Total Antioxidant Activity, Total Phenolic and Flavonoid Contents of Eggplant *(Solanum melongena L.),* and Six of its Wild Relatives in the Philippines

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In the Philippines, phytochemical study on eggplant and its wild relatives has not yet been explored. The total phenolic and flavonoid contents as well as the total antioxidant capacity of seven *Solanum* species in the Philippines were assessed through Folin-Ciocalteau assay, aluminum chloride colorimetic method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. *S. americanum* and *S. melongena* were noted to produce the highest amount of phenols (96.43 and 74.38 mg GAE/g DW, respectively) and flavonoids (33.58 and 31.61 mg RUE/g DW, respectively) in their fruits. These two *Solanum* species also revealed the highest radical scavenging activity (8.61% and 6.85%, respectively). On the other hand, *S. capsicoides* was observed to produce low amount of phenols (54.31 mg GAE/g DW) and flavonoids (12.54 mg RUE/g DW), and eventually showed weak radical scavenging activity (3.76%). Correlation analysis suggested that the antioxidant capacity of the seven *Solanum* species was positively correlated to their total phenolic and flavonoid contents.

Keywords: *Antioxidant, Flavonoids, Phenolics, Radical Scavenging Activity, Solanum species*

INTRODUCTION

The family *Solanaceae* is constituted of about 3,000 to 4,000 species (Gebhardt, 2016), which are significant sources of phytochemicals. This particular plant family includes eggplant (*Solanum melongena L.*) and its wild relatives. Eggplant is a high yielding vegetable crop in Asia, which is well adapted to various environmental conditions. With this unique environmental adaptability, the vegetable remains affordable for the consumer all year round. In the Philippine setting, it is one of the major vegetable crops with relatively high production rate because of its demand as a staple vegetable in every Filipino household (PSA, 2019).

Over the past decades, one of the top priorities of plant breeders has been the improvement of the agronomic characteristics of eggplant to generate high yielding varieties with pest and insect resistance (Rotino et al., 1997; Chattopadhyay et al., 2012; Plazas et al., 2016). However, the increased health consciousness of the growing population has shifted some researchers' interests towards the discovery and enhancement of the phytochemical compositions of potential eggplant accessions (Boubekri et al., 2012; Salerno et al., 2014; Djouadi & Boubekri, 2016).

It was reported that the whole eggplant fruit possesses antioxidant activity (Akanitapichat et al., 2010; Noda et al., 2000; Somawathi et., 2014). On the other hand, its wild relatives such as *S. aethiopicum* (Nwanna et al., 2019), *S. diphyllum* (Hossain et al., 2009), *S. mammosum* (Wetwitayaklung & Phaechamud, 2011), *S. nigrum* (Gbadamosi & Afolayan et al., 2016; Veerapagu et al., 2018; Aryal et al., 2019) and *S. torvum* (Rahman et al., 2013; Abdulkadir et al., 2016; Khatoon et al., 2018) were also found to produce weighty amount of antioxidants in their fruits. According to Nisha et al. (2009), the antioxidant level in eggplant varies according to its variety, fruit shape and size, and even according to its production management system. Furthermore, it is postulated that the antioxidant capacity of eggplant is positively correlated to its phenolic content (Okmen et al., 2009, Chumyam et al., 2013; Plazas et al., 2013).

Phytochemicals are thoroughly studied for human health benefits as well as for drug discovery and development (Koulora et al., 2014). The wide array of vegetable and its related species worldwide hold a great number of bioactive compounds. These compounds can be a great source of natural antioxidants which are perceived to be a less toxic, healthier, and cheaper alternative to synthetic ones (Krishnalah et al., 2011). In fact, countries like Turkey (Okmen et al., 2009), United States of America (Singh et al., 2009), Sri Lanka (Somawathi et al., 2014), Italy (Salerno et al., 2014), Puerto Rico (Zambrano-Moreno et al., 2015), Algeria (Djouadi and Boubekri, 2016), and India (Khatoon et al., 2018) have already initiated basic researches on the phytochemical potential of different eggplant varieties. This study was thought out to initiate phytochemical study on eggplant and its wild relatives found in the Philippines.

