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Evaluation of Antioxidant Activity and Polyphenolic Content of Commonly Consumed Egg Plant Varieties and Spinach Varieties in Sri Lanka

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Authors' contributions

This work was carried out in collaboration among all authors. Author HACOH designed the study, performed the statistical analysis, literature survey, wrote the protocol and wrote the first draft of the manuscript. Author KDPPG managed the analyses and supervised of the study, edited and reviewed the manuscript. Author SJ supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antioxidant capacity and the polyphenolic content of commonly consumed egg plant varieties and spinach varieties in Sri Lanka, in order to screen the potential for utilizing them in developing of functional food products.
Study Design: Complete randomized design was performed.
Place and Duration of Study: Department of Food Science & Technology, Faculty of Livestock, Fisheries & Nutrition, Wayamba University of Sri Lanka, between September 2020 and October 2020.
Methodology: Locally available, commonly consumed eggplant varieties *Solanum torvum, Solanum macrocarpon*, *Solanum melongena* BW 11, *Solanum melongena* and two spinach varieties *Basella alba* and *Talinum paniculatum* were collected, freeze dried & powdered. Methanolic extracts were prepared using 80% methanol.Total Antioxidant capacity (TAC), DPPH

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radical scavenging activity, Reducing power assay, Total Phenolic Content (TPC) and Total Flavonoid Content of fresh methanolic extracts were measured.
Results: *S. torvum*, *S. macrocarpon*, *S. melongena* BW 11, *S. melongena* showed more or less similar but remarkable DPPH radical inhibition activity ranging from 92.49 to 93.02%. TAC results of tested eggplant varieties ranged from 3.02to 8.98 mg AAE / g dw. From the two tested spinach varieties *B. alba* leaf extract had higher antioxidant activity but lower flavonoid content than *T. paniculatum* leaf extract. TPC of methanolic extractions of all tested vegetable sample was within the range of 2.49 to 670.00 µg GAE / g dw. There was a significant difference between the recorded TPC for all the tested samples and *S. macrocarpon* and two varieties of *S. melongena* showed much lower TPC when compared to TPC of *S. torvum*.
Conclusion: These are considerable sources of polyphenols and are having a remarkable antioxidant activity. The potential of them to be utilized in functional food industry is high and yet to be investigated.

Keywords: Flavonoid; antioxidant; Solanum spp.; Spinach; phenolic.

1. INTRODUCTION

The free radicals induced oxidative stress has been reported to be involved in several diseased conditions such as diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease and Multiple sclerosis), cardiovascular diseases (atherosclerosis and hypertension), respiratory diseases (asthma), cataract development, rheumatoid arthritis and in various cancers (colorectal, prostate, breast, lung, bladder cancers) [1]. It has been revealed that several compounds from plants which are called phytochemicals or plant bio actives are known potentials for antioxidant activity. Antioxidant activity denotes the ability of a bioactive compound to maintain cell structure and function by effectively clearing free radicals, inhibiting lipid peroxidation reactions, and preventing other oxidative damage [2].

Eggplant, with its numerous varieties, all of which are belonging to Family Solanaceae the same family to which tomatoes belong, are widely consumed all over the world as a vegetable. In Sri Lanka, the eggplant variety-brinjal which is scientifically known as *Solanum melongena* L. is the most extensively grown vegetable in low country [3]. The availability in the local markets and the consumption cannot be deemed inferior to the extent of its cultivation. Peel of brinjal having a noted blue colour which is called 'Nasu Blue' by Japanese, is said to have Anthocyanin compounds [4] which are categorized under flavonoids, a phytochemical. A previous study has reported that the antioxidant capacity of eggplant has been categorized among the top ten of 120 vegetables in the world [5]. Also, it has reported that eggplant flesh carries a lot of flavonoid compounds like kaempferol, quercetin, apigenin and isorhamnetin [5]. Even the African eggplant had high concentrations of rutin and quercetin and other eggplants may also have such flavonoids in high concentrations.

