid content ( $R^2 = 0.3496$ ; Figure 1).



**Figure 1.** Correlation between antioxidant activity and ascorbic acid content in *Alternanthera sessilis* leaf extracts

However, it was not possible to obtain a relationship between antioxidant activity and total phenolic content ( $R^2 = 0.0272$ ; Figure 2). Ascorbic acid and phenolic compounds, among other bioactive compounds, significantly contribute to the

antioxidant capacity of green leafy vegetables. Those compounds offer protection against critical illnesses including cancer (Kaur and Kapoor, 2001).



**Figure 2.** Correlation between antioxidant activity and phenolic content in *Alternanthera sessilis* leaf extracts

## CONCLUSION

Farmers tend to apply high doses of inorganic fertilizer in commercial leafy vegetable cultivation. The present study indicated that, *A. sessilis* grown with inorganic fertilizer and a mixture of inorganic and organic fertilizer contained significantly lower antioxidant levels compared to those grown organically and without fertilizer application (home-scale). Organic fertilizer application significantly increased the antioxidant activity of the variety Piliyandala but not in the variety, Colombo Selection. Out of the two varieties of *A. sessilis*, Colombo Selection had higher content of phenolic compounds which also contribute to total antioxidant capacity.

Home-scale cultivations and organic fertilizer application resulted in increased phenolic contents in *A. sessilis* compared to inorganic fertilizer and organic and inorganic integration. Ascorbic acid contents did not vary significantly between two varieties or among fertilizer treatments.

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