This study aimed to assess the total phenolic and flavonoid contents as well as the total antioxidant capacity of eggplant and its wild relatives found in the country.

MATERIALS AND METHODS

Plant Samples

Matured fruits of *S. aethiopicum*, *S. americanum*, *S. capsicoides*, *S. diphyllum*, *S. mammosum*, *S. melongena* and *S. torvum* (Fig. 1) were gathered from the collection garden at Barangay Batong Malake, Los Baños, Laguna for phytochemical analyses. These *Solanum* species were collected from various places in the country (Table 1). The fruits were collected from three different plant samples (replicates) of each *Solanum* species.

Sample Preparation

The fruits (peel and flesh) were cut into pieces and oven dried at 45^{o}C for two days. The dried samples were pulverized and eventually utilized for different phytochemical analyses.

Phytochemical Contents

A total of 50 mg of pulverized samples were added to 10 mL 50% methanol (1:1 vol/vol absolute methanol: distilled water). The mixture was immediately vortexed at medium speed of 3 minutes. It was then centrifuged at 3000 rpm for about three minutes. The supernatant was thoroughly collected and utilized for the determination of total phenolic and flavonoid contents as well as two 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays.

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using Folin-Ciocalteau assay (Velioglu et al., 1998). A total of 2.9 mL distilled water was added to 100 μL methanol extract. Then, 1 mL of 0.2 M sodium carbonate and 0.2 mL of 50% Folin-Ciocalteau reagent were added to the solution. The solution was mixed properly using a vortex mixer. The samples were thereafter placed in a water bath for 20 minutes. The absorbance was measured using spectrophotometer at 710 nm. Total phenolic contents in the studied Solanum species were expressed as percent gallic acid equivalent (GAE).

Determination of the Total Flavonoid Content (TFC)

Total flavonoid content was estimated using aluminum chloride colorimetic method (Chang et al., 2002). About 2.0mL dH20 was added in 0.5mL methanolic extract. After that, 7.5µL 5% NaNO2 was added to the methanolic extract and was left to stand for 5 minutes. Then, 7.5µL 10% AlCl3 was added in the mixture and was left again to stand for 6 minutes. Finally, 50µL 1.0 M NaOH was added in the mixture before the absorbance was determined. The absorbance of the mixture was measured at 510 nm, and the results were stated as Rutin equivalent (mgRUE/gDW).

Determination of the Total Antioxidant Activity (% Radical Scavenging Activity)

The antioxidant activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams et al., 1995). The DPPH assay was dissolved in 100 mL of absolute methanol for a final concentration of 10-4 M DPPH. About 2.9 mL aliquot of the DPPH solution was transferred in test tubes and eventually mixed with 25 µL of H2O. The solution was thoroughly mixed and incubated in the dark room at 300C for 40 minutes Absorbance reading was finally done at 517nm. The total antioxidant activity was expressed using % radical scavenging activity (%RSA).

STATISTICAL ANALYSES

Analysis of Variance (ANOVA) was used to statistically analyze the data. Tukey's HSD test was done to find out which specific group means were different. In addition, Spearman Correlation Analysis was conducted to determine the correlation between the total antioxidant activity and total phenolic content, and total antioxidant activity and total flavonoid content.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Figure 2a presents the total phenolic content of seven *Solanum* species. As noticed, *S. americanum* (96.43 mg GAE/g DW) showed the highest amount of total phenolic content followed by *S. melongena* (74.38 mg GAE/g DW), *S. torvum* (mg GAE/g DW), *S. aethiopicum* (60.35 mg GAE/g DW), *S. mammosum* (58.47 mg GAE/g DW) S. capsicoides (54.31 mg GAE/g DW) and *S. diphyllum* (52.51 mg GAE/g DW). The variation observed in the total phenolic content among the studied fruit samples was quite expected since the samples utilized in the analysis were of different species. Okmen et al. (2009) Djouadi and Boubekeri (2016) and Aryal et al. (2019) witnessed the same results while assessing the phenolic contents of different eggplant cultivars and some of its wild relatives in Turkey, Algeria, and Nepal, respectively. Apparently, these observations suggest that the total phenolic content of a certain plant sample can be governed by several factors. It was well-documented by Antolovich et al. (2000), Griffith and Collison (2001), Robbins (2003), Lee et al. (2004), Naczk and Shahidi (2004), Achouri et al. (2005) and Luthria (2006) that the quantity and quality of phenolic compounds present in fruits and vegetables were significantly influenced by cultivar, environment, soil type, and growing and storage conditions. Furthermore, it may even depend on the extraction procedures used (Luthria et al., 2010).