Solanum macrocarpon also known as African eggplant is used in the treatment if gout, rheumatism, angina and inflammatory tumours [6]. *Solanum torvum* is used as cough ailments and in cases of liver and spleen enlargement [7]. The methanolic extracts of S. torvum fruits have been identified to contain phytochemicals such as alkaloids, flavonoids, saponins, tannins and glycosides. A study has reported the presence of flavonoids, alkaloids, saponins and tannins in *Talinum* sp. Leaves [8]. Similar study done by [9] has identified the polyphenol concentration of Water Leaf plant. Lourith & Kanlayavattanakul in 2017 [10] reports about antioxidant, anticancer and antidiabetic properties of *Basella alba* leaf extract and the presence of phytochemical compounds as well. However, very limited amount of studies has been conducted about antioxidant properties of (*B. alba*) leaf. Therefore, the study focused on locally available, commonly consumed fruits of eggplant varieties - Turkey berry/Wild eggplant/Thibbatu (*S. torvum*), African eggplant/ Thalanabatu (*S. macrocarpon*), brinjal-Padagoda BW 11 (*S. melongena* BW 11) and Indian baby eggplant (*S. melongena*) and two leafy vegetables namely, Ceylon spinach (*B. alba*) and Water Leaf (*T. paniculatum*) for their Total Antioxidant capacity, Reducing power, DPPH radical scavenging activity, Total Phenolic Content andTotal Flavonoid content with the objective of investigating the potential antioxidant activity and polyphenolic content of the vegetables when consumed as a whole.

2. MATERIALS AND METHODS

2.1 Materials

Rutin, Gallic acid, Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and methanol were obtained from Sigma-Aldrich, St. Louis, MO, USA through Analytical Instrument Pvt Ltd, Colombo, Sri Lanka. All other chemicals used in this study were of analytical grade.

2.2 Sample Preparation

S. torvum, *S. macrocarpon*, *S. melongena* BW 11, *S. melongena*, *B. alba* and *T. paniculatum*were collected from Horticultural Crop Research and Development Institute (HORDI) Gannoruwa and local home gardens at Kurunegala and Gampaha areas of Sri Lanka. Vegetables were washed and lyophilized (Martin Christ, Freeze-dryer, Alpha 1-2/LD) and ground into fine powder before storing in amber colour bottles at -18°C for further studies.

2.3 Preparation of hydro-methanolic Extract

Lyophilized and ground samples were weighed and 0.50 gram of each sample were mixed with 20 mL methanol/water (80%, v/v) and vortexed at high speed for five minutes and then centrifuged (Hettich, EBA 20) at 4500 rpm for 10 min. The extracts were filtered through filter paper (Whatman No. 42; Whatman Paper Ltd, maidstone, UK) and the prepared extracts were stored at -18°C until further analysis.

2.4 Antioxidant Activity

2.4.1 Determination of DPPH radical scavenging ability

The assay was conducted according to Janarny and Gunathilake [11] to assess the capacity of prepared methanolic extracts to scavenge the stable free radical DPPH. Freshly prepared methanolic solution of DPPH (1 Mm, 3.9 mL) was added to 0.1 mL of sample and vortexed for 15 seconds before incubating at room temperature for 30 min in the dark. The absorbance was measured at 517 nm and the percentage scavenging ability was calculated using the following equation.

$$Scavenging\ ability\% = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} x\ 100$$

2.4.2 Total antioxidant capacity using phosphomolybdenum method

The total antioxidant capacity of methanolic extracts was analyzed according to the method described by Prieto et al. [12]. The antioxidant capacity was expressed as mg Ascorbic Acid Equivalents per g Dry Weight of sample (mg AAE per g DW).

2.4.3 Reducing power assay

Reducing power was determined using the method described by Mel et al. [13] with slight modifications. Briefly, 1 mL of methanolic extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (W/V) Potassium ferricyanide. Solution mixtures were incubated in a water bath at 50°C for 20 minutes. Then, 2.5 mL of 10% (W/V) trichloroacetic acid solution was added and centrifuged for 10 minutes at 3000 rpm. Finally, 2.5 mL of upper layer is mixed with 2.5 mL distilled water and 0.5 mL of 0.1% (W/V) Ferric chloride solution and the absorbance was measured at 700 nm using UV/Vis Spectrophotometer. Reducing power was expressed as mg Ascorbic Acid Equivalents per g Dry Weight of sample (mg AAE per g DW).