The growing significance of phenolic compounds in plant species is primarily attributed to its antioxidant capacity (Salerno et al., 2014). This particular phytochemical is one of the most important classes of secondary metabolites mostly found in plants with diverse structures (Cheynier, 2012; Tungmunnnithum et al., 2018). Polyphenol components commonly found in plant extracts are capable of protecting a system against free radicals that are associated with abnormal aerobic cell metabolism (Enein et al., 2009; Brewer, 2011; Tiong et al., 2013). These compounds are among the greatest electron donors that can secure the conversion of H2O2 and H2O in a short period. Hence, they are referred to as the powerful chain-breaking antioxidants (Bendary et al., 2013). Nonetheless, phenolic compounds are crucial for plant's growth and reproduction, which are produced as a response to environmental factors and to defend injured plants (Valentine et al., 2003).

Total Flavonoid Content (TFC)

Based on the analysis, the seven *Solanum* species exhibited different levels of flavonoid content (Fig. 2b). *S. americanum* (33.58 mg RUE/g DW), and *S. melongena* (31.62 mg RUE/g DW) were observed to be the top flavonoid producing species followed by *S. diphyllum* (29.51 mg RUE/g DW), *S. aethiopicum* (28.38 mg RUE/g DW), *S. torvum* (25.63 mg RUE/g DW), *S. mammosum* (21.61 mg RUE/g DW), and S. capsicoides (12.54 mg RUE/g DW). Trend wise, the *Solanum* species (i.e. *S. americanum* and *S. melongena*) that revealed the highest amount of phenols were the same species that produced a high amount of flavonoids. This particular trend was also witnessed from previous *Solanum* researches (Gbadamosi & Afolayan, 2016; Khatoon et al., 2018; Aryal et al., 2019). The observed trend might only be coincidental and not true to all plant species. According to Cotelle (2001), the production of phenols is not directly involved in the production of flavonoids; hence, there is no direct relationship between these two phytochemicals. Despite the various role of flavonoids in plant system, these phytochemicals are not understood completely yet (Abdulkadir et al., 2016). However, there are already few authors who reported that these phytochemicals were involved in plant-microorganism communication (Rice-Evans et al., 1996), plant's stimulation and protection (Wink, 2010), and even pigment and flavor expression (Harborne, 1976).

Total Antioxidant Activity (% Radical Scavenging Activity)

The total antioxidant activity of the seven *Solanum* species was expressed using percent radical scavenging activity (%RSA) (Fig. 2c). Remarkably, *S. americanum* (8.61%) and *S. melongena* (6.85%) exhibited the highest percentages of RSA. These results were consistent with the findings of Gbadamosi and Afolayan (2016), Veerapagu et al. (2018), and Aryal et al. (2019). On the other hand, *S. diphyllum* and *S. capsicoides* were found to express the lowest percentages of RSA (3.72% and 3.67%, respectively). The

% RSA of *S. torvum*, *S. mammosum* and *S. aethiopicum* showed no significant variation (4.79%, 4.67% and 4.58%, respectively) as far as statistical analysis was concerned.

The purple colored fruit of *S. melongena* (Fig. 1e) and *S. americanum* (Fig. 1f) is one of the primary reasons for their high antioxidant activities. In 2009, Nisha et al. reported that the purple fruits of *Solanum* species can express higher antioxidant capacity than other *Solanum* fruit types because of the presence of anthocyanin. Over the years, Singh et al. (2009), Akanitapichat et al. (2010) and Boubekeri et al. (2012) have proven that nasunin (delphinidin-3-p-coumaroylrutinoside-5-glucoside), the most common anthocyanin in *Solanum* species, is the key player in their total antioxidant activity.

Naturally, plant species can synthesize a variety of antioxidants that are efficient in neutralizing or even recycling free radicals. Phytochemistry wise, eggplant was ranked among the top ten vegetables with high antioxidant capacity (Cao et al., 1996; Nisha et al. 2009 ; Singh et al., 2009; Akanitapichat et al., 2010). However, like the phenolic compounds, the total amount of these phytochemical compounds varied according to variety, fruit shape and size, and even to the method of extraction (Medina et al., 2014). Moreover, antioxidant capacity and phenolic acid content in eggplant cultivars are positively correlated with each other (Okmen et al., 2009; Chumyum et al., 2013; Plazas et al., 2013).

Correlation between Total Antioxidant Activity and Total Phenolic Content, and Total Antioxidant Activity and Total Flavonoid Content

Phenolic and flavonoid molecules are important plant components that are responsible for deactivating free radicals based on their ability to donate hydrogen atoms. These molecules also have an ideal structural characteristic for free radical scavenging activity, which is the innate responsibility of antioxidants (Amarowicz et al., 2004). To further analyze the roles of phenolic and flavonoid compounds in the antioxidant activities of the studied *Solanum* species, correlation analysis was done.

Spearman correlation analysis was done to measure the degree of association between the total phenolic content (TPC) and antioxidant activity, and total flavonoid content (TFC) and antioxidant capacity of the seven

Solanum species. Figure 3a clearly displays a very strong, positive monotonic correlation between total antioxidant activity and TPC (rs=0.98; n=21; p< 0.0001). There was also a very strong, positive monotonic correlation between total antioxidant activity and TFC (rs=0.65; n=21; p<0.0023). It is worth noting that *S. americanum* and *S. melongena* which were observed to produce the highest amount of phenols and flavonoids were also the ones that showed the highest antioxidant capacity among the other studied species. On the other hand, S. capsicoides which was noticed to produce the least amount of phenols and flavonoids expressed a relatively low antioxidant capacity. Based on the results, the antioxidant capacity of the seven *Solanum* species was directly correlated to their total phenolic and flavonoid contents. This means that the antioxidant level of the studied *Solanum* species is a straight reflection of their phenolic and flavonoid contents. Previous studies in tart cherries (Wang et al., 1999) cranberry (Wang & Stretch, 2001), onion (Yang et al., 2004), guava (Kriengsak et al., 2006) and purslane (Aryal et al., 2019) have also demonstrated a strong association between their total phenolic content and antioxidant activities. Nevertheless, Shreshta and Dhillion (2006), Sharififar et al.(2009) and Abdulkadir et al. (2016) have also elaborated the involvement of flavonoids in the antioxidant activities of fruits, vegetables, herbs, and even medicinal plants.

The phytochemical activities of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh et al., 2006).

CONCLUSION

The total phenolic and flavonoid contents as well as the total antioxidant capacity of *Solanum aethiopicum*, *S. americanum*, *S. capsicoides*, *S. diphyllum*, *S. mammosum*, *S. melongena* and *S. torvum* were successfully assessed. As observed, all studied *Solanum* species contained different amount of phytochemicals.

Among the seven *Solanum* species, *S. americanum* and *S. melongena* were noted to produce high amount of phenols (96.43 and 74.38 mg/ GAE/g DW, respectively) and flavonoids (33.58 and 31.62 mgRUE/gDW, respectively). Eventually, these species were also detected to exhibit high antioxidant capacity (8.61% and 6.85%, respectively). This particular finding was believed to be influenced by the presence of nasunin in their purple fruit peel. Variously, *S. diphyllum* and *S. capsicoides*, as recorded, produced the least amount of phenols (52.51 mg/GAE/g DW) and flavonoids (12.54 mgRUE/gDW), respectively. *S. diphyllum* and *S. capsicoides* also showed weak antioxidant capacity (3.72% and 3.67%, respectively) as far as radical scavenging activity was concerned.