2.5 Polyphenolic Content

2.5.1 Total phenolic content

The total polyphenol content of methanolic extracts were assessed using Folin-Ciocalteu method of Singleton et al. [14] with some modifications as described by Gunathilake [15]. Folin-Ciocalteu reagent (0.5 N, 0.2 mL) was added to 1 mL of the sample and incubated in the dark for 15 min. Then 5 mL of Na_2CO_3 (7.5%) were added to each mixture and incubated in the dark for 2 h. The absorbance was measured using UV/VIS spectrophotometer (Thermo Scientific, Evolution 201 series, USA)and the concentration of total polyphenols was expressed as µg Gallic Acid Equivalents per g Dry Weight of sample (µg GAE per g DW).

2.5.2 Total flavonoid content

Total flavonoid content was measured according to a spectrophotometric method described by Gunathilake et al. [16], with minor modifications. Briefly, 1.5 mL of methanolic extract/ rutin standard solution were added to 3.5 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added and incubated for 5 minutes at room

temperature. About 0.3 mL of 10% $AlCl_3$ was added into each solution and again incubated for 6 minutes at room temperature. Then 2 mL of 1.0 M NaOH was added and immediately the solutions were made up to 10 mL by adding distilled water. The absorbance of samples was obtained at 510 nm using UV-Visible Spectrophotometer (Thermo Scientific, Evolution 201 series, USA). Total Flavonoid content was expressed as mg rutin equivalents (RE)/g of dry weight of sample.

2.6 Statistical Analysis

Significant differences between the results were calculated using ANOVA (General linear model and Tukey test) with the help of Minitab 18. Differences at $P < 0.05$ were considered to be significant. All the data collected were presented as the mean ± standard deviation for all assays where samples were analyzed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

3.1.1 DPPH radical scavenging activity

According to the results indicated in the Fig. 1, all eggplant varieties *S. torvum*, *S. macrocarpon*, *S. melongena* BW 11 and *S. melongena* showed more or less similar but remarkable DPPH radical scavenging activity. The DPPH radical scavenging ability of *S. torvum*, *S. macrocarpon*, *S. melongena* BW 11, *S. melongena* and two spinach varieties *B. alba* and *T. paniculatum* ranged from 21.83 ± 1.31 - 93.02 ± 0.30 %. Usually polyphenolic compounds are having the ability to quench DPPH radicals. The higher the percentage of inhibition of free radical activity, the more potent the antioxidant activity of the extract in terms of hydrogen atom-donating capacity [17]. It is apparent that *S. torvum* having the highest DPPH radical scavenging ability, also has the highest total phenolic content (Fig. 4). However, *T. paniculatum* has the lowest radical scavenging ability even though it possesses high amount of total phenolic content (Fig. 4) when compared to others.

Therefore, it does not always support the theory of having a positive correlation between the total phenolic content and DPPH radical scavenging activity, since there may be other compounds that can act as electron donors to DPPH radical which is indicated as an antioxidant activity [18]. The DPPH radical scavenging activity of the two tested varieties of spinach were significantly lower ($p<0.05$) when compared to eggplant varieties ($p<0.05$). Nevertheless, *B. alba* having a lesser total phenolic content than *T. paniculatum* (Fig. 4), has shown a higher DPPH radical scavenging ability. This may be due to the presence of other non-phenolic compounds that can neutralize the DPPH radical indicating an antioxidant activity. According to Foti & Amorati [19], there are few metal ions including $FeCl_3$ (Fe^{3+}) which are also the constituents of antioxidant enzymes such as catalase and superoxide dismutase, that can have an antioxidant effect and help to neutralize free radicals.