The values reported in the study were for uncooked fruits of eggplant and its wild relatives. If these were used for dietary purposes, the effect of processing on the compounds investigated must be taken into consideration.

Generally, the antioxidant capacity of the seven *Solanum* species was detected to be correlated to their total phenolic and flavonoid contents. These findings are instrumental in the selection of putative parent materials to be used in the improvement of the antioxidant activity of eggplant through breeding.

It is recommended that other wild *Solanum* species, which is also reported to be present in the country, be included in the future phytochemical research endeavors.

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Table 1

List of the Solanum species used in the phytochemical analyses

SCIENTIFIC NAME	COLLECTION SITE	TYPE
S. aethiopicum L.	Brgy. Balindog, Kidapawan City	Wild
S. americanum Mill.	Los Baños, Laguna	Wild
S. capsicoides Allioni	Pasar, Leyte	Wild
S. diphyllum L.	Pob. Compostela, Compostela Valley	Wild
S. mammosum L.	Brgy. Katangawan, General Santos City	Wild
S. melongena L.	San Fernando, La Union	Landrace
S. torvum Sw.	Brgy. Binoligan, Kidapawan City	Wild

Figure 1. Fruits of a) *Solanum aethiopicum* L., b) *S. capsicoides Allioni*, c) *S. diphyllum* L., d) *S. mammosum* L., e) *S. melongena* L., f) *S. americanum* Mill. and g) *S. torvum* Sw.

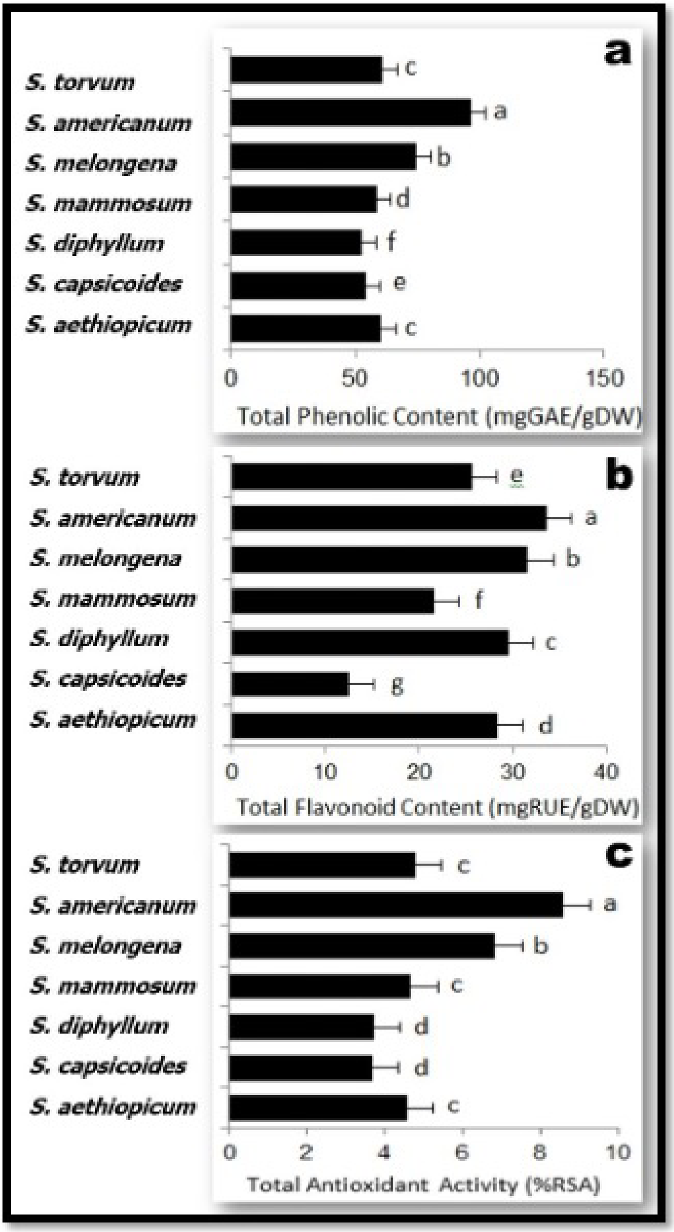

Figure 2. a) Total Phenolic Content b) Total Flavonoid Content and c) Total Antioxidant Activity of *Solanum aethiopicum* L., S. *americanum* Mill., *S. capsicoides Allioni*, *S. diphyllum* L., *S. mammosum* L., *S, melongena* L. and *S. torvum* Sw. (The values with error bars follow ed by different letters are statistically different at P< 0.0001).