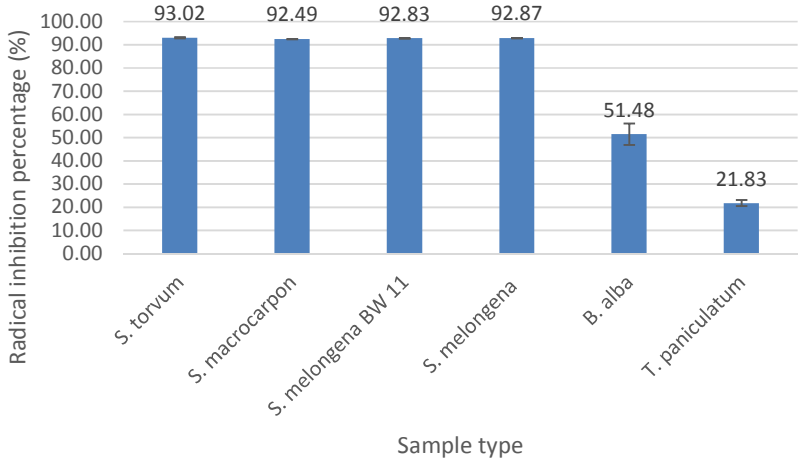

Fig. 1. DPPH radical scavenging ability of methanolic extracts of commonly consumed eggplant and Spinach varieties in Sri Lanka

3.1.2 Total antioxidant capacity

Phosphomolybdenum method was used to assess the total antioxidant capacity (TAC) and the assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [12]. The results ranged from 3.02 ± 0.79 to 9.34 ± 0.13 mg Ascorbic Acid Equivalent per g dry weight of samples. Among the tested eggplant varieties, *S. macrocarpon* has shown the highest TAC, 8.98 ± 1.77 mg AAE per g dw of sample (Fig. 2). However, among all the tested samples the highest TAC (9.34 ± 0.13 mg AAE per g dw) was recorded for *B. alba* leaf extract and the *T. paniculatum* leaf extract had significantly ($p<0.05$) lower TAC than *B. alba* leaf extract. Similar study done by Djouadi et al. [20] reported TAC of 8.50 - 10.91 mg AAE per g dw for Algerian *S. melongena* cultivars. More or less, there may be a synergistic effect between antioxidants such as ascorbic acid and tocopherol which may enhance the antioxidant capacity by regenerating the antioxidant which is oxidized by reducing free radicals [21]. Therefore, TAC should be assessed in terms of tocopherol, without considering only the individual antioxidant effect exerted by Ascorbic acid.

3.1.3 Reducing power assay

The ability of reducing any compound can be a significant indicator of potential antioxidant activity and it may represent the reducing action of phytochemical compounds upon radicals inside the body [22]. Assay is based on the conversion of potassium ferricyanide (Fe^{3+}) to potassium ferrocyanide (Fe^{2+}), by reducing compounds and reaction of potassium ferrocyanide with ferric chloride to form ferric-ferrous complex. Fe^{2+} is directly proportional to the reducing ability of the test substance. Fig. 3 shows a comparison among the reducing power of tested samples and the highest reducing power (111.49 ± 2.56 mg AAE / g dw) was recorded for *S. macrocarpon* extract and the lowest reducing power (4.28 ± 1.46 mg AAE / g dw) was recorded for *S. torvum* extract. Among the two tested spinach varieties *B. alba* leaf extract showed a significantly ($P<0.05$) higher reducing power (58.96 ± 1.38 mg AAE / g dw) when compared to *T. paniculatum* leaf extract (7.24 ± 1.38 mg AAE / g dw).

3.2 Polyphenolic Content

3.2.1 Total phenolic content

The total phenolic content (TPC) of samples was analyzed using Folin-Ciocalteu (FC) assay. Gallic acid, which is a phenolic acid was used as a standard for reference and the TPC was expressed in terms of Gallic acid equivalents. According to Fig. 4, *S. torvum* methanolic extract has shown the highest TPC (670.00 ± 23.00 µg GAE / g dw) and the second highest TPC (262.15 ± 39.40 µg GAE / g dw) was recorded by *T. paniculatum* methanolic extract among the analyzed vegetable samples. The lowest TPC content (2.49 ± 1.10 µg GAE / g dw) among the

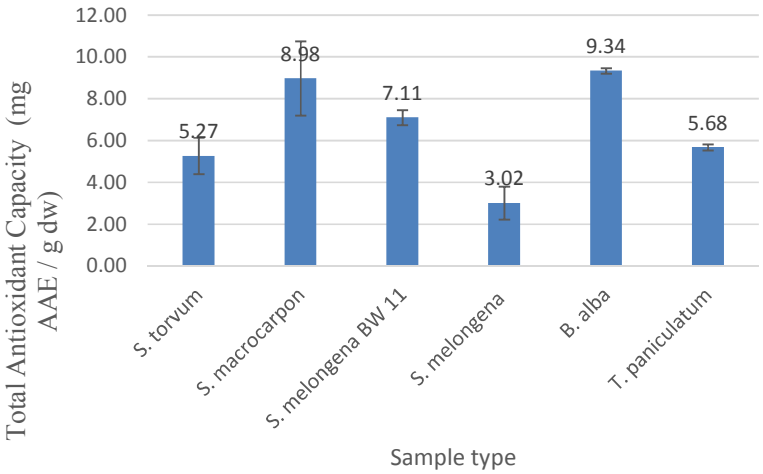

Fig. 2. Total antioxidant capacity of methanolic extracts of commonly consumed eggplant and Spinach varieties in Sri Lanka

tested was shown by methanolic extract of *B. alba* leaf. Tongco et al. [23] reported a TPC of 93.89 mg GAE/g extract for ethanolic extract of *B. alba* leaf. Nonetheless, the FC reagent can react with not only the phenolic compounds but also with the non-phenolic compounds that may be available in the extracts. There was a significant difference between the recorded TPC for all the tested samples and *S. macrocarpon* and two varieties of *S. melongena* L. showed much lower TPC when compared to *S. torvum.* Somawathi et al. [24] reported much higher values of TPC for *S. melongena* extracts (48.67±0.27 - 61.11±0.26 mg GAE/100 g fresh weight) and the variations may be due to the selected cultivars and their geographical state of growth. Djouadi et al. [20] reported 20.14- 30.51 mg GAE/g dw of TPC for ethanolic extracts of *S. melongena* cultivars. However, as reported by Šilarová et al. [25] and Lo Scalzo et al. [26], the most abundant phenolic compounds in *S. melongena* are chlorogenic acid and caffeic acid. Accordingly, the TPC recorded in terms of chlorogenic acid for *S. melongena* was 154 mg/100 g of dry matter and TPC in terms of caffeic acid was 12.8 mg/100 g of dry matter [26].

However, there were no sufficient evidences from previous studies to prove the highest TPC recorded for *S. torvum* in terms of Gallic acid, although several studies reported TPC in tannic acid equivalents [27]. Ve et al. [28] reported TPC of 2.357 mg Tannic acid equivalent/g extract of *S. torvum* fruit. Moura et al. [9] reported a TPC of 17.8± 0.90 mg GAE/100 g for *T. paniculatum* leaf extract.