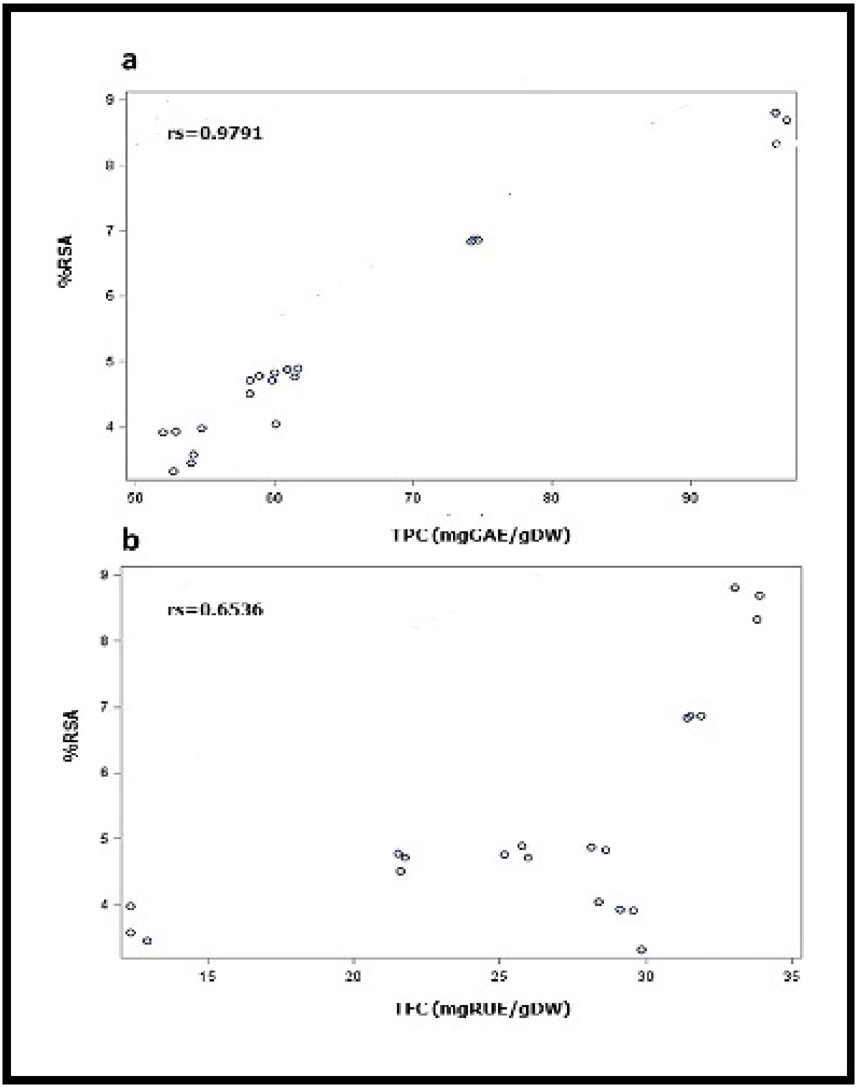

Figure 3. Correlation between (a) Total Antioxidant Activity (%RSA) and Total Phenolic Content (TPC) and, (b) Total Antioxidant Activity (%RSA) and Total Flavonoid Content (TFC) of *Solanum aethiopicum* L., *S. americanum* Mill., *S. capsicoides Allioni*, *S. diphyllum* L., *S. mammosum* L., *S, melongena* L. and *S. torvum* Sw.