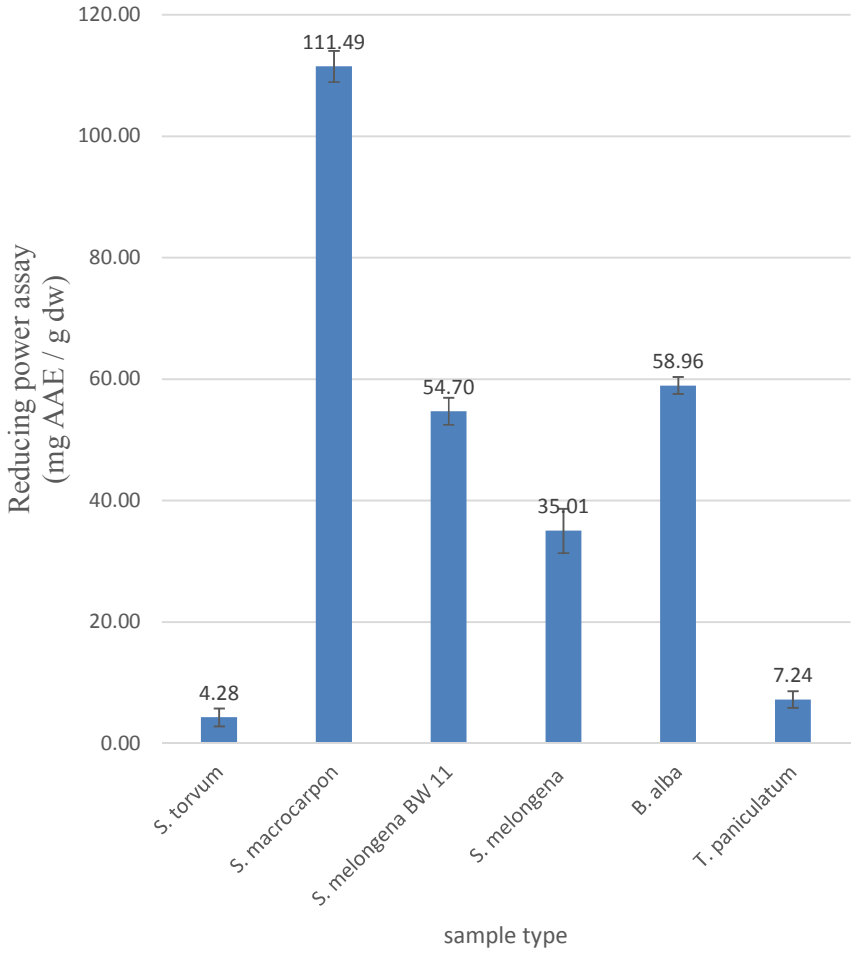

Fig. 3. Reducing power of methanolic extracts of commonly consumed eggplant and Spinach varieties in Sri Lanka

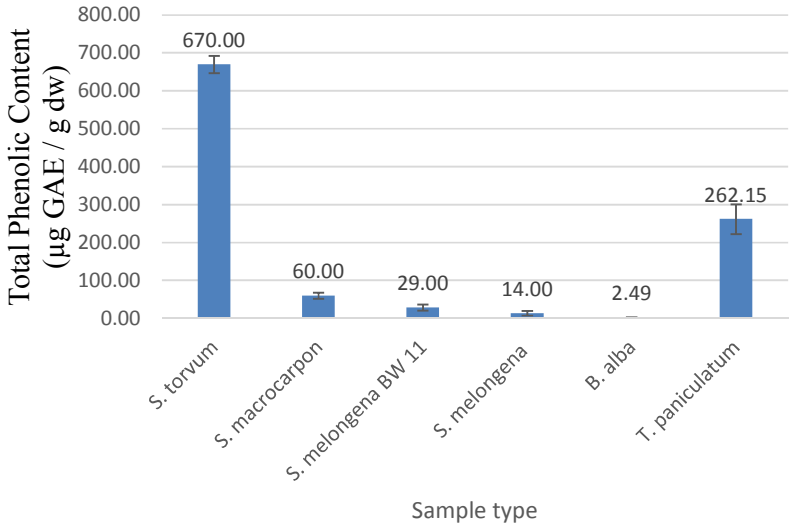

Fig. 4. Total phenolic content of methanolic extracts of commonly consumed eggplant and Spinach varieties in Sri Lanka

3.2.2 Total flavonoid content

Flavonoids are a sub group among diverse and vast phenolic compounds. The total flavonoid content (TFC) was evaluated using aluminium chloride method and expressed as Rutin equivalents. Rutin is a flavonol which is a sub class of flavonoid that is abundant in citrus fruits, berries, apples, peaches and green tea [29]. Among the vegetable samples tested, the TFC was very much higher in *S. torvum, S. macrocarpon* and the two tested varieties of *S. melongena* when compared to the TFC of two tested spinach varieties (Fig. 5). Among the tested varieties the highest TFC (62.51 ± 2.00 mg RE / g dw) was recorded for the methanolic extract of *S. melongena* BW 11 variety and the lowest TFC (1.55 ± 0.09 mg RE / g dw) was recorded for *T. paniculatum* methanolic extract. There was a significant difference in TFC of two spinach varieties, tested and *B. alba* leaf extract had the highest TFC among two varieties. Tongco et al. [23] reported TFC of *B. alba* leaf

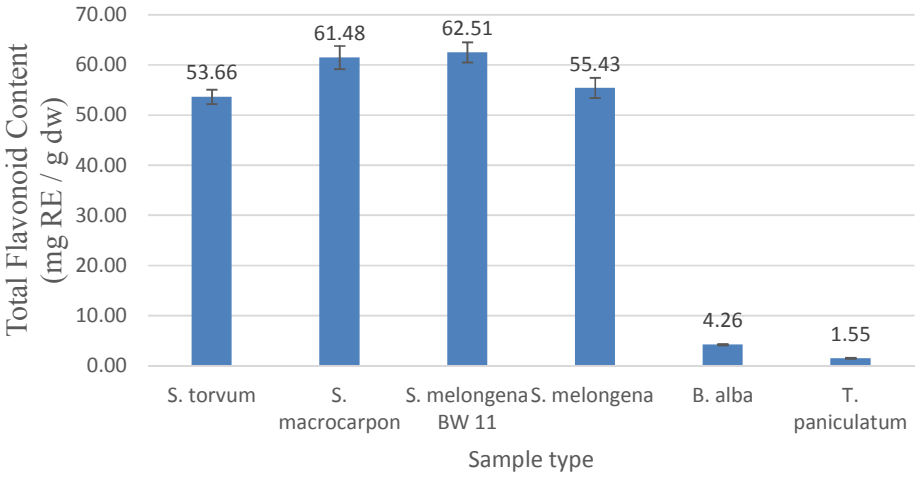

Fig. 5. Total flavonoid content of methanolic extracts of commonly consumed eggplant and Spinach varieties in Sri Lanka

extract in quercetin equivalents. Ve et al. [28] reported TFC of *S. torvum* fruits as 1.95 mg RE/g extract. However, in the present study, the cause for having high TFC and lower TPC for a same species (*S. torvum*– TFC 53.66 mg RE / g dw and TPC 670.00 µg GAE / g dw) or vice versa may be due to the presence of other phenolic compounds that is not accountable in Gallic acid equivalents or having other sub groups of flavonoid compounds such as C-4 sulfated isoflavonoids including torvanol A which is a prominent compound in *S. torvum* [30] and not accountable in Rutin equivalents. Nevertheless, previous studies reported high content of flavonoid compounds like rutin and quercetin in different eggplant species [5]. As indicated by Lo Scalzo et al. [26], *S. melongena* peel contains Delphinidin-3-rutinoside which is a major anthocyanin compound and one of the most important flavonoid. Singh et al. [31] was the first to report trace quantities of three additional flavonols, namely, quercetin-3-glucoside, quercetin-3-rhamnoside, and myricetin-3-galactoside in freeze-dried eggplant pulp. Chinedu et al. [32] reported the presence of saponins, flavonoids, tannins and ascorbic acid in *S. macrocarpon* fruits even though they were not quantified.

4. CONCLUSION

In conclusion, this study has evinced that locally available commonly consumed eggplant varieties and spinach varieties are considerable sources of polyphenols and having a remarkable antioxidant activity. Among the tested eggplant varieties *S. macrocarpon* methanolic extract had higher antioxidant capacity and flavonoid content. However, from the two tested spinach varieties *B. alba* leaf extract had higher antioxidant activity but lower flavonoid content than *T. paniculatum* leaf extract. The presence of higher flavonoid content in methanolic extracts of all eggplant varieties among the tested vegetable samples may be contributed by the anthocyanin compounds which is a sub class of flavonoids. Hence the study should be carried out further to examine and elucidate phytochemicals compounds available in tested vegetable samples. The potential of them to be utilized in food and nutraceutical industry is high and yet to be investigated.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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